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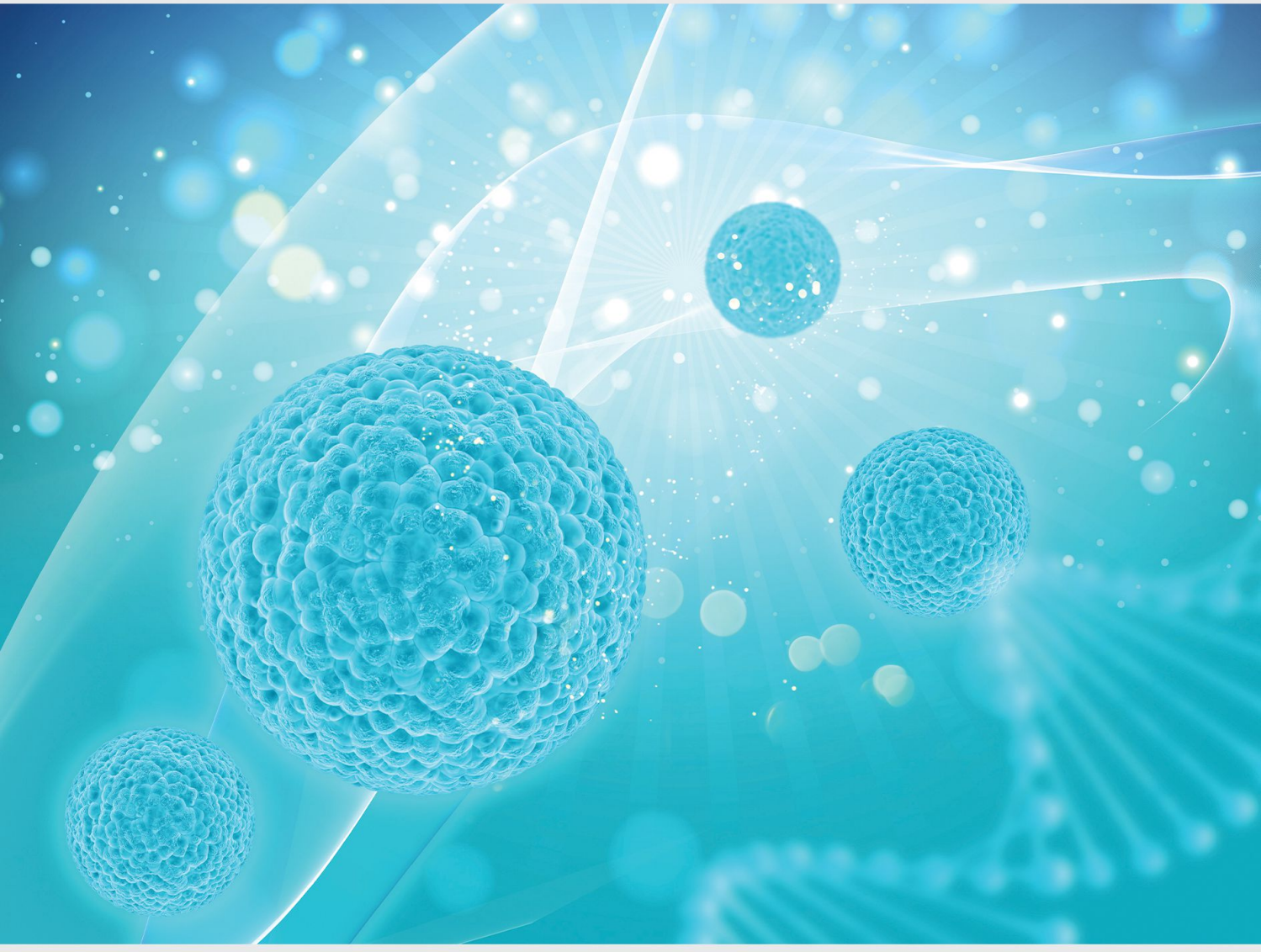

Editor
İnan KAYA

LIVRE DE LYON

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Cancer Biology

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Editor • Prof. Dr. İnan KAYA • Orcid: *0000-0003-2900-4067*
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website • http://www.livredelyon.com
e-mail • livredelyon@gmail.com

LIVRE DE LYON

PREFACE

Dear readers,

Cancer is a disease condition that involves the uncontrolled proliferation, growth and spread of cells in a living organism. There are many types of cancer according to the tissue in which it develops (lung, breast, colon, pancreas, etc.) or cell type (carcinoma, sarcoma, leukemia, lymphoma, melanoma, etc.). Today, the most common causes of deaths relation to cancer are lung, stomach, liver, colon and breast cancer. With the increase in the human population exceeding 8 billion in the 21st century, the most effective reasons for the development of cancer are the damage of genetic material inherited in the family or by carcinogenic factors such as toxins, chemicals, malnutrition, weakening of immunity, radiation or old age, depending on the range of products that are developed later. The predisposition of genetic material to cancer development varies among individuals in terms of acquisition. Long-term exposure of cells to carcinogenic substances causes mutations in the genetic material and uncontrolled proliferation in the tissue occurs if immunity is weak. Uncontrolledly proliferating cells become metastatic to spread to distant tissues. A total of 10 chapters in this book have been prepared by scientists who are experts in their fields to provide examples. We ask for the tolerance and feedback of the readers if there are any overlooked errors in the book. I would like to express my gratitude to publishing and authors who contributed to this book thinking that it will serve as an important example for readers and researchers about cancer studies conducted today.

Kind regards,
Prof. Dr. İnan KAYA, 2023

CONTENTS

CHAPTER I

THE RELATIONSHIP BETWEEN CANCER STEM CELLS AND EPİTHELİAL-MESENCHYMAL TRANSITION: IMPLICATIONS FOR TUMOR HETEROGENEITY, METASTASIS, THERAPY RESISTANCE AND RECURRENCE

HIBA HUSSEIN A. KHAIR[1] & IŞIK DIDEM KARAGÖZ[2]

[1] Gaziantep University, Department of Biology,
E-mail: hibakhair2@gmail.com,
ORCID: 0000-0001-8545-2425

[2]Gaziantep University, Department of Biology,
E-mail: *karagozid@gmail.com,*
ORCID: 0000-0001-6527-2750

1. Introduction

The discovery of cancer stem cells (CSCs) has significantly advanced our understanding of cancer biology and treatments. Cancer stem cells are a distinct subset of cells within tumors that possess self-renewal and differentiation capacities (Clarke et al., 2006). Epithelial-to-mesenchymal transition (EMT) is a crucial process in the invasion-metastasis cascade through which cancer cells acquire mesenchymal features such as increased motility and invasiveness (Brabletz et al., 2018). This process is closely linked to the generation and maintenance of cancer stem cells (Shibue & Weinberg, 2017). CSCs frequently undergo (EMT), its reverse process mesenchymal–epithelial transition (MET), and a hybrid or partial phase of EMT, which enhance their migration, invasion and dissemination to distant sites, and ultimately result in

poor patient outcomes (Ciriello & Magnani, 2021). The association between EMT and CSCs accounts for the plasticity of tumor cells and their ability to acquire stem cell-like properties, which contribute to tumor heterogeneity, therapy resistance, and recurrence (Kapoor-Narula & Lenka, 2022). Therefore, the elimination of CSCs presents a potential opportunity to not only eradicate the primary tumor but also to gain a prolonged tumor-free period and prevent cancer metastasis (Liang & Kaufmann, 2023). In this context, targeting the heterogeneity and plasticity of tumor cells is indispensable for designing effective therapeutic strategies (Yang et al., 2020).

In this section, we review the development of the CSC concept, focusing on its functional and molecular properties and their role in the metastatic process. We further recapitulate the link between CSCs and EMT/MET and their crosstalk within the tumor microenvironment. Finally, we highlight potential therapeutic strategies that target these critical drivers of cancer progression.

2. Emergence of the Cancer Stem Cells (CSCs) Concept

Early studies suggested that tumor growth is initiated by a rare population of cells with stem-like properties. However, this concept has gained increasing attention in the field of cancer research over the past few decades. Over 160 years ago in the 1860s, Rudolf Virchow and Julius Cohnheim proposed that cancer originates from the remnants of embryonic cells found in mature tissues (Capp, 2019). Since then, there have been accumulating observations that not all cells within a tumor possess the same ability to initiate and sustain tumor growth. In 1937, Furth and his colleagues conducted an experiment where they injected a small number of leukemic cells into mice and discovered that even one malignant leukocyte could initiate leukemia, unveiling the tumorigenic capability of particular tumor cells (Furth et al., 1937). The ‘embryonal carcinoma cells’ from teratocarcinomas were demonstrated to be multipotent (Pierce & Verney, 1961) with variable abilities both *in vitro* and *in vivo* (Kleinsmith & Pierce, 1964). Distinct subgroups of cells with sustainable tumorigenesis capacity were isolated from murine lymphoma tumor mass (Bruce & Van Der Gaag, 1963). Only a small number of hematopoietic cells with the ability to extensively proliferate, differentiate, and self-renew were found in the hematopoietic tissue, which further supports the concept of CSCs (Till et al., 1964).

Further research in the 1990s and 2000s provided more evidence to support the existence of cancer stem cells. For example, advanced techniques such as Fluorescence-activated cell sorting (FACS) and mouse xenograft models allowed

researchers to isolate and characterize cell subpopulations with stem cell-like properties from various types of tumors (Liang & Kaufmann, 2023). The first validation of the existence of cancer-initiating cells was the characterization of these cells in acute myeloid leukemia (AML) based on the expression of the $CD34^{+}CD38^{-}$ cell surface markers and their tumor-forming ability in immunocompromised mice (Lapidot et al., 1994). A small subpopulation of breast cancer cells expressing the $CD44^{+}CD24^{-/low}$ surface marker was identified that could form sustainable tumors, which enabled the differentiation between tumorigenic cancer cells and non-tumorigenic cells and highlighted the heterogeneity of breast cancer (Al-Hajj et al., 2003). These findings paved the way for the identification and isolation of CSCs in various cancers, such as brain, lung, prostate, pancreatic, head and neck, colon, melanoma, bone, ovarian and liver cancers (Liang & Kaufmann, 2023), confirming the significant role of these cells in the initiation, progression, and recurrence of tumors and supporting the concept of CSCs.

2.1. Characteristics of CSCs

CSCs typically exhibit many embryonic or tissue stem cell properties, such as self-renewal and differentiation (Paul et al., 2022). The asymmetric division of a CSC produces two cells with unequal sizes and different fates; one of these cells is a daughter CSC that can proliferate indefinitely through self-renewal, while the other cell is a phenotypically distinct cancer cell that has a limited lifespan and proliferative capacity and integrates into the tumor mass through a differentiation-like mechanism (Conde et al., 2022).The abnormal increase in self-renewal, in combination with the inherent extensive proliferation capability of cancer stem cells, may explain much of what is considered as a malignant phenotype (Jordan et al., 2009).

In vitro clonogenic assays, such as sphere formation, organoid culture, and co-culture experiments, have been developed to study tumor heterogeneity and proliferation, differentiation, and self-renewal capacities (Liang & Kaufmann, 2023). Previous studies used a sphere-formation assay to study neuronal stem cells from adult brain tissue that can form neurospheres in a serum-free culture medium (Reynolds & Weiss, 1992). In another study, human skeletal muscle cells were expanded in vitro as myospheres that maintained proliferative capacity and expressed skeletal progenitor cell markers ALDH1, Myod, Desmin, and Pax7, as well as stem cell markers Sox2, Oct3/4, and Nanog and spontaneously differentiated into myotubes and other mesodermal cell lineages (Wei et al., 2011).

Aldehyde dehydrogenase 1 (ALDH1), a detoxifying enzyme involved in retinoic acid production by the oxidation of all-trans-retinal and 9-cis-retinal (Marcato et al., 2011), is used as a reliable CSC marker in various types of cancers including head and neck cancer (Yao et al., 2021), throat cancer (Qian et al., 2013), and ovarian cancer (Guo F. et al.,2019). For instance, high activity of the ALDH enzyme was reported in breast cancer stem cells (BCSCs) spheroids (Yang et al., 2019).

Expression of specific cell surface markers such as CD44, CD133, CD117, CD166, Nestin, ABCG2, ESA, Ep-CAM, CXCR4, and CXCL12 has been identified as CSC markers in various tumor types (Agliano et al., 2017). Stemness-related transcription factors (TFs) Nanog, Oct3/4, and Sox2 are also overexpressed in CSCs (Agliano et al., 2017; Paul et al., 2022).

CSCs also share with normal stem cells the activation of one or more of the highly conserved signaling pathways implicated in development, tissue homeostasis, self-renewal and differentiation such as the Wnt, BMP, Notch, and Hedgehog pathways (Wang et al., 2015). Deregulated expression of these pathways plays a crucial role in enabling CSCs to retain stem-like features and the capability of tumor initiation. Such pathways may represent important therapeutic targets for disrupting self-renewal and proliferation of CSCs and subsequently blocking tumor progression (Merchant & Matsui, 2010).

Dormancy or quiescence allows CSCs to grow slower than other tumor cells, enter a state of low metabolic activity, and evade the effects of repeated cycles of chemotherapy and/or radiation therapy by retaining proliferative, metastatic, and tumor regrowth capacities (Talukdar et al., 2019; Phan & Croucher, 2020). They can remain undivided and quiescent until they are triggered by favourable conditions to proliferate (Liang & Kaufmann, 2023). Therefore, CSCs have a slow cycling pattern when migrating, trafficking through blood and/or lymph vessels, and invading target sites during the invasion–metastasis cascade (Talukdar et al., 2019; Phan & Croucher, 2020); and, once adapted to the new tumor microenvironment, they can rapidly reproliferate (Conde et al., 2022). The recurrence of a tumor at the primary site or in other parts of the body, which may occur years after the initial diagnosis, can be explained by this phenomenon (Talukdar et al., 2019). CSCs have been shown to be arrested in the G0/G1 phase of their cell cycle, allowing them to survive and undergo cellular reprogramming as an adaptive response to the tumor microenvironment (Phan & Croucher, 2020).

Elevated expression of specific markers associated with CSCs, including CD34, Nanog, Sox2, and Oct4, has only been observed in a subset of dormant

cancer cells (Phan & Croucher, 2022). Furthermore, a partial overlap has been observed between quiescent slow-cycling cells and CSCs in various solid tumors, including pancreatic cancer, colorectal cancer (CRC), melanoma, and glioblastoma (Liang & Kaufmann, 2023). These studies demonstrate that CSCs can survive under stressful conditions, for example, hypoxia and exposure to traditional cancer treatments, by remaining dormant with low energy consumption and slow cell division. Therefore, new treatment modalities that selectively target these cells are needed to control stem cell survival, proliferation, and differentiation (Leon et al., 2016).

Multiple drug resistance is another characteristic of CSCs that enables them to survive when exposed to toxic substances, including many currently used conventional therapies to treat cancer (Shibue & Weinberg 2017; Ciriello & Magnani, 2021). CSCs resistance to therapy can be attributed to a combination of mechanisms, including protection from oxidative stress (Yoshida & Saya, 2021), increased expression of the antiapoptotic proteins such as Bcl-2 family members (Castelli et al., 2021), increased expression of the multi-drug resistance (MDR) proteins and the drug efflux pumps, adenosine triphosphate–binding cassette (ABC) transporters, such as ABCG2 or BCRP which efflux the drugs out of the cell (Frank et al., 2005), cell cycle dormancy as well as high activity levels of aldehyde dehydrogenase 1 (ALDH1) (Raha et al., 2014). In addition, CSCs have great capacity to evade immune responses via downregulating the expression of tumor-associated antigens or specific ligands and thus escaping from T cells or natural killer cells, respectively (Wang et al., 2014).

These stemness phenotypic characteristics allow CSCs to resist traditional cancer treatments and contribute to tumor recurrence and metastasis. Since drug-resistant tumors are attributed to the presence of CSCs, revealing the biological nature of those cells and developing novel therapeutic strategies aimed at specifically targeting and eradicating them is of tremendous importance to prevent metastasis and tumor recurrence (Liang & Kaufmann, 2023).

3. Epithelial-Mesenchymal Transition (EMT)

Metastasis is the process by which tumors spread from their primary site to distant sites via lymph and/or blood (Chaffer & Weinberg, 2011). About 90% of all tumor mortalities occur as a result of metastasis as opposed to primary tumors (Mehlen & Puisieux, 2006). It is a complex and multi-step process involving primary tumor growth, angiogenesis, epithelial-to-mesenchymal transition (EMT), invasion through the extracellular matrix, intravasation into the blood and/or lymphatic vessels, survival during systemic circulation, endothelial cells

docking at the secondary site, trans-endothelial migration/extravasation and invasion through the basal lamina, tissue parenchyma, and subsequent secondary tumor growth in distant organs (Lambert et al., 2017).

Epithelial cells, typically organized in tightly packed layers and maintaining cell-cell junctions, undergo a biological process known as epithelial-mesenchymal transition (EMT), where they transform into mesenchymal cells, characterized by elevated production of extracellular matrix (ECM) components, enhanced motility, invasiveness, and apoptosis resistance (Kalluri & Weinberg, 2009). A schematic representation of the characteristics of epithelial and mesenchymal cells is given in Figure 1. Loss of cell adhesion occurs when the epithelial marker and adherent junction molecule E-cadherin is downregulated, and the mesenchymal markers, vimentin and N-cadherin, are upregulated (Ciriello & Magnani, 2021). Similarly, mesenchymal cells transform back to epithelial ones by undergoing the reverse process, known as mesenchymal-epithelial transition (MET) (Marconi et al., 2021). EMT involves a complex network of epigenetic and transcriptional reprogramming processes that can be induced by different signaling pathways and interactions within the tumor microenvironment (Tsai & Yang 2013).

Regulation of EMT occurs at multiple levels, including transcriptional, differential splicing, post-translational modification, and non-RNA regulation (Li et al., 2023). EMT is primarily induced by transcription factors such as TWIST, E47, SNAIL, SLUG, ZEB1, and SIP1, which exert their function by suppressing the expression of E-cadherin (Peinado et al., 2007). Additional EMT-related transcription factors (EMT-TFs) have been identified, including E2-2, E47, Gata3, HMGA2, Zeppo, Goosecoid, KLF8, FOXC2, Prrx1, Six1, Pit-1, and Brachyury (Nieto & Cano, 2012). Besides E-cadherin, EMT-TFs also target genes encoding essential proteins for maintaining tight junctions or desmosomes in epithelial cells, such as occludin, claudin, plakophilin, and desmoplakin (Sato et al., 2016). Furthermore, EMT-TFs also induce the expression of mesenchyme-related markers such as fibronectin, N-cadherin, and vimentin, resulting in a "cadherin switch," an EMT hallmark characterized by the decrease in E-cadherin expression and the increase in N-cadherin expression (Sato et al., 2016). As an EMT suppressor, NKX6.1 promotes E-cadherin transcription by directly recruiting the coactivator BAF155 and recruiting the corepressor retinoblastoma binding protein 7 (RBBP7) to repress the transcription of the vimentin and N-cadherin genes (Li et al., 2016). These various transcription factors act cooperatively and are activated in a coordinated

manner but not necessarily at the same time (Shibue & Weinberg, 2017; Ciriello & Magnani, 2021).

Several pathways, including TGFβ-SMAD, ECM-integrins, canonical and non-canonical Wnt, Notch, NF-κB, and growth factor (such as EGF, PDGF, HGF, IGF, and FGF)-tyrosine-kinase receptors, are involved in EMT regulation (Ye et al., 2015; Shibue & Weinberg, 2017). Various dynamic cues from the surrounding microenvironment, such as cytokines, growth factors, contact with the extracellular matrix (ECM), and hypoxia, activate these pathways (Liang & Kaufmann, 2023). Functional interaction between these pathways might result in signal amplification, inducing EMT and metastasis (Wang et al., 2015).

The miR-200 family, a well-known example of miRNAs that regulate the EMT program, downregulates the expression of ZEB1 and ZEB2, whereas the expression of miR-200 miRNAs is reciprocally repressed by ZEB1 and ZEB2, generating a double-negative feedback loop (Burk et al., 2008; Gregory et al., 2008). MiR-34 family members downregulate snail expression, while snail inhibits miR-34 expression, establishing a double-negative regulatory feedback loop (Siemens et al., 2011).

EMT plays pivotal roles in both physiological processes such as embryonic development, wound healing, and tissue regeneration, and pathological events such as fibrosis and cancer progression (Dongre & Weinberg 2019). Therefore, cancer cells can hijack cell plasticity as a "pre-built system" required for physiological processes to promote their invasive and metastatic potential (Ciriello & Magnani, 2021).

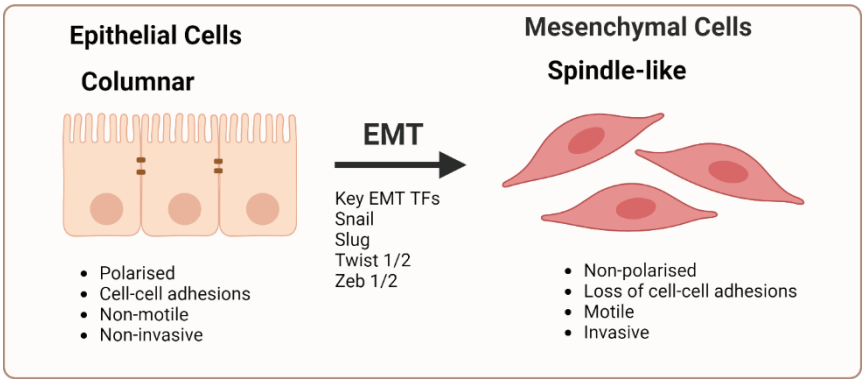

Figure 1. A schematic representation of the characteristics of epithelial and mesenchymal cells and the key transcription factors involved in the EMT process (Shu, 2023).

3.1. Link between CSCs and EMT

Several lines of evidence suggest that EMT promotes the acquisition of CSC properties, resulting in increased tumor-initiating capacity, invasion, metastasis, resistance to therapy, and cancer recurrence in many types of solid tumors (Lytle et al., 2018). The link between CSCs and the EMT is complex and multidirectional. It involves reciprocal interactions and feedback loops, with EMT promoting stem cell-like properties in cancer cells and cancer stem cells undergoing EMT to acquire invasive and migratory characteristics (Wang & Unternaehrer, 2019). This crosstalk contributes to tumor heterogeneity, therapy resistance, and metastasis, highlighting the importance of targeting CSCs and EMT for developing effective therapeutic strategies.

EMT-associated markers such as TGF-β, vimentin, ZEB1, ZEB2, TWIST1, SNAI1 and SNAI2 are highly expressed in CSCs (Polyak & Weinberg, 2009) and promote their maintenance in various types of cancers (Lytle et al., 2018). EMT-inducing transcription factors have been linked to the acquisition of stem cell properties such as tumorsphere formation (Mani et al., 2008; Morel et al., 2008), and activation of the expression of transcription factors involved in stem cell programs such as SOX2 and KLF4 (Wellner et al., 2009).

Mammary epithelial cells that have undergone EMT by overexpression of TWIST, SNAIL (Mani et al., 2008), or TGF-β treatment (Morel et al., 2008) have acquired a $CD44^{+}/CD24^{-}$ phenotype, stem cell expression profile, the capacity to form mammospheres (Mani et al., 2008; Morel et al., 2008), and to differentiate into mammary ductal outgrowths in xenotransplantation assays (Mani et al., 2008). $CD44^{+}/CD24^{-}$ cell subpopulations isolated from normal breast tissues and primary breast cancer tissues expressed high levels of mesenchymal markers such as TWIST1, TWIST2, SNAI1, SNAI2, ZEB2, FOXC2, N-cadherin, fibronectin, and vimentin, as well as low levels of E-cadherin compared to the more differentiated $CD44^{-}/CD24^{+}$ epithelial cells (Mani et al., 2008).

The transient coexpression of Slug and Sox9 converted differentiated mammary luminal cells into stem-like cells (Guo et al., 2012). In addition, it was revealed that Slug stabilizes Sox9 and thus enhances the expansion of lung cancer stem cells (Luanpitpong et al., 2016). Cancer stem cells enriched from breast cancers and metastatic breast pleural effusions express the same markers as EMT-undergoing cells (Mani et al., 2008; Morel et al., 2008). In addition, EMT and CSC markers are frequently linked with metastatic-prone cancers, for example, basal-like breast cancer (Blick et al., 2010) and metaplastic breast cancer (Imani et al., 2016).

The overexpression of Snail in the subpopulation of prostate cancer cells with epithelial characteristics resulted in the suppression of stem cell features; conversely knockdown of EMT-TFs such as Twist2, Snail, and ZEB1 in the subpopulation with mesenchymal characteristics promoted the growth of cells independent of anchorage and the expression of gene network related to self-renewal and epithelial phenotype (Celià-Terrassa et al., 2012). Snail expression has also been associated with the undifferentiated phenotype in cancer types such as gastric, breast, pancreatic, and colon, indicating that differentiated cancer cells undergoing EMT display less differentiation and a more CSC-like phenotype (Wang & Unternaehrer, 2019). The $EpCAM^-$ subpopulation expressed a higher level of Snail, which was associated with an increase in the expression of mesenchymal markers N-cadherin and Vimentin and a decrease in the expression of the epithelial marker E-cadherin, whereas the $EpCAM^+$ subpopulation expressed higher level of Slug expression, indicating that Snail, rather than Slug, was associated with the CSC phenotype (Ye et al., 2015).

Sox2 has been shown to promote the expression of Snail and induce the expression of mesenchymal markers N-cadherin and vimentin but reduce the epithelial cell marker E-cadherin, increase cell proliferation, enhance cell motility, chemoresistance, and sphere-forming ability in vitro in colorectal cancer (CRC) cells by activating the Sox2-β catenin/Beclin1/autophagy signaling axis (Zhu et al., 2021). Another study found that CRC cells with high expression of Sox2 exibited stem-like features, such as a $CD133^+$ phenotype, enhanced sphere formation, increased migratory and invasive activity, and poor differentiation (Chen et al., 2020).

CSC formation and acquisition of the EMT phenotype have been shown to be regulated by some miRNAs (Wang & Unternaehrer, 2019). It has been reported that pancreatic cancer cells with the EMT phenotype display stem-like cell features, which is associated with the downregulation of miR-200 and/or the let-7 family, suggesting that miR-200 and let-7 could link cancer stem-like cells with EMT (Wang et al., 2015). Self-renewal genes such as Myc, Sall4, and Lin28 are suppressed by Let-7 (Melton & Blelloch, 2010). The miR-200 family regulates EMT via targeting of ZEB1 and ZEB2 (Gregory et al., 2008) and also contributes to CSCs regulation by targeting Bmi-1, Sox2 and KLF-4 (Wellner et al., 2009), a CSC-related marker. MiR-93 induces MET associated with a decrease in TGFß signaling and downregulates the expression levels of some genes involved in stem cell regulation, such as JAK1, SOX4, AKT3, HMGA2, and EZH1, causing depletion of CSCs (Liu et al., 2012). Another study

showed that blocking miR-21 can reverse the EMT and CSC characteristics by targeting PTEN and inactivating the AKT and ERK1/2 pathways (Han et al., 2012). The repression of miR200c mediated by the loss of tumor suppressor p53 in mammary epithelial cells has been demonstrated to cause EMT induction and CSC enrichment, indicating that activation of the p53-miR200c pathway could be a therapeutic strategy for inhibiting EMT-related CSCs (Chang et al., 2011). The acquisition of EMT and CSC phenotypes in pancreatic cancer is assocaited with the overexpression of FoxM1, which is partly mediated through miR-200b regulation (Bao et al., 2011). EMT-related factors also contribute to stemness regulation by inhibiting various differentiation pathways, including tumor necrosis factor alpha (TNF-α), NF-kB, p53 and β-catenin (Wang & Unternaehrer, 2019).

CSCs have been reported to undergo MET at distant sites without losing their stemness features, suggesting that the EMT and stemness regulatory feedback loops, such as Lin28/let7 and Zeb1/miR-200/BMI1 or Zeb1/miR-200/Notch axis might confer stemness to tumor cells by controlling EMT decision making and determining the position of a "stemness window" on the EMT axis (Jolly et al. 2015). Other examples of association between EMT and stemness include Slug-induced CSC self-renewal ability and transcriptional activation of BMI1 by Twist (Zhang et al. 2021). However, the molecular mechanisms by which EMT-TF activation is linked to cellular stemness and chemoresistance need further elucidation in future studies.

3.2. Phenotypic Plasticity, Partial EMT, and Tumor Heterogeneity

There are multiple dimensions for intratumoral heterogeneity, including diverse states of drug sensitivity/resistance, CSCs/non-CSCs, and spectrum of hybrid-epithelial/mesenchymal phenotypes (Jain et al., 2022). Genetic mechanisms such as mutations, and chromosomal instability and epigenetic factor as well as the tumor microenvironment cues drive both dynamic phenotypic plasticity and intratumoral heterogeneity (Biswas & De al.,2021).

EMT and its reverse, MET, are the most well-known and thoroughly studied examples of "cancer cell plasticity" (Ciriello & Magnani, 2021). The invasive front of the primary tumor in various types of carcinomas frequently shows indications of EMT activation, such as the decreased expression of E-cadherin, whereas cells that trail behind typically exhibit multiple epithelial characteristics and maintain strong cell-to-cell adhesion (Shibue & Weinberg, 2017). During the process of carcinoma invasion, there is a dynamic exchange

between the cell phenotypes of 'leaders' and 'followers', suggesting phenotypic platicity and EMT reversibility in cancer cells (Shibue & Weinberg, 2017).

Hybrid EMT states and phenotypic plasticity play crucial roles in tumorigenicity, stemness, heterogeneity and the progression of tumors (Kröger et al., 2019). Cells with hybrid E/M phenotype express epithelial and mesenchymal markers concurrently indicating a partial activation of the EMT program and exhibiting remarkable diversity even within the same tumor (Shibue & Weinberg, 2017: Brabletz et al., 2018). A small subset of triple-negative breast cancer (TNBC) cells with the $ITGB4^{+}CD44^{+}CD24^{-}$ phenotype was reported to exhibit a hybrid/partial E/M phenotypic state with the potential for use as a prognostic marker (Bierie et al., 2017). Snail and canonical Wnt signaling can drive the acquisition of a highly tumorigenic E/M hybrid state in breast cancer cells (Kröger et al., 2019). However, transitioning from this state to a fully mesenchymal phenotype via constitutive ectopic expression of Zeb1 resulted in a significant loss of tumorigenicity and a shift from canonical to noncanonical Wnt signaling (Kröger et al., 2019). Moreover, Zeb 1 expression has been shown to induce stemness, tumorigenicity, and tumor cell plasticity in pancreatic cells, and higher tumorigenic capacity was observed in differentiated epithelial cells compared to undifferentiated mesenchymal cells, indicating that the dynamic EMT-MET transitions contribute to EMT plasticity and stemness (Liu et al., 2021).

Phenotypic plasticity allows CSCs to transition between stem cell and non-stem cell states, further contributing to the heterogeneity observed within tumors (Kapoor-Narula & Lenka, 2022). It enables the dynamic evolution of cancer cell populations, allowing for adaptability and survival in different microenvironments (Biswas & De, 2021). Furthermore, extrinsic or intrinsic cues including EMT induction and microenvironmental stimuli such as hypoxia, cytokines and cell-cell interactions can trigger the transition of non-CSCs to CSCs, acquiring stem cell-like properties and promoting tumor invasion and metastasis (Tanabe et al., 2020; Ciriello & Magnani, 2021). CSCs are, in turn, capable of modulating their respective microenvironment and employing various signaling pathways to maintain their homeostasis through hypoxia, inflammation, EMT, and angiogenesis processes (Tanabe et al., 2020).

Phenotypic plasticity involves various mechanisms, including stochastic gene expression, chromatin reprogramming, asymmetric cell division, and destabilization of specific gene expression patterns that correspond to a specific cell state in a multi-dimensional space known as "attractors" (Jain

et al.,2022). For example, a computational modeling technique revealed that epithelial-mesenchymal heterogeneity can arise due to the noise in partitioning biomolecules, including RNAs and proteins, among the resulting daughter cells during cancer cell division (Tripathi et al., 2020). A mathematical modeling framework developed by Jain et al. (2022) suggests that spontaneous phenotypic switching and resulting dynamic heterogeneity in PMC42-LA cells, which have been observed experimentally at both single-cell and bulk-level analysis, can be explained by fluctuations or noise in the duplication and partitioning of SNAIL, a transcription factor that induces EMT, during cell division, indicating that asymmetric cell division could be a mechanism for phenotypic heterogeneity.

Hybrid EMT states and phenotypic plasticity endow cancer cells with the ability to adapt, survive, and resist therapy, ultimately leading to treatment failure. Therefore, unveiling the cellular and molecular mechanisms of hybrid EMT states, CSC plasticity, and tumor heterogeneity is crucial to overcoming therapy resistance and improving the clinical outcomes of patients.

3.3. Interplay between CSCs and EMT in the Tumor Microenvironment (TME)

The tumor microenvironment (TME) consists of cancer cells surrounded by stroma cells, extracellular matrix (ECM) components, and blood and lymphatic vessels interacting with each other (Liang & Kaufmann, 2023). Accumulating evidence suggest that the activity of carcinoma cells is highly influenced by both resident and recruited non-neoplastic cell types present within the tumor stroma (Shibue & Weinberg, 2017). These stromal cells can be classified into three primary categories: vascular cells that facilitate angiogenesis, immune cells that infiltrate the tumor, and fibroblasts that are associated with carcinomas (CAFs) (Hanahan & Coussens, 2012). There are other components in the TME, such as exosomes and microvesicles, and soluble factors, such as cytokines, growth factors, and hormones (Liang & Kaufmann, 2023). These various biological, chemical, and mechanical factors available in TME endow CSCs with the essential elements to mediate the ECM remodeling and regulate proliferation and self-renewal associated signals by transcription factors and microRNAs, thereby sustaining their stemness (Prager et al., 2019). A schematic representation of the components of tumor microenvironment is given in Figure 2.

CSCs and EMT can be triggered by stress factors in the tumor microenvironment (Liang & Kaufmann, 2023). The factors that can cause such stress include hyoxia, high levels of reactive oxygen species, insufficient

nutrients, acidic pH, mechanical pressure, the presence of pro-inflammatory cytokines such as TGF-β, TNF-α, IL-1B, and IL-6 and treatment with genotoxic agents (Zhang et al., 2021). CSCs transform the tumor microenvironment (TME) into a favorable niche that is closely associated with hypoxia, acidosis, remodeling of the extracellular matrix (ECM), alterations in nutrients, and necrosis to support their survival by maintaining stemness and regulating dormancy (Liang & Kaufmann, 2023).

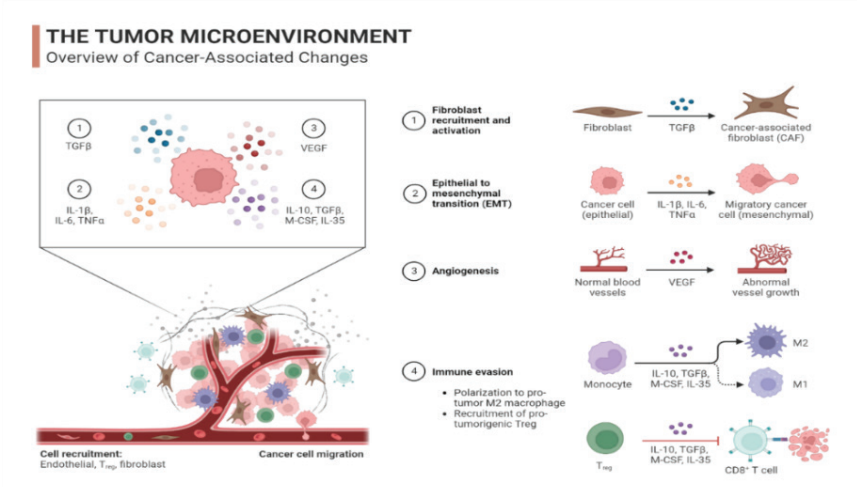

Figure 2. A schematic representation of the tumor microenvironment (Ramadan, 2020).

3.4. Therapeutic Approaches to Target Cscs and EMT

CSCs and EMT are key factors contributing to therapeutic resistance, disease progression, and metastasis in cancer (Liang & Kaufmann, 2023). Therapeutic approaches to target CSCs including targeting CSC surface markers, EMT markers, drug efflux transporters and modulating CSC- and EMT-related signaling pathways, such as TGF-β, Wnt/β-catenin, Hedgehog, and Notch pathways, as well as PI3K, NF-κB and JAK-STAT signaling pathways, have shown promising results and currently being tested in clinical trials (Walcher et al., 2020; Tanabe et al., 2020; Yang et al., 2020).

Monoclonal antibodies (mAbs) to target specific surface biomarkers of CSCs is a recently emerging approach for cancer treatment (Yang et al., 2020). Bivatuzumab (targeting CD44v6), catumaxomab, and adecatumumab (targeting EpCAM) are some examples of monoclonal antibodies used to target CSC

surface markers in different phases of clinical studies for treating multiple types of cancer (Yang et al., 2020).

Small molecule drugs inhibiting Snail-induced EMT, such as GN 25 and GN 29, have been reported (Kaufhold & Bonavida, 2014). In addition, other small molecule drugs that inhibit histone deacetylases and histone demethylases LSD1 and LSD2, such as trichostatin A, tranylcypromine, entinostat, pargyline, and LBH589, were also shown to block the Snail-mediated EMT (Sato et al., 2016). Small molecule inhibitors of cyclin-dependent kinases (CDKs) have been demonstrated to attenuate ZEB1 expression, hence inhibiting the expression of EMT markers in breast cancer cells (Arima et al., 2012). The CDK4/6 inhibitor PD0332991 (palbociclib) was reported to reduce $CD44^{+}$ $CD24^{-}$ CSC population percentage in breast cancer cells (Sato et al., 2016).

Inhibitors directed at targeting EMT inducers, such as the TGF-β signaling pathway, are currently being subjected to both preclinical and clinical evaluation (et al., 2015). Several small-molecule inhibitors targeting the enzymatic function of the hepatocyte growth factor receptor (HGFR) tyrosine-kinase domain are currently under investigation in clinical trials as potential therapeutic options for inhibiting the induction of EMT (Shibue & Weinberg, 2017). Two multi-targeted kinase inhibitors that have demonstrated effectiveness against HGFR protein have been authorized by the FDA: crizotinib, for treating non-small cell lung cancer, and cabozantinib, for treating medullary thyroid cancer and renal cell carcinoma treatment (Scagliotti et al., 2013).

Targeting plasticity and heterogeneity within a tumor may also be a promising strategy (Kvokačková et al., 2021). Inhibition of mesenchymal transition by targeting its stimulation, preventing MET at secondary sites by targeting mesenchymal or hybrid state cells, and reprogramming of mesenchymal cells to epithelial are among the strategies suggested to block plasticity (Shibue & Weinberg, 2017; Kvokačková et al., 2021). Another therapeutic approach is to inhibit plasticity by "fixing cells at a specific point along the epithelial-mesenchymal axis", potentially by disrupting positive feedback loops that regulate state switching (Hari et al., 2020).

Immunotherapeutic approaches to target CSCs have also recently been investigated by many research groups (Tanabe et al., 2020). Immune checkpoint inhibitors, oncolytic viruses, dendritic cell (DC)-based vaccines, and chimeric antigen receptor-modified T (CAR-T) cell therapies are some examples of immunotherapy-based strategies that are being evaluated to target CSCs (Yang et al., 2020). For example, drugs targeting some immune checkpoint receptors, such

as pembrolizumab, nivolumab, and cemiplimab (targeting PD-1), durvalumab, atezolizumab, and avelumab (trageting PD-L1) are currently under clinical trials (Yang et al., 2020). CD133-directed CAR T-cell therapy has demonstrated encouraging results as both a monotherapy and in combination with cytostatic agents in clinical trials on hepatocellular carcinoma (Walcher et al., 2020).

Nanotechnology-based therapy is another promising approach to targeting CSCs (Tanabe et al., 2020). A nanomedicine consisting of polymeric micelle incorporating cisplatin (CDDP/m) has demonstrated efficacy in eliminating both undifferentiated and differentiated cancer cell populations in head and neck tumors (Wang et al., 2016), and it is in the third phase of clinical trials for pancreatic cancer treatment (Cabral & Kataoka, 2014). Multifunctional magnetic nanoparticles have been used in combination with thermo- and chemotherapy to target CSCs effectively (Liu et al., 2020). A nanomedicine containing miR-125b-5p, an EMT, and CSCs targeting microRNA showed significant tumor suppression in vivo (Guo R. et al., 2019). Another nanomedicine approach for targeting CSCs is the utilization of liposomes co-loaded with cabazitaxel and the CSC inhibitor silibinin to target CD44 receptors (Mahira et al., 2019). In addition to peptides or small molecules, other ligands such as aptamers, antibodies, and antibody fragments can be used to modify the surface of nanomedicines for targeting CSCs (Tanabe et al., 2020).

4. Conclusions and Future Perspectives

The interplay between CSCs and EMT is a dynamic and multifaceted process that plays a critical role in tumor heterogeneity, metastasis, and therapy resistance. Therefore, further investigation is needed to fully comprehend this interplay and to provide insights into the molecular mechanisms underlying therapy resistance, tumor dissemination, and recurrence. Furthermore, exploring the role of EMT in the crosstalk between CSCs and the tumor microenvironment and modulating host immune response to treatments is an active research area. Lineage tracing methods, spatial genomics, and single-cell multi-omics are some of the most promising advanced techniques for studying genetic and non-genetic factors that control the interaction between CSCs, EMT, and the tumor microenvironment (Ciriello & Magnani, 2021).

Investigation of the origins of phenotypic plasticity and heterogeneity is another research avenue that has great potential for mapping the landscape of phenotypic plasticity in various types of cancer and ultimately could shift cancer treatment approaches from being reactive to being proactive and predictive (Jain

et al. 2022). The heterogeneity of CSCs poses challenges for clinical trials as their surface phenotypes can vary among different patients and cancer types, and multiple CSCs populations with various phenotypes may coexist within the same tumor. Furthermore, CSCs can also evolve into different cancer cells, developing unique characteristics upon relapse. As a result, it is crucial to tailor treatment strategies based on the phenotypic diversity in different types of cancer (Yang et al., 2020).

Integration of multi-scale computational modeling, multi-omics, single-cell based approaches along with the development of experimental tools such as inducible reporter systems or intra-vital imaging is required to capture dynamics of the entire EMT/MET spectrum in a time-course manner and to unveil the implications of epithelial-mesenchymal plasticity (EMP) in experimental models and clinical specimens (Kvokačková et al., 2021).

Overall, future research in this field should aim to develop targeted therapeutic strategies that can effectively inhibit EMT and eliminate CSCs, ultimately improving patient outcomes and combating cancer.

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CHAPTER II

THE ROLES OF MATRIX METALLOPROTEASES IN DIFFERENT STAGES OF CANCER

***MÜGE MAVİOĞLU KAYA**

**(Asst. Prof. Dr.), Department of Molecular Biology and Genetic, Faculty of Art and Science, Kafkas University, Kars, Turkey*
E-mail: m.mavioglu@hotmail.com
ORCID: 0000-0003-1276-3745

1. Introduction

The matrix metalloproteases (MMPs) were first observed in the tail tissues of *Rana catesbiana* tadpoles in 1962 by Jerome Gross and Charles Lapiere (Srivastava et al., 2019). MMPs belong to a class of endopeptidases that are zinc and calcium dependent and facilitate the degradation of extracellular matrix proteins, thus playing an important role in human physiology and pathology (Zhang et al., 2023). A certain group of MMPs that can digest collagen are called collagenase 1 or MMP 1. This collagenase contributes to metamorphosis by ensuring collagen distribution in the tadpole tail during the transition to adulthood (Srivastava et al., 2019).

MMPs are found in invertebrates, vertebrates and plants (He, 2023). MMPs are secreted by living cells such as macrophages, fibroblasts, neutrophils, chondrocytes, smooth muscle cells of the vascular wall, connective tissue, keratocytes, lymphocytes, osteoblasts, cytotrophoblasts, uteroplacental and proinflammatory cells. It hydrolyzes procollagens of the extracellular matrix and connective tissue proteins such as collagens, elastin, proteoglycans, laminin, fibronectin (Popov et al., 2023, He et al., 2023). They can also degrade insoluble extracellular matrix components into hydrolyzable fragments. When they encounter autocrine or paracrine signals, they affect membrane-bound proteins and cause the formation of soluble ectodomains (Niland et al., 2021). MMPs regulate the functions of cells and tissues that need to be healthy, including

the development and restructuring of tissues consisting of various cells within the living organism, ossification, angiogenesis and wound healing in damaged cells or tissues (Zhang et al., 2023; Apte and Parks, 2015). Although MMPs are proteolytic organic catalysts that act on different substrates, they have common structural features. Zinc and three histidine residues are important in the stability of the active center (Figure 1) (Laronha and Caldeira, 2020).

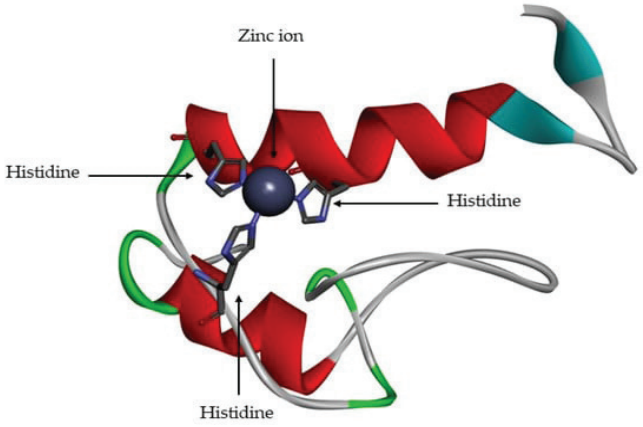

Figure 1. Active domain of MMP 1 (Laronha and Caldeira, 2020).

The expression levels and functions of MMPs which are responsible for maintaining homeostasis in normal tissues may change in disease cases. Some MMPs which are not expressed under healthy conditions may be overexpressed in some disease states such as cancer. Excessive MMP expression can contribute to malignant cell proliferation, invasion, migration and angiogenesis. Since the expression levels of MMPs change in cancer cases, the ability to use MMPs as biomarkers to detect these changes early is very valuable in terms of prognosis and prediction of the disease.

2. The Roles of MMPs In Cancer Development

MMPs are vital for invasive cancer cells to overcome extracellular matrix barriers (Niland and Eble, 2020). Excessive extracellular matrix proteins accumulation occurs as a result of various injuries of tissues and this condition causes loss of function in organs. During the process of healing and renewal, fibroblasts which fibrous cellular substances that connects tissues and organs as one of the important cells of the connective tissue are activated and differentiated into myoblasts. Myoblasts also play a key role in the formation

and secretion of extracellular matrix proteins content to renormalize the tissue. The first stage of regeneration is the remodeling of the extracellular matrix proteins by proteolytic enzymes that cause molecular changes in the extracellular matrix. At the end of the regeneration process, fibroblasts encounter apoptotic mechanisms or become inoperable. However, in the persistence of the fibrosis state, fibroblasts remain constantly active and produce abundant extracellular matrix. Similarly, disruption of balance among tissue inhibitors of metalloproteinase and MMPs makes fibrotic condition even more severe. For all these reasons, extracellular matrix degradation by MMP is closely related to invasion of cells, migration of cells and tissue remodeling (Zhao et al., 2023). In general, deterioration and re-normalization of extracellular matrix integrity is tightly regulated; however, under pathological conditions, hyperactivation of MMPs causes tendon extracellular matrix degradation by excessive collagen proteolysis (Mohindra et al., 2022). Moreover, under pathological conditions, excessive and constantly generated signals by extracellular matrix remodeling and degradation lead to loss of polarity of the cell and loosening of intercellular tight junctions, generation of mesenchymal protein molecules and induction of an invasive phenotype. As a result, it performs a very critical function in the epithelial-mesenchymal transition of malignant cells (Niland and Eble, 2020). Any change in the integrity of the extracellular matrix results in an imbalance in the rate of production and destruction of proteins. These enzymes enable molecular and physiological processes such as tissue remodeling, migration and adhesion (Popov et al., 2023). In the process of cancer or cancer, the extracellular matrix, which is at the forefront in almost all processes including cell survival, cell migration, cell adhesion and cell proliferation, undergoes biomechanical, biochemical and topographic changes (Niland et al., 2021). While MMP functions are regulated according to the type and intensity of stimuli in tissues with healthy physiological properties, the balance begins to be lost in pathological or pathophysiological conditions. Highly stimulated and overactivated MMPs are associated with diseases caused by many pathological conditions such as arthritis, periodontal diseases, cardiovascular disease, central nervous system disorder, tumor metastasis, tumor invasion, and osteogenesis imperfecta. Different MMP activities such as MMP1-3 and MMP 7-11 increase in the microenvironment conditions where the tumor is located. MMPs involved here mediate the increase in tumor number, angiogenesis, and preservation of tumor viability, thus ensuring tumor cell migration, immune evasion, continuity of replication, and similar processes. They also play a role in tumor cell

growth by activating various signaling mechanisms related to growth. MMPs also play a role in the migration of tumor cells, which is an important step in cancer development. The MMP 14 enzyme has the largest share in contributing to tumor invasion. ProMMP 2 with MMP 14 is activated and the extracellular matrix contents promoted tumor migration break down. MMP 7 also affects the initiation and progression of the metastatic process for the tumor cell by stimulating the signaling pathways JNK 1/2 and ERK ½ (He, 2023).

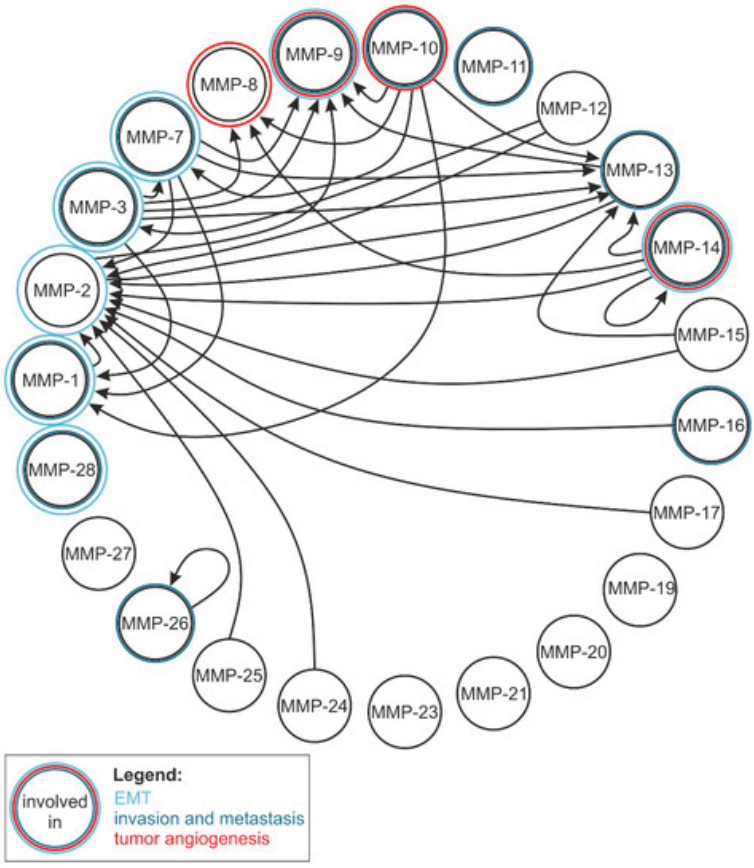

Figure 2. Human MMP activation. Many MMPs function at stages of tumor or cancer development, such as epithelial-mesenchymal transition, metastasis and invasion, as well as tumor angiogenesis. MMP mediated activations are indicated by arrow signs. Metastasis, tumor angiogenesis and invasion states, including the epithelial-mesenchymal transition, are implied by colors. MMPs in the upper half are very effective in cancer development or cancer progression (Niland et al., 2021).

According to the idea that encompasses the tumor microenvironment, a tumor is like a wound that cannot transform into normal tissue. When injuries occur in healthy tissues, clotting factors are concentrated and delivered to the relevant area for healing. The fibrin-dense network formed in the relevant area stabilizes thrombosis to ensure hemostasis, and the extracellular matrix environment in which immune cells and fibroblasts proliferate for damaged tissue repair is also provided. The solidified tumor mass develops and grows using environmental conditions and comes into contact with blood vessels, and the tumor cells migrate to the endothelial cell layer and initiate the process of infiltration of many components in the blood into the nearby tissue area. Before this stage, healthy cell behavior becomes abnormal in the relevant region. Cancer-associated fibroblasts are formed, after which stromal cells are cancer cells that extensively synthesize core matrix protein molecules such as glycoproteins, proteoglycans and collagens. The combination of cancer and immune cells mediates the transformation of fibroblasts into cancer-associated fibroblasts by increasing soluble contents such as cytokines and growth factors. MMP enzymes cause changes in the immune system in tumor formation and the spread of cancer. For example, the MMP 9 enzyme acts on the interleukin-2 receptor-α, preventing the increase in the number of T-lymphocyte cells, and thus the defense mechanism against cancer is negatively affected. The sensitivity of natural killer cells to cancer-causing cells and cancer cells is down-regulated as a result of the release of α1 proteinase inhibitor through some MMP (1, 3 or 11) enzymes. MMP enzymes (such as 7 and 8) also play a role in the infiltration of leukocyte cells by affecting the cleavage and mobilization mechanisms of chemokines. MMP enzyme synthesis and concentrations increase depending on the stages and malignancy, depending on the onset and progression of cancer. For these reasons, the importance of MMP enzymes as diagnostic and prognostic biomarkers attracts attention (Niland et al., 2021).

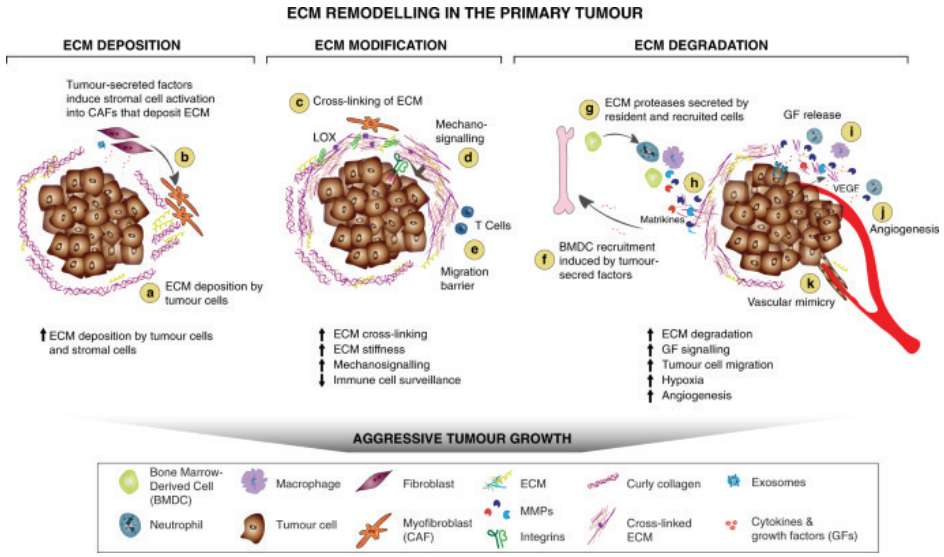

Figure 3. Restructuring of the extracellular matrix in the primary tumor. (a and b) In cancer, molecules released into the tumor microenvironment by malignant cells mediate the intense release and increase of extracellular matrix content together with cancerous cells by activating cancer-related fibroblasts. (c) Enzymes that change the extracellular matrix such as lysyl-oxidases synthesized by tumor cells and cancer-related fibroblasts, support the thickening of the matrix that forms the tumor environment by regulating the cross-linking of connective tissue element collagen fibers. (e) T cells promote an inhibitory moiety to ensure they can escape immune control. (d) When the consistency of the matrix is hard, the interaction of the extracellular matrix content and receptors of tumor cell is supported, highlighting the signaling in which integrin molecules play a role. (f) It enables the synthesis of cytokines, growth factors and chemokines that cause the differentiation and recruitment of cells produced from the bone marrow under the influence of tumor cells and resident immune cells to ensure the continuity of the tumor microenvironment. (g) Proteolytic enzymes which are found on the cell surface of cells produced from bone marrow, cancer-associated fibroblasts and tumor cells including MMPs synthesize and release into the environment by these cells and negatively affect the extracellular matrix. (h) Bioactive matrixins are produced by negative alteration of the proteolytic extracellular matrix. (i) Proteolytic degradation of the extracellular matrix releases growth molecules bound to the matrix. Extracellular matrix signaling is stimulated

for the proliferation, invasion, migration and angiogenesis of tumoral cells. (j) Changes in the extracellular matrix lead to the formation of an area with low oxygen levels. Excessive MMP 9 content, which causes disruptions in the extracellular matrix, is synthesized and released by neutrophils. Thus, a concentration gradient emerges that supports the maintenance of angiogenesis. (k) Tumor cells stimulated by the dense extracellular matrix can follow metabolic pathways similar to those of normal endothelium and integrate into essential nutrient blood vessels, positioning themselves as if they were normal vascularity (Winkler et al., 2020)

Metastasis consists of steps in the malignant cell journey that ensure tumoral continuity such as intravasation, extravasation and localization in metastatic areas. Sometimes, the division of extracellular matrix molecules causes the formation of shorter fragments of extracellular matrix components which are matricines that contain intensely cytokine/chemokine-like molecular structures. Transforming growth factor-beta (TGF-β) is a very familiar molecule that functions in proliferation, metastasis and angiogenesis. Latent TGF-β protein-1 (LTBP-1) is a TGF-β binding protein molecule, and when it undergoes proteolytic change, TGF-β passes into the extracellular matrix and tumorigenesis is maintained (Tanaka and Sakamoto, 2023).

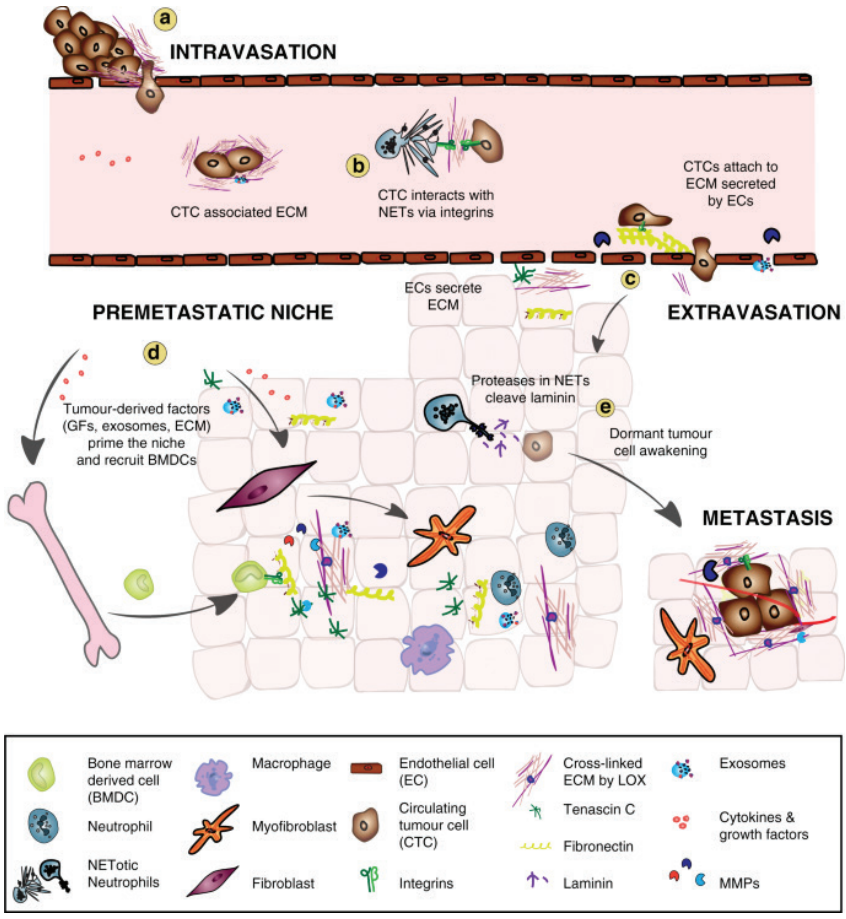

Figure 4. Restructuring of the extracellular matrix (ECM) in metastatic cascade (a) Angiogenesis in the primary tumor area and high levels of MMP activity cause negative changes in the vascular system, which supports the involvement of malignant tumor cells in the vascular system and circulation. Circulating tumor cells can form and release extracellular matrix that can evade immune system defenses. (b) It can bind to neutrophil extracellular traps using integrins secreted by circulating tumor cells and neutrophils. (c) Endothelial cells proliferate and assemble fibrillar fibronectin, which supports the attachment of circulating tumor cells to endothelial wall of distant organs. High levels of MMP activity modify the vascular structure, which causes malignant cells in the circulation to pass into the surrounding tissue. Endothelial cells also aid in the formation of a metastatic niche by contributing to distant regeneration and increasing the content and width of the extracellular matrix. (d) Various molecules or structures generated from primary malignant

cells, such as extracellular matrix proteins and exosomes, create a distant pre-metastatic stage niche to make the new tissue compatible with the metastatic process. The stromal cells in this niche are activated by malignant cell-derived factors. Myofibroblasts cause the extracellular matrix to be shaped for a new suitable life by the accumulation of components such as tenascin C, versican, fibronectin and osteopontin, depending on the tissue type. Cells produced from the bone marrow are incorporated into the niche before metastasis. It is integrated into the new extracellular matrix using integrins, and the extracellular matrix base is thoroughly harmonized by helping the contact of disseminated malignant cells. (e) Circulating malignant cells that spread to integrate into distant tissue by compromising the vasculature may be stable. Proteolytic molecules synthesized extracellularly in neutrophils including neutrophil elastase and MMP 9 degrade laminin resulting in the formation of specific matrix that can induce stable malignant cells. It supports the continuity of metastatic activity by being involved in the occurring extracellular matrix regeneration processes (Winkler et al., 2020).

2.1. MMPs and Angiogenesis

Angiogenic homeostasis depends on the balance between anti-angiogenic and pro-angiogenic components that enable new vessel formation or existing vessel silencing/regression. Not only healthy cells but also cancerous cells require angiogenesis for growth and development. In fact, malignant cell angiogenesis is an essential stage of metastasis. Factors secreted from malignant cells promote new vessel formation in these cells and contribute to the overexpression of proliferation-inducing factors and the formation of the tumor microenvironment by restructuring extracellular matrix (Bellon et al., 2004). MMPs, which play a key role in restructuring the extracellular matrix, can induce the release of GFs, angiogenesis, tissue repair and regeneration (Chen, K., 2023). As a result of all these, malignant cell proliferation, invasion and metastasis have a direct relationship with MMP expression and activity (Niland et al., 2021; Carminati et al., 2023). Some members of the matrix metalloproteases have an initiating or inhibiting effect on the formation of new vessels. Although MMP 1, MMP 2, MMP 7, MMP 9 and MMP 14, which are extracellular matrix MMPs, regulate the extracellular matrix restructuring process, especially the first three of them play a critical role in angiogenesis (He, 2023). Specifically, MMP 2 and MMP 9 are indispensable proteases in both angiogenesis and neurogenesis (Rashid and Bardaweel, 2023). As a

result of the hydrolytic protease activity of MMP 2 gelatinase, secretion and activation of angiogenic growth factors such as basic fibroblast growth factor (bFGF) and TGF-β and vascular endothelial growth factor (VEGF) occur. In connection with this, according to the results of a clinical study, it was reported that TGF-β and VEGF were also up-regulated in metastatic lesions, showing positive correlation with the up-regulation of MMP 2 gelatinase. Biologically active factors such as VEGF, bFGF, and TGF-β can induce angiogenesis by using the signaling pathways of relevant intravascular epithelial cell receptors and can also promote angiogenesis by stimulating vascular epithelial cells to express MMP 2 and MMP 9. In a study showing parallel results with these data, a connection was established with overexpression of MMP 3 resulting from increased secretion of MMP 9 and TNF-α in mice with endometrial damage and the resulting increased angiogenesis (Chen et al., 2023).

A study examining mice lacking the MMP 9 or MT1-MMP nucleotide sequences against antibodies showed reduced angiogenic responses. Recent findings show that MMP 9 can also induce angiogenesis by ensuring the secretion of VEGF, one of the most important angiogenic factors, by extracellular matrix elements (Bellon et al., 2004). In a study conducted in mice to determine the function of MMP 14, complete knockout of MMP 14 causes delayed ossification, reduced new vessel formation, severe fibrosis, and early death. It has been found that cancer cell invasion is reduced by silencing MMP 14 at the mRNA level through RNA interference or knockdown of the MMP 14 ectodomain. Contrary to these results, the invasion of malignant cells increases with the increasing expression level of MMP 14. CAFs within the tumor microenvironment, which directly contribute to tumorigenic development, also express MMP 14. In connection with this, increased MMP 14 expression level in the mouse breast cancer model contributes to malignant cell invasion and metastasis (Niland et al., 2021).

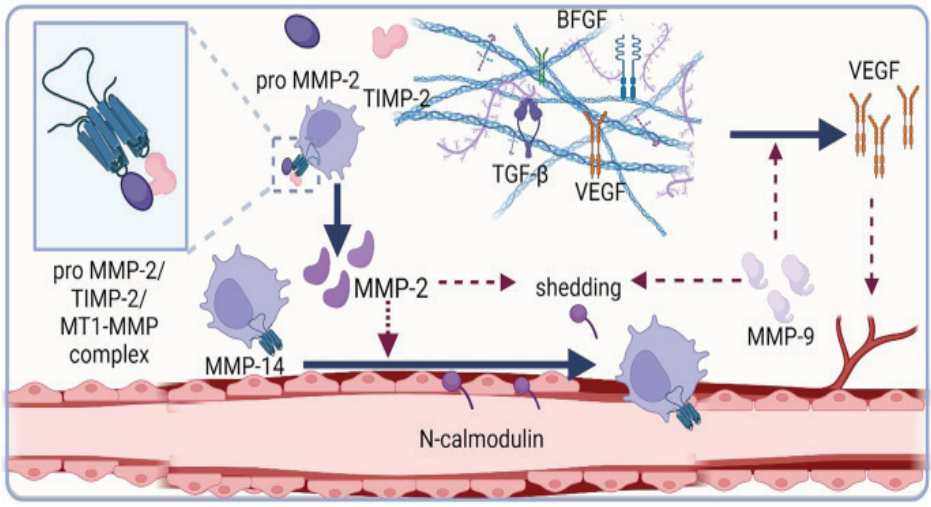

Figure 5. Malignant cell migration along with new vessel formation plays a key role in extracellular matrix restructuring. MT1-MMPs, also called MMP 14, located on the outer surface of the cell membrane, degrade extracellular matrix elements. Cell migration is supported by the formation of a ternary complex of MT1-MMP, TIMP-2 and pro-MMP 2, which activates MMP 2. Because MMP 2 breaks down type IV collagen, which is a component of the basement membrane (BM), which facilitates cell migration. Therefore, activation of MMP 2 activation is considered a critical step in malignant cell invasion. MMP 2 may also contribute to malignant cell proliferation and migration by degrading N-calmodulin and loosening connections between VSMC. Moreover, the hydrolytic protease activity of MMP 2 gelatinase also promotes angiogenesis by inducing the release and activation of angiogenic molecules such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (BFGF), and TGF-β. Dashed red lines represent the function of MMPs, solid lines represent the physical origin of the molecule (Chen et al., 2023).

2.2. MMPs and Inflammation

MMPs have very important roles in regulating the functioning of immune cells during inflammation (He, L, 2023). When antigen invades the body, immune cells-macrophages or epithelial cells—secrete chemokines in response. The secretion of chemokines also causes vasodilatation by increasing the interstitial epithelial cell space in blood vessels. As a result, large numbers of eosinophils

mast cells, neutrophils and basophils pass from blood vessels into the tissue fluid and cause regional fever, redness, swelling and pain. All these phenomena are indicators of inflammation. The most effective proteins in the immune response are cytokines and chemokines. They play a key role in directing immune cells to the site of inflammation, cell proliferation, apoptosis, interstitial cell junctions and communication. Not every immune response produces inflammation, but inflammation often causes an immune response. In this case, a large number of immune cells such as macrophages and neutrophils are directed to the inflamed area to immediately release inflammatory factors (such as IL-6, IL-1β and TNF-α) (He, 2023)

MMPs are secreted by inflammatory cells that are activated in response to cytokines and chemokines abundant in the tumor microenvironment. They serve as both regulators and effectors of inflammation. MMPs participate in the following events in case of inflammation: (1) disruption of the blood vessel basal lamina and migration of leukocytes to the area of inflammation, (2) inactivation of serpins, which are serine proteinase inhibitors involved in tissue degradation and restructuring, (3) TNF-α and IL- in the area of inflammation. Activation of pro-inflammatory cytokines such as 1β, (4) activation of pro-inflammatory cytokines such as TGF-β, stimulation of the synthesis of chemokines such as IL-8 to increase the inflammatory response, and (6) stimulation of the synthesis of anti-inflammatory chemokines such as MCP-3 to reverse the inflammation phenomenon. In the malignant microenvironment, inflammatory cells secrete mostly MMP2 and MMP9. They have both pro-inflammatory and anti-inflammatory properties. How MMP will affect the cancer phenomenon also depends on the stage of malignant development. TGF-β suppresses immunity in malignant cells by causing inactivation of innate and adaptive immune factors. MMPs play a role in the escape of malignant cells from immune cells, both by activating TGF-β and by degrading protein-based IL receptors or by disrupting the cytokine signaling pathway in T lymphocytes by interfering with alkyl cytotoxicity (Quintanilla et al., 2012).

Constant inflammation in the organism creates an environment that supports tumor formation and metastasis by inducing immune suppression, malignant cell proliferation, invasion and new blood vessel formation (He, 2023). For example, people with chronic ulcerative colitis or Crohn's disease are more prone to developing colon cancer. People with pancreatitis are also more likely to develop pancreatic carcinomas. Therefore, these data suggest that persistent inflammation can transform a normal tissue into a neoplastic tissue.

In the case of chronic inflammation, the continuous release of pro and anti-inflammatory cytokines and free radicals can induce malignant cell formation by causing DNA damage, increased cell proliferation, inhibition of apoptosis and new blood vessel formation (Quintanilla et al., 2012). Another example where chronic inflammation promotes cancer is the significantly increased risk of colitis-associated cancer (CAC) in people with inflammatory bowel disease (IBD) compared to other healthy people. In environments shaped by chronic inflammation, extracellular matrix is an important factor in malignant cell proliferation and maintenance of this state. MMPs located in extracellular matrix are the main protease involved in the development of IBD. MMP 9, a member of MMPs closely associated with cancer, plays an important role in the progression of ulcerative colitis (UC) and in creating an environment of chronic inflammation due to its effects on the immune system. therefore, MMP 9 can be used as a biomarker of prognosis of cancer and metastasis. MMP 19, on the other hand, regulates the host immune response against colon tissue antigens. MMP 9 and MMP 10 are synthesized at significant levels in colon tissue with inflamation and this level begins to decrease as healing begins. They are not expressed in healthy colon tissues. MMP 10 is frequently synthesized by macrophages from immune cells. In cases of ulcerative colitis, it is expressed at high levels in enterocytes and granulation tissue cells at the edges of the ulcer. Some studies show that increased MMP 10 expression in bone marrow cells reduces inflammation. Additionally, when MMP 10 deficiency is induced experimentally, dextran sodium sulfate (DSS)-induced colitis increases and dysplasia may occur as a result of chronic irregular bowel syndrome. Ultimately, MMPs appear to play a key role in the induction of the host immune response, recovery, and epithelial cell regeneration. With all this, MMPs have bidirectionally effects in irregular bowel syndrome. In addition, they play a role in terms of irregular bowel syndrome by contributing to the formation of chronic inflammation and its specific environment, moreover, some members from MMP enzymes carry inhibitory properties on the inflammatory conditions (He, 2023).

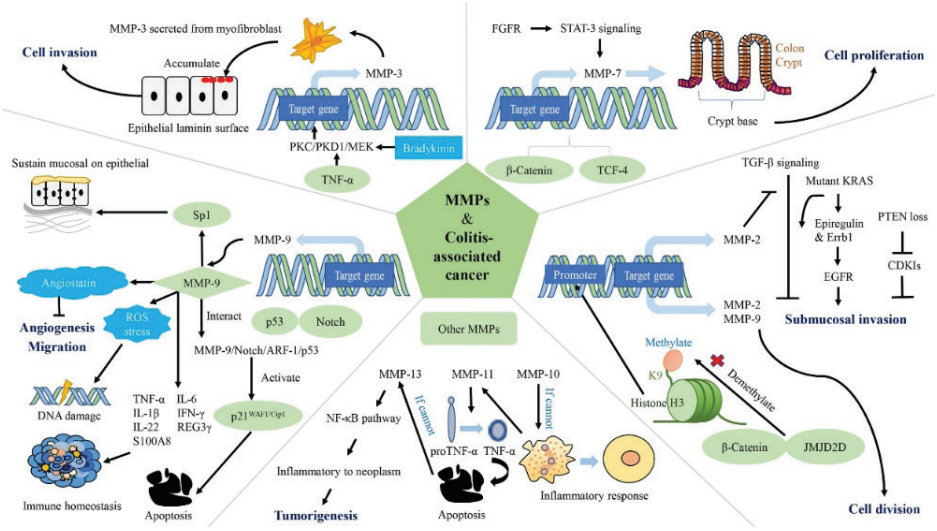

Figure 6. Network of MMPs involved in colitis-associated cancers. MMP 2, MMP 3, MMP 7, MMP 9, MMP 10, MMP 11 and MMP 13 play a role in the induction, development and maintenance of CAC. Increased MMP 2 and MMP 9 levels in the setting of chronic inflammation in CRC cases promote CRC cell proliferation. MMP 2 and MMP 3 contribute to malignant cell invasion. While increased expression of MMP 9 leads to increased apoptosis, decreased ROS accumulation, and inhibition of tumor angiogenesis, it may also cause DNA damage. MMP 10 plays an anti-inflammatory role in reducing the severity of inflammation. MMP 11, which causes increased malignancy, reduces apoptosis in cancer cells. TCF 4: T cell factor-4, PKC/PKD1/MEK: protein kinase C/ protein kinase D1/mitogen-activated protein, EGFR: epidermal growth factor receptor, PTEN: phosphatase and tensin homolog deleted on chromosome 10, CDK: cyclin-dependent kinase, FGFR: fibroblast growth factor receptors, ROS: reactive oxygen species Sp1: specificity protein 1, (He, 2023).

5. The Dual Role of MMPs İn Cancer

MMPs disrupt the basement membrane proteins, the base on which the cells sit, and extracellular matrix components in the tumor microenvironment, allowing malignant cells to invade the vessels and lymph systems and achieve metastasis. During the proliferation of malignant cells, MMPs are highly expressed, increasing the intravascular intercellular space, increasing the permeability of endothelial cells and contributing to cell proliferation, invasion, angiogenesis and cell migration (He, 2023). In addition, MMPs shape the environment in which malignant cells in the premetastatic period are located in

the direction of the tumor microenvironment, contributing to the re-proliferation of tumor cells in the stationary phase. (Carminati et al., 2023).

An increase in MMP protein expression levels does not always mean tumor growth, angiogenesis or metastasis. It has even been shown that some MMPs play a protective role in cancer cases. The extracellular matrix remodels in the malignant cell niche, and these changes can both promote and suppress tumorigenesis. There is also evidence that MMP 1, -3, -7, -9, -14, -16, and -19 regulate endothelial vascular growth factor (VEGF) synthesis and suppression in cancer. Exposure of extracellular matrix elements such as collagen -4, -18, perlecan and heparan sulfate proteoglycan 2 (HSPG2) to MMP -1, -2, -3, -9 or -13 leads to neovascular neovascularization such as endostatin, tumstatin, angiostatin and endrepellin. It can initiate the synthesis of molecules that prevent its formation. MT1-MMP and MMP 2 are involved in the degradation of extracellular matrix proteins, but in the malignant cells and tumor microenvironment these enzymes induce their proliferation and invasion. In healthy cells, MT1-MMP and MMP 2 protein expression levels are low or not expressed, but these values may increase in cancer cases (Popov et al., 2023).

6. Conclusion

MMPs have remarkable roles in pathology of human diseases such as cancer. According to literature reviews, it is understood that effective MMP inhibitors which have the capacity to interfere with many MMP enzyme activities in terms of both initiating and preventing tumor development in cancer cases, have not yet been sufficiently introduced to this field. In this context, a vital question is how to harmonize the dual role of MMP enzymes and their activities in different tissues, especially in cancer researches and treatments. When targeting therapeutics with MMP, it seems that larger and more detailed research materials and techniques are especially must be revealed exact function and activity of a particular MMP and which MMP types work together or separately in which tissue and which agents will inhibit different MMP activities.

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CHAPTER III

NEW APPROACH TO CANCER TREATMENT "PHOTODYNAMIC THERAPY"

DILEK SAĞIR

(Assoc. Prof. Dr.), Sinop University, Faculty of Health Sciences
E-mail: dileks@sinop.edu.tr,
ORCID: 0000-0002-6862-988X

1. Introduction

Cancer development occurs with the abnormal increase of a number of cells with structural differences and their spread throughout the body. The formation of cancer cells is usually a result of gene mutations resulting from intrinsic or exogenous factors. Cancer, which has become a serious problem worldwide, is expected to continue to increase in the future (Zhou, 2014). Although some standards have been established, different approaches and treatments are applied to each type of cancer. In addition to treating cancer, identifying factors causing cancer, preventing cancer before it develops, and providing social and psychological support to alleviate patients and their families make cancer an extremely complex and significant problem. In addressing these challenges, oncology utilizes various branches of medicine. Consequently, radiotherapy, chemotherapy, and surgical methods are employed alone or in combination to work towards the treatment of cancer. However, these well-known methods, considered as the gold standard, have both advantages and disadvantages. These procedures often lead to numerous complications associated with the deterioration of healthy tissues. Recurrence is frequently observed in patients, or the tumor progresses towards secondary diseases through metastasis (Gavas, Quazi, Karpi´nski, 2021; Kubrak et al., 2022). The inadequacy of conventional procedures to selectively impact malignant tumors motivates the search for more innovative cancer treatments (Kubrak et al., 2022). Therefore, in addition to these conventional methods, various immunological and biological treatment

methods are being developed (Fitzmaurice et al., 2013; Pavlopoulou and Spandidos, 2015). One of these innovative treatments is Photodynamic Therapy (PDT), which involves the use of light-activated drugs (photosensitizers).

Photodynamic therapy (PDT) is a technique that utilizes a combination of a drug that is made light-sensitive in the target tissue to cause selective damage and appropriate light (Figure 1). Adequate concentration of molecular oxygen is also required to cause damage in the tissue. If any of these three components is missing, the therapy has no effect. Therefore, careful planning of both drug and light doses is required for the effectiveness of the treatment. While drugs are given systemically to patients in treatment, light is usually applied locally, precisely targeting the affected area, using a laser source (Hooper, 2000).

Understanding the mechanism of PDT is crucial because, like other methods, it has both advantages and disadvantages (Hooper, 2000; Oleinick, Morris RL and Belichenko, 2002). One disadvantage of PDT is its inability to treat advanced and disseminated diseases due to the current technology's inability to irradiate the entire body at appropriate doses. However, although this method may not provide a complete cure for advanced diseases, it can improve the patient's quality of life and extend survival. For diseases detected in early stages or those without metastasis, PDT can be a more advantageous, selective, and curative treatment compared to other treatment methods. In such cases, PDT alone can completely cure the disease.

Despite the long-known clinical potential of PDT in cancer treatment, its use in clinical settings has only recently begun. The significant advantage of PDT, which is causing damage or destruction to the target tissue, stems from the energy of the light used in therapy. Through a photosensitizer (PS), energy is absorbed from a light source and then transferred to molecular oxygen to create an activated form of oxygen called singlet oxygen. This resulting singlet oxygen is a potent cytotoxic agent. This oxygen reacts with cellular components, leading to cell death and, consequently, the destruction of the tumor (Brown, Brown and Walker, 2004).

Different types of PS, synthetic inorganic and organic, have been investigated for the last 10 years. Among these, only some have been converted into clinical studies (Oniszczuk, 2016).

There are three different basic interrelated cell death pathways:

(1) Pathways that directly kill cancer cells;

(2) Those that damage the vascular system and prevent the supply of oxygen to the cells,

(3) Stimulants of systemic immunity.

PDT uses these three pathways to kill cancer cells (Agostinis et al., 2011; Mroz et al., 2011)

2. Principles of Photodynamic Therapy (PDT)

PDT generally consists of two stages. In the first stage, a light-sensitive photosensitizer (PS) is applied, and then tumor areas are irradiated with light at an appropriate wavelength. In the second stage, it is transmitted to the organs in the body through a flexible fiber optic device (Figure 1). The selectivity feature, which is the most important advantage of this treatment, is obtained from both the ability of the used PSs to localize in neoplastic lesions and the precise transmission of light to the treated areas. However, this selectivity is also a disadvantage of PDT, as it is ineffective against metastatic lesions, which are the most common cause of death in cancer patients (Brown , Brown, and Walker, 2004).

PDT can be used before or after chemotherapy, radiotherapy, or surgery, utilizing all of its features. Clinically approved PSs do not accumulate in the cell nucleus, showing no carcinogenic effect. They also do not cause DNA damage that could lead to the development of resistant clones. Additionally, they do not cause the negative effects induced by chemotherapy or radiotherapy. Resistance to radiation or chemotherapy does not affect the sensitivity of PDT (Agostinis et al., 2011).

PDT does not cause a change in tissue temperature and leads to fibrosis to preserve the connective tissue. This ensures the mechanical integrity and function of the hollow organs where PDT is applied. These features make PDT particularly suitable for patients with skin cancer. This treatment method is entirely patient-friendly. The only negative effect of PDT on the patient is that it can cause permanent skin photosensitization (Figure 1) (Agostinis et al., 2011).

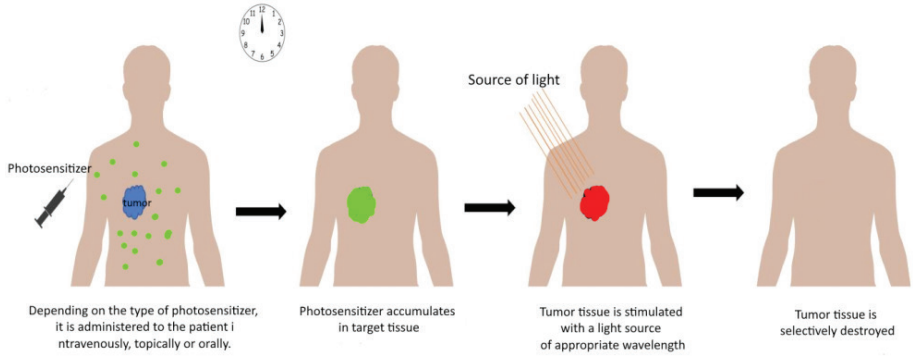

Figure 1. Application of photodynamic therapy.

2.1. Light Sources

Light sources, one of the three components of photodynamic therapy (PDT), enable the activation of the light-sensitive agent accumulated in the targeted region. As new light-sensitive agents are developed, light sources and light distribution systems are also being improved. The selection of the light source to be used during PDT depends on the spectrum in which the photosensitive agent absorbs, the characteristics of the tumor (location, size, accessibility, and tissue characteristics), the size, and cost of the light system (Brancaleon and Moseley, 2002; Tong and Kohane, 2012). Optical filters associated with lamps are used for the treatment of dermatological diseases where access to the target area is easier. With the development of semiconductor materials over time, Light Emitting Diodes (LEDs) have become an alternative to lamps with a wide wavelength range. LEDs have become a good alternative to lamps due to their low cost, reduced size, constant narrow-band emission, no need for optical filters, and the ability to be mounted to cover large irradiation areas or complex anatomical shapes (Triesscheijn et al., 2006; Brown et al., 2004; Trachtenberg et al., 2008; Erkiert-Polguj et al., 2016). Laser systems integrated with optical fibers that ensure the correct transmission of light for photodynamic therapy of deeply located or larger tumors where light cannot easily reach are used. Diode lasers, the most reliable and cost-effective lasers, are preferred. Diode lasers emit light with a fixed wavelength. The lasers most commonly used in photodynamic therapy applications today are helium-neon lasers (633 nm), gallium-aluminum-sequential diode lasers (630–690, 830, or 906 nm), and argon lasers (488–514 nm). In addition to the light source, the application protocol is also important. Different irradiation protocols with the same light source lead to different results in photodynamic therapy. Light dose regimens such as total light dose (J), fluence (J/cm2), and fluence rate (W/cm2) affect antitumor reactions. Especially high fluence rates (W/cm2) lead to a rapid decrease in the amount of oxygen in the tumor tissue. This limits the tumor volume reached during therapy. However, the most appropriate dose regimens depend on the case. Therefore, a thorough understanding of light dosimetry is an important part of photodynamic therapy (Henderson et al., 2006).

2.2. Photosensitizers (PS)

Photosensitizers (PS), one of the three components of photodynamic therapy, are compounds with a natural or synthetic structure that accumulate

in the targeted area. After accumulation, they absorb energy from an appropriate wavelength light source and transfer this energy to biomolecules in the surrounding environment. The chemical, biological, and photo-physical properties of the chosen light-sensitive agent are crucial for the success of photodynamic therapy. The key features that an ideal photosensitizer should possess include:

- It should be easily obtainable, have a low production cost, and maintain stability in a pure chemical structure when stored for an extended period.
- It should accumulate selectively in tumor tissue compared to surrounding tissues, demonstrating selectivity.
- It should have a hydrophobic structure for easy internalization into cells, but it is preferable for systemically administered light-sensitive agents to have an amphiphilic structure. This facilitates the unhindered delivery of the light-sensitive agent to the tumor tissue (Calzavara-Pinton et al., 2007; Kessel and Woodburn, 1993)
- It should not exhibit toxicity in the dark, but it should demonstrate a toxic effect when stimulated by an appropriate wavelength light source.
- It should be rapidly cleared from the skin and epithelial tissues.
- It should have strong absorption in the red and infrared spectrum (600-800 nm) because wavelengths longer than 800 nm do not provide sufficient energy for single-photon absorption to activate oxygen.
- To increase the penetration depth of light into tissues, it should have high absorption at wavelengths above 700 nm. This is important because endogenous molecules such as hemoglobin and water strongly absorb below 700 nm, limiting the penetration depth of light
- It should have a long lifetime in the excited triplet state (T1).
- It should have a high quantum efficiency for singlet oxygen, allowing for the production of a large amount of reactive oxygen species after the stimulation of the light-sensitive agent (Meisel and Kocher, 2005; DeRosa and Crutchley, 2002; Pinheiro et al., 2009; Sigusch et al., 2005; Allison et al., 2004).

Finding and developing an agent with all these diverse characteristics to qualify as a photosensitizer is quite challenging. However, many light-sensitive agents that do not meet all these requirements have received approval for clinical use. These agents are often classified as first and second-generation photosensitizers.

2.2.1. Types of Photosensitizers

Photosensitizing agents are classified based on their generations, chemical purity, target tissue, and chemical structures. First-generation photosensitizers are porphyrin-based, named as hematoporphyrin and its derivatives. Second-generation photosensitizers have been developed to address the shortcomings of the first generation.

Second-generation photosensitizers include expanded porphyrins, chlorophyll derivatives, and dyes with different structures. Dye-based photosensitizers are approved dyes used in clinical settings. Third-generation photosensitizers are developed by conjugating first and second-generation agents with nanoparticles or various biological substances (such as antibodies, amino acids). Many second and third-generation photosensitizers are not clinically approved or commercially available. Therefore, predicting their clinical success has not been fully realized (Juzeniene et al., 2007; Wilson and Patterson, 2008).

The classification based on the chemical purity of photosensitizers considers the purity of the components they contain (Dougherty et al., 1992). Photosensitizers are also classified based on the tissues they accumulate in. There are photosensitizers targeted for accumulation in vascular tissue, cell membranes, and intracellular compartments. Porphyrins accumulate in vascular tissue, hematoporphyrin derivatives accumulate in cell membranes, phthalocyanines accumulate in mitochondria, and benzoporphyrin derivatives accumulate in the Golgi apparatus (Scourides et al., 1987; Berg and Moan, 1997).

In recent years, there has been increasing emphasis on research involving nanomaterial-based inorganic photosensitizers (PS). Nano-inorganic PS demonstrate greater efficacy in PDT applications compared to organic PS. One primary reason is the stability of most inorganic PS even under laser irradiation. Another reason is the modifiability of inorganic PS, providing them with targeting capabilities, which is beneficial for achieving precise cancer treatment and reducing side effects. A third reason is the widespread use of some inorganic PS as carriers for photothermal reagents and chemotherapeutic drugs, allowing for the simultaneous application of multiple therapeutic approaches. The disadvantages of inorganic PS include low singlet oxygen (1O2) quantum yield, weak biological degradability, and biocompatibility. Therefore, further research is needed to explore the clinical use of inorganic PS (Hu et al., 2021).

Table 1. Photosensitizers approved in oncological PDT

Photosensitizers (Chemical Nomenclature and Trademark)	**Approved Applications (Approved Countries)**
Porfimer Sodyum (Porfirin)	Basal cell skin cancer (AB) Bladder cancer (Canada) Squamous cell carcinoma in situ (AB) Cervical cancer (Japan) Esophageal cancer and dysplasia (Canada-EUUS Japan) Gastritis cancer (Japan)
5-Aminolevulinic acid -ALA (Levulan)	Actinic keratosis (Canada-USA)
Methyl ester 5-ALA (Metvix, Metvixia)	Actinic keratosis (Canada-USA)
Meta-tetrahydroxyphenylchlorin (mTHPC) (Foscan)	Head and neck cancers (EU)
Taporfin sodium (Talaporfin), mono-(L)- aspartylchlorin-e6 (MACE, NPe6, LS11) (Laserphyrin)	Lung cancer (Japan)
Meta-tetrahydroxyphenylchlorin (m-THPC) (Foscan)	Head and neck cancers (EU)
Taporfin sodium (Talaporfin), mono-(L)- aspartylchlorin-e6 (MACE, NPe6, LS11) (Laserphyrin)	Lung cancer (Japan)
Chlorin e6 + polyvinylpyrrolidone (Fotolon)	Skin, vulva, cervix and oral cancer (Russia)
Chlorin ash + chlorine psh (Photoditazine)	Skin cancer (Russia, South Korea)
Aluminum sulphonated phthalocyanines (Photosense)	Skin, vulva, oral, esophagus, stomach breast cancer and breast metastases (Russia)

2.3. Singlet Oxygen

The efficacy of photodynamic therapy is directly linked to oxygen. The presence of hypoxic regions in solid tumors poses a significant obstacle to treatment (Vaupel et al., 2001; Fuchs and Thiele, 1998). Damage to tumor vascularization is one of the reasons for hypoxia (Casas et al., 2011). Other causes of hypoxia include the effects of radiotherapy and certain chemotherapy drugs (Fingar et al., 1992). Tissue oxygenation is crucial for the formation of singlet oxygen (Al-Waili et al., 2005). While hypoxia is less common in tumors located more superficially, it is more widespread in deep-seated tumors. In deep tumors, when photosensitizers are activated by light, the rapid depletion of oxygen in the tissue, faster than diffusion, leads to hypoxia (Henderson et al., 2006). For the success of photodynamic therapy, not only the selection of photosensitizers and light but also the presence of oxygen in the environment is crucial (Chen et al., 2002).

3. Mechanisms of Photodynamic Therapy

Studies have demonstrated that photodynamic therapy can be effective with various antitumor mechanisms on tumor tissue (Morgan and Oseroff, 2001; Schmidt-Erfurth and Hasan, 2000; Moor, 2000; Kessel and Reiners, 2007). These mechanisms are divided into three categories: cellular, vascular, and immunological action mechanisms (Figure 2). In the first mechanism, direct cell death is achieved by the localization and activation of the photosensitizer within tumor cells. In the second mechanism, tumor tissue vascularity is damaged, preventing the supply of oxygen and essential nutrients, resulting in cell death through vascular effects. In the third mechanism, the immune system is stimulated, creating an inflammatory and immune response to induce cell death. In photodynamic therapy applications, these three main action mechanisms can be effective either together or separately. These mechanisms are explained in Figure 2 (Moor, 2000; Kessel and Reiners, 2007).

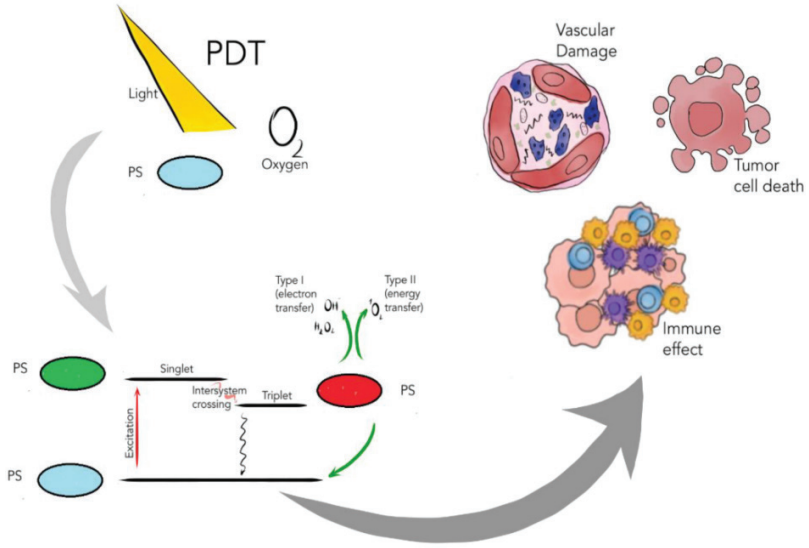

Figure 2. The mechanism of action on tumors in photodynamic therapy (Kubrak et al., 2022).

3.1. Direct Death of Tumor Cells

The type of cellular response that will occur after photodynamic therapy application depends on many factors. The most important factor determining the response that will occur is the localization where the photosensitive substance accumulates within the cell (Henderson and Dougherty, 1992; Kessel and Reiners, 2007). Depending on their biological and physico-chemical properties, photosensitive substances are generally localized in intracellular organelles such as mitochondria, plasma membrane, lysosome, Golgi apparatus, endoplasmic reticulum (Juarranz et al., 2008). The cytoskeleton and other components that hold the cell together have also been identified as targets of photosensitive substances. Although the photodynamic reaction affects different components, there are three main death mechanisms that can occur in the cell (Castano et al., 2004). Direct cell death mechanisms are apoptosis, necrosis and autophagy (Foote, 1991). The death mechanisms of cells are determined programmed or non-programmed, depending on morphology, enzymatic activity, functional and immunological responses (Kroemer et al., 2009; Oliveira et al., 2011).

3.1.1. Apoptosis

Apoptosis is defined as a programmed cell death mechanism (Oleinick et al., 2002). Following photodynamic therapy, photosensitizers are mostly

localized in the mitochondria and endoplasmic reticulum, leading the cell to apoptosis. Intracellular factors affecting apoptosis include cytokines, an increase in intracellular calcium ratio, tumor necrosis factor, activation of the tumor-suppressing p53 gene, and cytotoxic anticancer drugs (Kroemer et al., 2009; Yuan and Kroemer, 2010). It is known that photosensitizers in photodynamic therapy applications do not directly cause DNA damage, hence do not have a mutagenic effect.

Numerous studies have shown that photosensitizers accumulating in the mitochondria or endoplasmic reticulum induce apoptosis better than those found in the Golgi apparatus, lysosomes, plasma membrane, or distributed in the cytosol. Accumulation of photosensitizers in the endoplasmic reticulum alters membrane permeability, leading to the transfer of calcium ions from the endoplasmic reticulum to the mitochondria, consequently inducing apoptosis. The density of calcium ions causes morphological changes in the mitochondrial membrane, leading the cell to apoptosis (Giorgi et al., 2015; Ouyang et al., 2012; Lamkanfi et al., 2007).

3.1.2. Necrosis

Necrosis is characterized by the absence of signaling pathways causing programmed cell death and the generation of an inflammatory response (Kroemer et al., 2009). During necrosis, mitochondrial reactive oxygen species production intensifies, non-apoptotic proteases activate, ATP production levels decrease, and calcium channels open. The most important factor causing necrotic cell death is hypoxia. Necrosis can also occur in response to external stimuli such as heavy metals, infection, toxic substances, or trauma.

There are seven types of necrosis, and each involves consecutive stages. These include disruption of plasma membrane permeability, movement of calcium ions along the endoplasmic reticulum, swelling of organelles and plasma membrane, activation of calcium-dependent calpains, lysosomal breakdown, degradation of cellular structures, and the occurrence of inflammation and/or inflammation (Lemasters, 2005; Cho et al., 2011).

Necrosis is frequently observed in cell death with photosensitizers accumulating in the plasma membrane. In vitro and in vivo photodynamic efficacy of liposomal aluminum chloro-phthalocyanine has been investigated in Ehrlich tumors, and it has been reported that this photosensitizer destroyed the tumor with 90% necrosis and partial vascular effects (Mroz et al., 2011; Conrad et al., 2016; Longo et al., 2009).

3.1.3. Autophagy

Autophagy is the lysosomal degradation of intracellular macromolecules engulfed in a vacuole. Autophagy is a cell death mechanism that balances anabolic and catabolic intracellular functions and enables the recycling of damaged intracellular structures. A portion of the cytoplasm or relevant organelles is enveloped by the extracellular membrane of the endoplasmic reticulum. Primary lysosomes merge with this structure to form a secondary lysosome (autophagic vacuole=autophagosome). Autophagosomes are double-membraned structures. Autophagosomes, when broken down by hydrolytic enzymes, lead to condensation in the nucleus. (Jain et al., 2013; Michaud et al., 2011; Di et al., 2013; Nikoletopoulou et al., 2013).

Autophagy acts as a protective mechanism in low-dose PDT applications, while it drives cells to death in high-dose applications. Autophagic cell death can occur when photosensitizers accumulate in the mitochondrial and/or endoplasmic reticulum (Xue et al., 2010; Garg et al., 2015; Separovic et al., 2011).

3.2. Vascular Effect

Angiogenesis, named as the formation and development of new blood vessels, not only provides oxygen and nutrient sources to cancer cells but also allows cells to spread to distant organs (Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011). Therefore, targeting the tumor vascular system is considered a promising approach in cancer treatment.

Selective vascular damage and the occurrence of an anti-angiogenic effect enhance the effectiveness of photodynamic therapy applications. For example, hematoporphyrin derivative (HPD) induces therapeutic effects by causing damage to the vascular structure of tumors and causing problems in blood flow (Star et al., 1986). It has been noted that the duration between the application of the photosensitizer and light activation affects the damage to the target vascular system in PDT applications. In a study where Verteporfin was used as a photosensitizer in PDT applications to rat chondrosarcomas, it was recorded that vascular damage increased when the duration between drug-light applications was shortened (Fingar et al., 1999).

Endothelial and subendothelial cells are damaged in PDT applications with photosensitizers exhibiting vascular effects. Endothelial cells round up, and connections between cells widen (Ben-Hur et al., 1998). This situation causes the tissue under damaged endothelial cells to be exposed. Damaged endothelial

cells lead to the activation of coagulation factors such as Von Willebrand factor and the activation of platelets. Activated platelets pass into subendothelial tissue, leading to platelet aggregation, thrombus formation, and vessel occlusion. Active platelets also cause vasoconstriction after PDT, further reducing blood flow (Foster et al., 1991). Thus, during photodynamic therapy application and after application, damage such as vascular narrowing, vascular permeability, and leukocyte adhesion occurs in the target tissue.

After treatment, leakage of macromolecules and fluid from the vessels occurs, and tissue edema is observed in the target area (Nelson et al., 1987; Fingar et al., 1997). This slows down blood flow to the tumor tissue, leading to regional hypoxia. Hypoxia and lack of nutrients trigger the destruction of tumor cells. Vascular effect is one of the most important components of the anti-tumor effect of photodynamic therapy (Chen et al., 2005; Kurohane et al., 2001; Krzykawska-Serda et al., 2014).

3.3. Immunological Effect

The immunological effect is the third tumor destruction mechanism induced by photodynamic therapy and is based on the creation of an inflammatory response (Castano et al., 2006). The fundamental feature of the inflammatory process is the release of inflammatory factors in the treated area (Korbelik et al., 2005). Oxidative stress resulting from photodynamic therapy regulates the release of transcription factors for heat shock proteins and the release of inflammatory cytokines (Gollnick et al., 2003). The release of proteins and damage-associated molecular patterns (DAMPs) associated with damage contributes to the formation of the inflammatory response (Garg et al., 2010; Matroule et al., 2006).

Studies have shown that photodynamic therapy induces the expression and release of heat shock proteins after PDT applications (Reginato et al., 2014). Heat shock proteins interact with Toll-like receptors by binding to tumor antigens (Korbelik and Sun, 2006). These interactions regulate the expression of inflammatory and immune response genes. The source of heat shock proteins and damage-associated molecular patterns can vary depending on the cellular effects of FDT (Cecic and Korbelik, 2002; Gollnick et al., 2002). Consequently, an inflammatory response occurs in tumor tissue induced by PDT (Garg et al., 2010; Zhang et al., 2009).

4. Conclusion

PDT is considered a new and promising method for cancer treatment. Its potential when applied alone or in combination with other therapeutic approaches

is still being investigated. The most important advantages of PDT are that it is reproducible compared to surgery, chemotherapy or radiotherapy, that it has the ability to increase the immune response rather than suppressing the immune system, and that mutations that may occur cannot affect the antitumor properties of PDT. Due to all these promising therapeutic properties in the treatment of cancer, more interdisciplinary studies covering fields such as physics, chemistry, biology and medicine are required to develop PDT and use it in the clinic.

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CHAPTER IV

POMEGRANATE (PUNICA GRANATUM L.) AND CANCER RESEARCHES

İNAN KAYA*

** (Prof. Dr.), Department of Biology, Faculty of Art and Science, Kafkas University, Kars, Turkey*
E-mail: inankaya_@hotmail.com
ORCID:0000-0003-2900-4067

1. Introduction

Many plant species have been evaluated through scientific studies in the field of health to date, and the number of more detailed studies for the treatment of cancer which has emerged as the most important health problem in the world is increasing. Cancer treatments performed with developing medical techniques provide very successful results. The most common types of treatment used in advanced stages of cancer are chemotherapy and radiotherapy. These treatments have serious side effects. For this reason, there is an increase in the demand for natural and carcinogen-resistant foods before, during or after the disease. Among these demands, the pomegranate plant stands out as a plant species that can be very advantageous in terms of its easy and large quantity availability and the many bioactive components it contains.

2. Pomegranate

Pomegranate (*Punica granatum* L.), a perennial species belonging to the Punicaceae family from Myrtales order of Magnoliopsida class of plant Kingdom since ancient times has never lost its importance as a symbol of health, especially since ancient times, due to its taste, appearance and easy collection. Pomegranate originates from Southeast Asia and is widespread in tropical and subtropical regions (Çiçek 2019; Aşasın and Kaya, 2023). More than 1000 species of *Punica granatum* L. are reported to exist throughout the Mediterranean, east to China and India, and in the New World to the Southwest

America, California and Mexico (Levin, 1994; Lansky and Newman, 2007). While the juicy grain clusters in the pomegranate fruit are separated by membranes between the endocarp and exocarp, there is plenty of water in the grains around the core. The peel, root, flower and leaf parts of the plant are also important while these parts are important for the content of pomegranate juice. It is stated that pomegranate tree roots have anthelmintic properties, its flowers have antidiabetic properties, and its barks have astringent or germicidal properties, especially against aphtha, diarrhea and ulcers (Lansky and Newman, 2007). Figure 1 shows the weight percent composition of pomegranate pieces. On average 46 % of the pomegranate by weight is used as juice, and the rest is used as waste. Pomegranate peel is the waste component of the fruit, accounting for 43% by weight. Seeds, another waste component of pomegranate, have a share of 11% by weight (Ko et al., 2021).

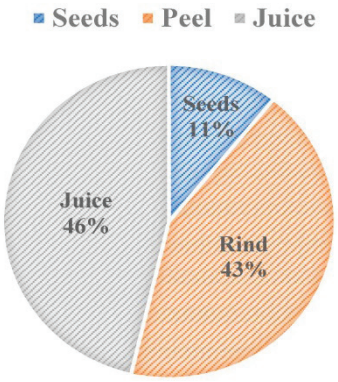

Figure 1. Weight percent composition of pomegranate (Ko et al., 2021)

The importance of this plant increases day by day due to its minerals and natural compounds such as phenolic compounds in pomegranate juice and polyunsaturated fatty acids in the seed, and their widespread use in areas such as cosmetics, medicine, nutrition, chemistry and coloring (Mansour et al., 2013; Dathan et al., 2023). It has been noted that there are significant improvements in the digestive system, growth and meat yield of livestock by giving pomegranate peel or pomegranate by-products to experimental animals (Imbabi et al., 2021). It is reported that the whole fruit including its water, seeds and peels should be used to maximize the effects of the bioactive compounds contained in pomegranate on health and living development (Imbabi et al., 2021), and studies involving different parts of this fruit are intensively applied (Kim et al., 2002; Lansky et al., 2005; Kaya et al., 2022; Dathan et al., 2023).

Polyphenols are bioactive components naturally found in high concentrations in plants (Kılıç et al., 2022), and the richness of polyphenols in pomegranate means that this plant will maintain its place as a functional food. The structures of phenolic compounds consist of at least one aromatic ring with one or more hydroxyl substituents, and the main phenolic substances among them are tannins, flavonoids and phenolic acids (Babbar and Oberoi, 2013). Considering their chemical structures, the tannins naturally found in pomegranate peel can be classified into four groups: ellagitannins, gallotannins, condensed tannins and complex tannins (Sieniawska and Baj, 2017). Punicalagin (Figure 2) (Valero-Mendoza et al., 2023), a type of ellagitannin and the characteristic main component of pomegranate peel tannins can produce ellagic acid through spontaneous endo-esterification hydrolysis of the hexahydroxybenzoic acid structure (Larrosa et al., 2006). Flavonoids are a class of compounds derived from the flavanone (2-phenylchromanone) commonly found in pomegranate peels. The chemical structure has two aromatic A and B rings, usually in the form of a heterocyclic ring attached to a third carbon chain (C) (Dua et al., 2016). The differentiation of localization on the ring give rise to different subclasses as flavonoids, flavonols, proanthocyanidins, and anthocyanidins. The density of phenolic acids, which include caffeic, ellagic, gallic, butyric, chlorogenic, cinnamic, ferulic and erucic acids, identified in pomegranates, varies depending on the climate and place where they live. Phenolic acids, divided into two groups as hydroxybenzoic acid and hydroxycinnamic acids (Figure 3) , consist of a phenolic ring and an organic carboxylic acid (Kaderides et al., 2020). Dietary fiber is the most abundant component in pomegranate peels, ranging from 33 % to 62 %. It contains the most dense lignin, followed by cellulose and uronic acids and neutral sugars such as xylose, arabinose and galactose (Hasnaoui et al., 2014; Mo et al., 2022).

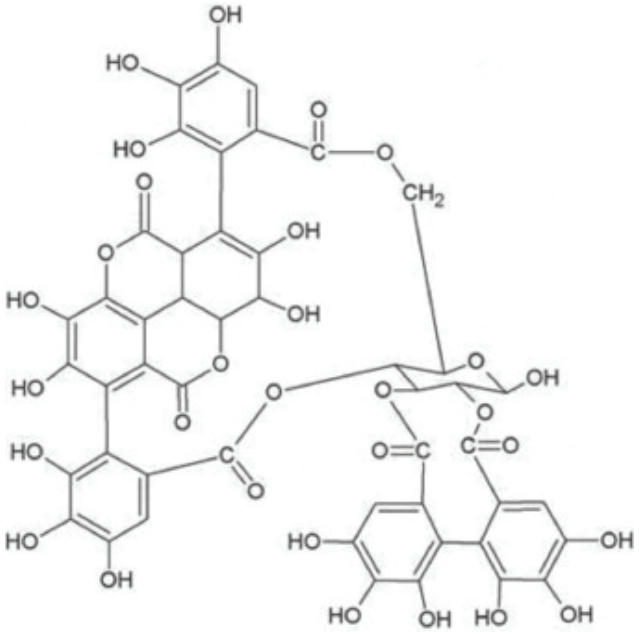

Figure 2. Molecular structure of punicalagin (Valero-Mendoza et al., 2023).

Some basic compounds of pomegranate include simple sugars, aliphatic organic acids, hydroxybenzoic acids, hydroxycinnamic acids, cyclitol carboxylic acids and their salts, flavan-3-ols, flavonols, flavonol glycosides, flavones, flavone glycosides, anthocyanidins, anthocyanins, ellagitannins, amino acids, indolamines, pelletierine alkaloids, piperidine alkaloids, pyrrolidine alkaloids, conjugated fatty acids, unconjugated fatty acids, sterols, steroids, tocopherols, triterpenoids, glycolipids and phenyl aliphatic glycosides (Lansky and Newman, 2007). According to different parts, it was reported that pomegranate contained anthocyanidins, phenolic punicalagins, gallic acid, catechin, flavones and flavonones in peel and pericarp, rutin, quecertin, anthocyanins, glucose, ascorbic acid, ellagic acid, gallic acid, catechin, minerals and amino acids in pomegranate juice, sterols, ellagic acid and 95% punicic acid in seed oil, gallic acid, urosolic acid, triterpenoids including maslinic and asiatic acid in pomegranate flower, piperidie alkaloids, ellagitannins, punicalin and punicalagin in roots and bark, apigenin, tannins, flavone glycosides and luteolins in leaves (Jauhar et al., 2018). The preventive effects on bladder cancer, inflammation of prostatic cells and testicular damage of ellagic acid as a compound belonging to the hydroxybenzoic acid class found naturally and extensively in pomegranate are quite remarkable in terms of anticarcinogenic, anti-inflammatory and

antioxidative mechanisms (Li et al., 2005; Masamune et al., 2005; Kaya et al; 2015). Bioactive components and their concentrations in pomegranate content and therefore in its peel vary according to analysis methods. General bioactive components found in pomegranate peel are in Figure 3 (Mo et al., 2022).

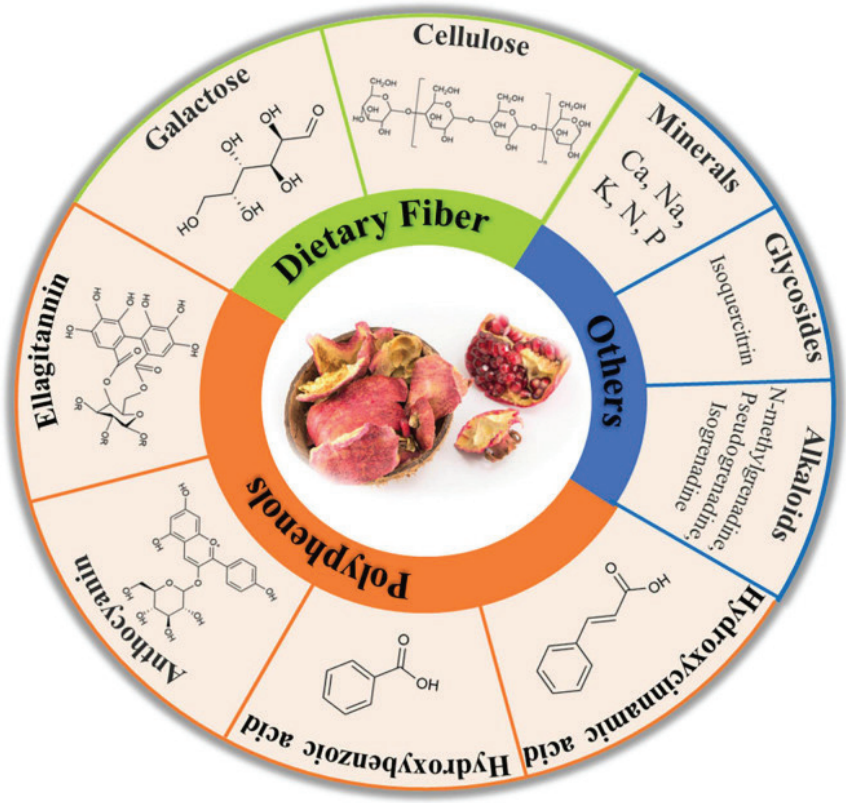

Figure 3. Main bioactive compounds of pomegranate peels (Mo et al., 2022)

3. Cancer

"Cancer", also known as neoplasm or malignant tumor, is a disease that manifests itself with the uncontrolled development of a cell or cells that have a way of life outside of their normal vitality functions, invading tissues and spreading. Approximately 10 million human deaths occurred in 2020 due to cancer, and this number corresponds to approximately 1 in every 6 deaths, and it can be estimated that deaths similar to these numbers and rates may occur at the end of 2023. It constitutes 50-55% of the total deaths due to cancer in the world according to tissues, and the top 6 death rates are lung, liver, stomach, prostate,

pharynx and colon in men, and breast, lung, cervix, colon, stomach and liver in women. Pancreatic, bladder, cervical, ovarian, leukemia, rectum, thyroid, etc. cancer types are also encountered with lower incidence and mortality rates. While the survival rate is high in countries with developed health systems due to faster access to early diagnosis and treatment, the opposite of this situation is observed in underdeveloped countries (Ferlay et al., 2021; WHO 2022).

While new studies on the treatment of cancer are rapidly continuing, plants rich in bioactive or phytochemical content continue to attract more attention. These plants are generally fruits with red or crimson and purple color tones when viewed macroscopically. Among these fruits, the use of pomegranate and grape herbs is intensively evaluated in terms of components, dosage and time for living tissues or cells.

4. The Effect of Pomegranate on Cancer and Its Stages

The process by which normal cells turn into cancerous cells in living organisms is known as "carcinogenesis". It is suggested that cancer cases will increase by approximately 70% by 2030 (Dathan et al., 2023). Inherited or acquired mutations that develop in living cells in a process associated with chemical, physical or biological carcinogens cause cancer development by affecting the activation of growth-promoting oncogenes, inactivation of tumor suppressor genes, changes in genes regulating apoptosis and damaged DNA repair genes (Langie et al., 2015). The main stages leading to this process are changes related to chronic inflammation and may continue for several years or decades with different periods such as tumor transformation, survival, proliferation, migration, adhesion, invasion, metastasis and angiogenesis. The most important way to control cancer seems to be possible by preventing tumor formation, resistance and spread, known as tumorigenesis. Figure 4 has been shared to emphasize the importance of this. Studies increase the importance of pomegranate as a functional food against cancer or tumorogenesis (Kim et al., 2002; Lansky et al., 2005; Adams et al., 2006; Zraikat et al., 2020; Kaya et al., 2022; Dathan et al., 2023).

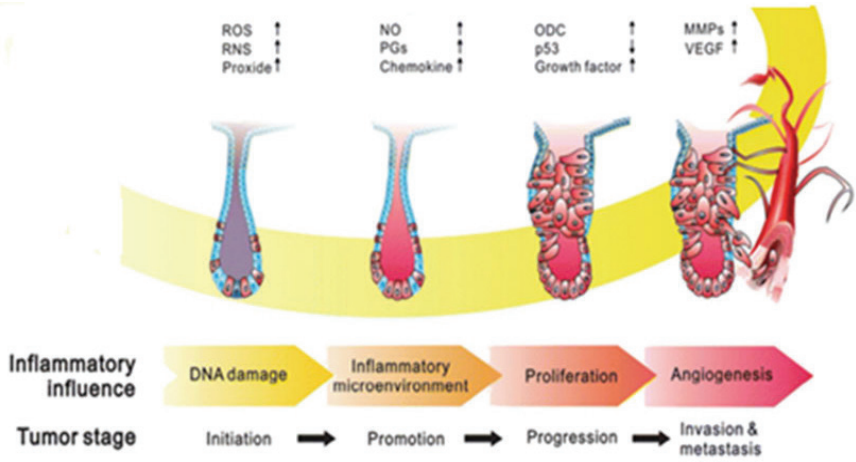

Figure 4. Inflamattory influence and tumorogenesis (Pan et al., 2010; Hussein et al., 2020)

4.1. Effect of Pomegranate on Inflammation

Deteriorations in tissues as a result of inflammatory reactions in living organisms can cause genetic material damage, cell cycle disorder, tumorigenesis and cancer along with metabolic differentiation. Studies show that the phytochemicals naturally found in pomegranate have important effects on the expression of inflammatory proteins that lead to cancer. It has been reported that tannins and punicalagin found in pomegranate juice increase the synthesis of prostaglandins responsible for inflammation that develops as a result of direct injuries and significantly reduce the expression of cyclooxygenase-2 (COX-2), an enzyme responsible for pain and the target of nonsteroidal anti-inflammatory drugs (Adams et al., 2006; Dathan et al. ., 2023). It has been noted that ellagic acid, a metabolite of pomegranate ellagitannins, inhibits intestinal inflammation by reducing the synthesis of some inflammation-mediating compounds such as COX-2 and iNOS and by blocking the nuclear factor kappa B (NF-kB), p38, mitogen-activated protein kinase (MAPK), interleukin 6 (IL6) and signal transducer and activator of transcription 3 (STAT3) signaling pathways of cells in colon tissues (Marin et al., 2013; Kaya et al., 2019; Dathan et al., 2023). It has been shown that pomegranate fruit extract may be an important modulator of apoptosis and inflammatory response by reducing oxidative stress in mice with induced colorectal cancer, suppressing the expression of transient receptor potential (TRP) ankyrin 1 and melastatin 2 protein channels, and the synthesis of caspase-3 and tumor necrosis factor-α (TNF-α) (Kaya et al., 2022).

4.2. Effect of Pomegranate on Proliferation

In experimental studies, it has been proven that extracts prepared with pomegranate peel, juice or oil can inhibit cell proliferation in different human cancer cell lines to different degrees when applied in certain doses and times (Kim et al., 2002; Lansky et al., 2005; Dathan et al., 2023). It has been reported that androgen-independent DU-145 cells in human prostate cancer cells are more sensitive to pomegranate peel and juice than cold-pressed pomegranate seed oil, while normal prostate epithelial hPrEC cells are significantly less affected than androgen-sensitive LNCaP cancer cells when pomegranate peel and juice are applied (Lansky et al., 2005). It was reported that pomegranate seed oil inhibits 90% of MCF-7 proliferation from human breast cancer cell lines in a medium with a concentration of 100 μg/ml (Kim et al., 2002). It has been claimed that fermented pomegranate juice polyphenols show antiproliferative properties that are approximately twice as effective as fresh pomegranate juice polyphenols in both MCF-7 and MB-MDA-231 human breast cancer cells (Kim et al., 2002; Lansky et al., 2005). .

4.3. Effect of Pomegranate on Invasion

In carcinogenesis, local invasion of a tumor and metastases are the most relevant and most difficult to target processes required for medical treatments. Invasion is a very critical stage of the tumor spread process which mechanistically involves the change of many proteins. Due to this diversity of factors, it is difficult to prevent invasion by using different treatment methods. It has been noted that a 10 μg/ml concentration of pomegranate seed oil inhibited 75% of invasion through the Matrigel membrane of MCF-7 (Kim et al., 2002). It has been reported that when any two of pomegranate peel, juice and cold-pressed seed oil are applied in equal proportions, the invasion suppression rate exceeds 90%, and when all three are given in equal proportions, this rate exceeds 99% (Dathan et al., 2023). It was noted that fresh pomegranate juice at doses of 1.7%, 3.3% and 5% (v/v) significantly prevented invasion depending on dose and duration in a study conducted with U87 glioma cells used in carcinogenicity studies for brain and glioma cell lines (Zraikat et al., 2020). Aqueous pomegranate fruit extract (50-300 μg/mL) was found to dose-dependently reduce RhoA and RhoC protein expression, which are increased in carcinogenesis, while inhibiting NF-kB-dependent reporter gene expression associated with proliferation, invasion and motility in aggressive breast cancer phenotypes (Khan et al., 2009).

4.4. Effect of Pomegranate on Metastasis

It has been suggested that approximately 90 % of deaths due to cancer are associated with metastatic spread of primary tumors (Lansky and Newman, 2007). Studies on the pomegranate plant indicate its significant potential in preventing metastasis which is a very important goal in cancer treatment.

Integrins are responsible for the adhesion of the extracellular matrix. In a study conducted with mice, it was reported that pomegranate peel extract whose polyphenolic component content corresponds to 10 mg/kg per day significantly reduced the expression of integrin β3, an important biomarker of metastasis and a subunit of integrins (Spilmont et al., 2015). Claudins are a family of transmembrane proteins that, along with occludins, are the most important components of tight junctions. It has been demonstrated that pomegranate increases the gene expression of claudin-1 as a member of the claudin family, and it has been stated that pomegranate juice components may be a potential inhibitor of prostate cancer metastasis (Wang et al., 2012; Ahmadiankia, 2019).

It is suggested that some molecules (hyaluronic acid-mediated motility receptor, tenascin C, matrix metalloproteinase etc.) in living cells and extracellular matrix adhesion may be significantly affected by pomegranate components. It has been reported that the targets of pomegranate components may include modulators of cytoskeletal dynamics (nexilin, fascin, vimentin, anillin etc.) and regulators of cancer cell anoikis and chemotaxis (caspase-3, stromal cell-derived factor 1 etc.). In addition, it has been stated that the antimetastatic effect of pomegranate components depends on molecular changes in the extracellular matrix, and pro-inflammatory and proangiogenic molecules (induced protein-10, interleukin, macrophage inflammatory protein-1, tumor necrosis factor-α etc.) may be other targets of pomegranate components related to the metastasis stage in cancer (Wells et al., 2013; Guan, 2015; Ahmadiankia, 2019).

4.5. Effect of Pomegranate on Angiogenesis

Oxygen and nutritional factors required for tumor formation, growth and metastasis among the carcinogenesis processes are provided by angiogenesis. Therefore, angiogenesis inhibition, first proposed by Folkman (1972), has become a nontoxic and promising therapy for the treatment of sick individuals exposed to solid tumors. It was later noted that pomegranate has the ability to inhibit angiogenesis doe to its bioactive components such as dense ellagitannins, flavonoids and anthocyanins (Lansky and Newman, 2007; Dathan et al., 2023).

Purified pomegranate fruit extracts or juices are reported to contain polyphenols that show many anticarcinogenic activities such as arresting the cell cycle with strong antioxidative effects, antiangiogenesis, inducing apoptosis, and antimutagenesis activities (Gil et al., 2000; Dathan et al., 2023).

Using approximately 50 µg/ml concentration of pomegranate seed oil and fermented pomegranate juice polyphenols, proangiogenic vascular endothelial growth factor (VEGF) expression was strongly reduced in estrogen-dependent breast cancer cells (MCF-7) and human umbilical vein endothelial cell (HUVEC) proliferation and tubule formation were recorded to be moderately suppressed while pomegranate peel and juice were reported to strongly inhibit human myometrial fibroblast proliferation (Toi et al.; 2003; Khan et al., 2013).

It has been noted that tumor formation initiated by the chemical carcinogen 7,12-dimethyl-enz[a]anthracene (DMBA) in mouse mammary organ culture was reduced by 46% with fermented pomegranate juice, and this rate reached 87% with seed oil (Mehta and Lansky 2004). In a study, 0.1, 0.3 and 0.5% dilutions of the extract prepared with 10 g of pomegranate powder dissolved in 100 ml of water significantly suppressed *in vivo* angiogenesis in the chicken chorio allantoic membrane with fermented pomegranate juice, but this suppression was not observed with pomegranate peel extract (Khan et al., 2013). The anti-angiogenic potential of pomegranate fractions attracts attentions according to numerous studies.

5. Conclusion

In this section, it was tried to present some general informations about how pomegranate affects the stages of cancer. It is still not fully understood which components should be used in combinations for how long and in concentration to determine the protective effects of pomegranate against different types of cancer and for treatment. In order to understand the molecular or biochemical mechanisms by which the effects of pomegranate will operate, current information containing many detailed results is required.

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CHAPTER V

BIOACTIVE COMPONENTS OF MUSHROOMS AND THEIR PLACE IN CANCER STUDIES

ŞULE İNCİ[1] & IŞIK DIDEM KARAGÖZ[2] & SEVDA KIRBAĞ[3]

[1] (PhD) Department of Biology, Science Faculty,
Gaziantep University, Gaziantep, Turkey,
E-mail: sule.inci@hotmail.com
ORCID: 0000-0002-4022-5269

[2](Prof. Dr.) Department of Biology, Science Faculty,
Gaziantep University, Gaziantep, Turkey,
E-mail: karagozid@gmail.com
ORCID: 0000-0001-6527-2750

[3](Prof. Dr.) Department of Biology, Science Faculty,
Firat University, Elazığ, Turkey,
E-mail: skirbag@firat.edu.tr
ORCID: 0000-0002-4337-8236

1. Introduction

Cancer has existed for centuries and poses a significant threat to humanity. It would not be wrong to say that throughout the history of medicine, no disease has been subject to as much research as cancer. Nowadays, with the knowledge of ways to protect against cancer, early diagnosis methods, and the increasing success of treatment, people have many reasons to get rid of their fear of cancer. However, although it is possible to cure some types of cancer or to live longer with this disease, cancer has not ceased to be a feared disease and is synonymous with death (Atıcı, 2007).

It was observed that the term cancer was first used by Hippocrates (460-377 BC) for new structures of the organism that could not be cured. These

swellings, which grow on the body's surface and are generally hot, painful, and have a different character than others, were called "karkinos" or "carcinoma" by Hippocrates and "cancer" by Galen (2nd century AD) because of their appearance, which he likened to a crab (Sigerist, 1960).

For the continuation of life, every cell in the body grows, divides, ages, and dies regularly. On the other hand, cancerous cells constantly grow, divide and do not die. Some proteins regulate the life cycle of normal healthy cells and genes that synthesize these proteins. These proteins and genes play important roles in the control mechanism of cells. These are known as tumor suppressor genes and proto-oncogenes. Any damage or mutations that may occur in these genes cause cancer (Topal et al., 2009).

The disease group caused by a group of cells that form abnormally in this way and have the ability to grow and spread uncontrollably is called cancer. Mere spread and uncontrolled proliferation are not sufficient for the disease to occur. The cell must also carry other malignant features such as invasion and metastasis (Başpınar, 2015).

Cell proliferation and metastasis, which are characterized as irregular abnormal cell growth, are observed in all cancer cells. In normal cells, cell proliferation and spread are controlled by some expressed genes, but, these genes are mutated in cancerous cells. In this way, the cells in the cancerous areas increase in number. However, they begin to spread to other tissues through blood and lymph to meet the necessary nutritional needs. As a result, abnormal tissue masses are formed, both structurally and functionally (Aslan, 2011).

The basic features that distinguish a cancer cell from a normal cell include the ability to escape from apoptotic mechanisms, the cancer cell's ability to provide growth signals for itself and its insensitivity to signals that inhibit development, angiogenetic capacity, unlimited proliferation power, ability to tissue invasion and metastasis. When evaluated in this way, tumor formation can be expressed as chromosomal changes that will initiate carcinogenic events, tumor proliferation and metastasis due to disruption of the cell cycle and apoptotic process (Özen et al., 2017).

Although the exact cause of cancer has not been fully revealed, current research shows that the incidence of cancer is increased by radiation exposure, chemicals, unhealthy diet, being overweight, aging, infections, family history, etc., suggests that it may be related to Today, considering the type, location, stage, and size of the cancerous cell, cancer treatments include chemotherapy, surgery, hormone therapy, radiotherapy, polytherapy or biological therapy (Xu et

al., 2022). However, considering the recurrence of the disease, drug resistance, hepatotoxicity and other side effects of these treatments, it is essentional to develop alternative treatment methods (Zhou et al., 2016). It is known that some dietary natural nutrients and their bioactive components have various mechanisms that inhibit cancer cells, prevent metastasis, affect apoptosis and cell cycles, and make tumor cells sensitive to radiotherapy and chemotherapy (Li et al., 2017). For this reason, studies on the anticancer activities of natural products have gained momentum in recent years. In this sense, due to the gradual increase in the disease and considering the inadequacy of synthetic drugs, the tendency towards natural products such as plants, animals, and mushrooms is increasing (Bal 2018).

2. Fungi and Their Bioactive Components

Fungi are organisms that produce distinctive fructification structures, eukaryotic, non-photosynthetic, and aerobic (Chugh et al., 2022). More than 14,000 mushroom species have been described so far, 2000 of which are stated to be edible (Chugh et al., 2022). Edible mushrooms have been consumed as food worldwide since ancient times due to their unique aroma and texture (Nowacka-Jechalke et al., 2018; Wieme et al., 2022). In addition to being low in calories, they are nutritional sources sought in the diet because they are rich in carbohydrates, essential amino acids, fibers, minerals and some crucial vitamins (Öztürk and Çopur, 2009). In addition, they contain many secondary metabolites such as alkaloids, terpenoids, lectins, steroids, and phenolic compounds (Wasser, 2010; Borthakur et al., 2020).

Mushrooms, which have an essential place in the ecosystem, are known as medicinal food because they contain both food and biologically active components (Sarıkürkçü et al., 2004), and as a result of the research, it has been confirmed that mushrooms are used for medical and therapeutic purposes (Amirullaha et al., 2018). The medicinal properties of mushrooms have been known to people since ancient times (Li et al., 2023). Its oldest uses, especially for its medicinal properties, come from Asian countries. Additionally, Poland and other Slavic countries also have a history of collecting and consuming mushrooms growing in the wild (Nowacka-Jechalke et al., 2018). For this reason, in recent years, interest in the consumption of mushrooms and their use for health has been increasing (Panda and Lutyen, 2022; Li et al., 2023).

It is known that many mushroom species have potential pharmaceutical and neutraceutical effects in the prevention and treatment of different life-

threatening diseases, thanks to the components they contain (Verma et al., 2023). In particular, it exhibits important biological activities against life-threatening diseases such as bioregulation, hepatoprotective, homeostasis, antimicrobial, antidiabetic, antioxidant, hypolipidemic, hypotensive activities, anti-inflammatory, and HIV-1 (Bladogatski et al., 2018).

Biologically active components in mushrooms are mainly obtained from fruit organs, called fructification organs (Mizuno, 1996; Borchers et al., 1999). Therefore, these components vary depending on the type of mushroom, growing conditions, developmental stage, storage status, processing and cooking of the mushroom (Barros et al., 2007; Barros et al., 2008; Mattila et al., 2001). In order to examine the biological activities of the components contained in mushrooms, it is necessary to obtain them essentially pure. Sometimes applying a single polysaccharide type gives better results, sometimes and raw mushroom extracts can also be applied (Borchers et al., 2004; Vickers, 2002). After the concentrations of these components are obtained under appropriate conditions, they are tested in animal models for certain types of cancer. These extracts have been shown to be more effective in cancer treatments compared to other anti-cancer agents (Sheikh et al., 2017).

It is explained by studies that these components of fungi exhibit anticancer activity through multiple mechanisms, including the regulation of immune responses by inhibiting cell proliferation and metastasis, inducing apoptosis and autophagy, and activating T lymphocytes, macrophages, and NK cells (Xu et al., 2022)). Therefore, instead of attacking cancer cells directly, they show this effect indirectly by stimulating the immune systems (Chakraborty et al., 2019).

Mushrooms have bioactive components such as glycoproteins, proteins, polysaccharides, lipids, and secondary metabolites (Lull et al., 2005). Polysaccharides are generally cell wall components such as β-glucans (Sánchez, 2017). Proteins obtained from fungi are lectins, ribosome-inactivating proteins, proteases and protease inhibitors, and fungal immunomodulatory proteins, which are also bioactive components of fungi (Erjavec et al., 2012).

3. Anticancer Role Of Bioactive Components of Mushrooms

3.1. Polysaccharides

The main active components of mushrooms are polysaccharides. Polysaccharides are large chains or branched biopolymers formed by the combination of many monosaccharide monomers. They exist mainly as glucans with different types of glycosidic bonds. Monosaccharide units in

polysaccharides can be linked to each other at various points in their linear or branched structures (Sharon and Lis, 1993). This great potential variability in polysaccharide structure also contributes to biodiversity.

Fungal polysaccharides can be classified as α-glucans (starch, cellulose, chitin), β-glucans and their derivatives. While biological activity is rare in α-glucans, β-glucans have been shown to be responsible for various biological properties (Grienke et al., 2014). They are also metabolites that have antioxidant, DNA damage-preventing and carcinogenic properties (Friedman, 2016). β-glucans have been shown to affect both pro- and anti-inflammatory cytokines. One of its other mechanisms of action is its ability to bind to immune cell surface receptors (Muta, 2006). Thus, β-glucans activate proliferation and maturation of immune cells, while also stimulating the activation of macrophages (Akramiene et al., 2007).

There are mainly fungal β-glucans used for pharmaceutical applications. These are schizophyllan isolated from *Schizophyllum commune* (Kumari et al., 2008), Grifolan compounds isolated from *Grifola frondosa*, lentinan isolated from *Lentinula edodes* (Nakano et al., 1999), and krestin (PSK) from *Coriolus versicolor* (Pang et al., 1998) (Okazaki et al., 1995). Lentinan and schizophyllan are pure β-glucans, while PSK is protein-bound β-glucans (Sivanesan et al., 2022).

3.1.1. Lentinan

This compound was first isolated by Chihara (Chihara, 1992). It is a fungal polysaccharide, one of the β-glucan types and the longest used for therapeutic purposes. Lentinan is a water-soluble homogeneous β-glucan. It has a molecular weight of 500 kDa and consists of β-(1-3) linked D-glucan as the main chain and β-(1-6) linked glucose units as side branches (Lv et al., 2009). Lentinan has immunomodulatory, anticancer, anti-inflammatory, and anti-diabetic effects (Sheikh et al., 2017; Wang et al., 2016; Ruthes et al. 2016). In clinical studies, it is known to have biological response-modifying and immune-stimulating effects in HIV (Gordon et al. 1998), hepatitis (Wang 1994; Wu et al. 1993) cancer treatments (Ina et al. 2013; Wang et al. 2012), and malignant pleural effusion (Yoshino et al. 2010; Chang et al., 2013). It is used in cancer treatments especially in Japan and China and has been approved as an adjuvant. It is available in capsules, tablets and injections. Lentinan capsules and tablets are taken orally as a traditional medicine. Intravenous lentinan injection is clinically approved and used in hospitals at a dose of 1-1.5 mg/day/patient (Zhang et

al., 2018). Although Lentinan does not directly kill the cancer cell, it has an antitumor effect by affecting the immune system through multiple signals.

It is known to have antitumor activity against Sarcoma 180 type tumors and several types of tumors containing methyl-chloroethane. Lentina obtained from shiitake mushrooms has been shown to prolong life in patients with stomach cancer and colorectal cancer (Sheikh et al., 2017). In one study, HT-29 was reported to significantly inhibit the proliferation of colon cancer cells and suppress tumor growth (Wang et al., 2017). In a study conducted in China between 2004 and 2016, it was reported that the use of lentinan ingredients in 9474 cancer cases had significant effects on improving the quality of life and increasing the effectiveness of chemotherapy and radiation therapy during cancer treatment (Zhang et al., 2019). It is known that lentinan can directly inhibit liver cancer (HepG2), breast cancer (MCF-7), and colon cancer (HT-29) in vitro (Zhang et al., 2021). In a different study of the same compound, it was found to inhibit Sarcoma 180 type tumor growth by ~75%. Additionally, lentinan has been shown to promote cell apoptosis and the development of immune cells in tumors during tumor development (Xu et al., 2016). Bao et al. (2015) reported that lentinan could induce apoptosis via intracellular reactive oxygen species (ROS) when treating human bladder cancer T24 cells. Similarly, lentinan has been reported to cause apoptosis in S180 cells via mitochondrial pathways by upregulating Bax and downregulating Bcl-2 (Zhang et al., (2015).

3.1.2. Schizophyllan

It is a polysaccharide first discovered by Kikomoto (1970) and Kikomoto (1971). This compound is a nonionic, water-soluble homoglucan with a β-(1→3)-linked backbone with β-(1→6)-linked glucose side chains (Zhang et al., 2013). Its molecular weight is approximately 450,000 daltons (Sheikh et al., 2017). Studies conducted in recent years have proven that it has immunomodulatory, anticancer, antineoplastic, anti-inflammatory, antimicrobial and antioxidant activities (Zhang et al., 2013; Chen et al., 2020; Yelithao et al., 2019). The most promising among these activities are anticancer and immunomodulatory activities. Schizophyllan is currently produced commercially by several Japanese companies as an immune enhancer in patients undergoing cancer treatment because it is a biological response modifier (Zhang et al., 2013). In a study, schizophyllan was tested against both acid and solid forms of four types of tumors (sarcoma37, sarcoma-180, Ehrlich carcinoma, and Yoshida sarcoma). The growth of acid-form tumors could not be prevented, and the most effective results were obtained at a dose of 0.5-10 mg/kg in solid-form

tumors (Komatsu et al., 1969). In a different study, the same compound was found to significantly reduce tumor incidence (from 75% to 15%) in mice with mammary tumors (Mansour et al., 2012). Schizophyllan has been reported to have significant cytotoxic effects (30.8-55.3%) on glioma cells. This compound has been reported to induce apoptosis at dosages of 40 and 60 mg/L, block the cell cycle, significantly increase the proportion of cells in the G0/G1 phase, and significantly reduce the proportion of S phase cells (Zhou et al., 2015). Zhong et al. (2015) reported that ultrasonographically treated schizophilan showed a high (32%) cytotoxic effect against human breast carcinoma T-47D cells at a concentration of 400 μg/mL compared to the normal schizophyllan compound. It is known that schizophyllan suppresses the growth of tumor cells when combined with different anticancer drugs (Sasaki et al., 2020). It has been reported that schizophyllan at a concentration of 1500 μg/ml reduces cell viability by arresting the G2/M cell cycle of MCF-7 cells (Aleem et al., 2011).

3.1.3. Krestin

It is another β-glucan with a molecular weight of 100 kDa, containing 25-38% protein and used in cancer treatments. Krestin has been shown to be used in the treatment of digestive organs, lungs and breast cancers (Mizuno, 1999) and is also known to strengthen the immune system through the induction of IL-6 (interleukin6) and TNF (Tumor Necrosis Factor) in various animals (Price et al., 2010). Clinical use of Krestin's began in Japan in 1977. After re-evaluation in 1989, this compound was approved for use in combination with chemotherapy to prolong the survival of patients with gastric cancer or colorectal cancer (Maehara et al., 2012). In a study, it was reported that orally taken krestin prevented the apoptosis of T cells in cancer patients receiving chemotherapy after 5 weeks (Kono et al., 2008). It is known that krestin, especially isolated from *Coriolus versicolor*, has a preventive effect on the development of cancer (Khan et al., 2017). Kawaratake (*C. versicolor*) is the source for Polysaccharide-K (PSK, Krestin). The drug obtained from the PSK active ingredient of *C. versicolor*, which was developed in the late 1970s, is known as one of the most popular cancer drugs in Japan and is taken orally for the treatment of stomach and other types of cancer (Öztürk and Çopur, 2009).

3.1.4. Pleuran

It is 1,3/1,6-β-glucan, weighing 600-700 kDa and isolated from *Pleurotus ostreatus*. This compound has immunomodulatory (Liu et al., 2015), anti-tumor (Jose et al., 2002) and anti-diabetic effects (Chu et al., 2005). Pleuran was found

to significantly reduce (>50%) precancerous abnormal crypt-focus lesions in the colon of male Wistar rats (Bobek and Galbavy, 2001).

3.1.5. Grifolan

It is a β-glucan obtained from the fructification or mycelium of Grifola frondosa, known as the maitake mushroom. Its molecular weight is between approximately 450 and 500 kDa. It is a polymer of glucose molecules linked by (1 → 6) branched (1 → 3) β-glucan and its structure is similar to schizophyllan. Grifolan is used in the treatment of HIV, hyperlipidemia, hypertension and viral hepatitis (Khan et al., 2017). It was determined that it showed a strong antitumor activity of over 95% when given to mice bearing grifolan sarcoma 180 tumors, which are known to have significant effects in cancer treatments, at a dose of 25-200 μg/mouse for 10 days (Suzukı et al., 1987).

3.1.6. D-fraction

It is a β-glucan with a β-1,6 chain and a β-1,3 side chain, isolated from the fructification structures of the fruit bodies of *G. frondosa* (Kodoma et al., 2005). It has been reported that D-fraction substance prevents tumor development by injection or oral administration. It has been determined that the D-fraction substance found in Maitake mushroom strengthens the immune system and reduces cancer development, as well as preventing the recurrence and spread of cancer (Nanba et al., 1997). In a pilot study conducted on 63 cancer patients in China, it was reported that the total effectiveness rate of Maitake D-Fraction against solid tumors was over 95% and the effective rate against leukemia was higher than 90%, but unfortunately they did not disclose the dose amount used (Sheikh et al., 2017). It has been emphasized that combining D-Fraction with other types of chemotherapy will increase the outcomes and quality of life of cancer patients (Kodoma et al., 2005). Maitake D-Fraction has been reported to suppress cancer progression and exert its effect primarily through stimulation of NK activity (Kodoma et al., 2003). In recent studies, D-fraction has been reported to be effective on T24 (bladder cancer), PC3 (prostate cancer), MCF7, LM3 (breast cancer), U89 (brain cancer), HL60 (leukemia), HepG2 (liver cancer), three canine cancer cell lines, and ACHN cells (kidney cancer), but has not affect the viability of AGS cells (gastric cancer) cells and A549 (lung cancer) (Fullerton et al., 2000; Konno, 2009, Alonso et al., 2017; Alexander et al., 2013; Degen et al., 2013; Konno et al., 2004; Soares et al., 2011).

3.2. Proteins

Fungi produce many types of proteins that contain bioactive properties and are of potential importance for pharmaceutical applications. The anti-cancer effects of these components attract attention, especially considering the increasing number of cancer cases in recent years. These compounds show their anticancer activity by binding to cell membranes, leading cancerous cells to apoptosis (Rezvani et al., 2020). Fungi produce proteins and peptides with biological activities that include lectins, fungal immunomodulatory proteins (FIPs), ribosome-inactivating proteins (RIPs), ribonucleases, and laccases (Xu et al., 2011).

3.2.1. Lectins

They are proteins or glycoproteins that can bind with carbohydrate membranes. These protein groups support the cell adhesion process and also contribute to the activation of lymphocytes (Hassan et al., 2015). Lectins are the most researched fungal protein and have been isolated from some fungal species (*Pholiota adiposa*, *Hericium erinaceus* and *Russula lepida*) in the last few years. Lectins have been determined to have antiproliferative, antitumor and immunomodulatory activities (Li et al., 2010; Zhang et al., 2009). Cytotoxic studies have proved that lectins isolated from *Agaricus bisporus* have a strong inhibitory effect against tumor cells, and are especially effective against the proliferation of breast cancer cell lines (Singh et al., 2016). It has been determined that lectins isolated from *Tricholoma mongolicum* significantly inhibit the development of tumor cells and also show activating properties of mouse macrophages by affecting the production of the oncogene homologous transcription factor, tumor necrosis factor α (Wang et al., 1996). It was determined that lectin B16 isolated from *Phellodon melaleucus* showed a 47.8% inhibition effect in melanoma mice at a dosage of 1.0 mg/kg bw (Li et al., 2023). It was determined that lectins isolated from *Agrocybe aegerita* and *Russula rosea* showed 36.4% and 67.6% inhibition, respectively, in Sarcoma 180 tumor-bearing mice (Zhao et al., 2003; Zhang et al., 2010). It is known that lectin isolated from Pholiota adiposa, Lactarius flavidulus and *H. erinaceus* inhibits the growth and proliferation of MCF-7, HepG2, leukemic (L1210) cells (Zhang et al., 2009; Wu et al., 2011; Li et al., 2010). *Coprinopsis cinerea* galectins have been reported to exhibit cytotoxic effects (IC_{50}: 22.13 μg/mL-29.09 μg/mL) against HCT116 colorectal cancer cells (Yan et al., 2022).

3.2.2. Fungal Immunomodulatory Proteins (FIPs)

A new family of bioactive proteins isolated from some medicinal and edible fungi. Various fungal immunomodulatory proteins with molecular weights of 12.4–15 kDa have been reported in GenBank. They are known to be used in the treatment of asthma, allergies, autoimmune diseases and cancer (Chu et a., 2015; Wang et a., 2012). It has been reported that the immunomodulator protein isolated from *Ganoderma tsugae* shows antitumor activity against urothelial cancer by inhibiting telomerase activity and downregulating Beclin-1 in cancer cells (Li et al., 2014). It has been reported that the fungal protein isolated from *Flammulina velutipes* increases the migration of A-549 cells and inhibits the proliferation and development of cells through p53 gene activation (Chang et al., 2013). It was determined that the fungal protein isolated from *Volvariella volvacea* stimulated the maximum proliferation of human peripheral blood lymphocytes at a concentration of 5 µg / ml (Hsu et al., 1997). Similarly, the immunomodulatory protein isolated from Tratemes versicolor has been reported to increase the proliferation of human peripheral blood lymphocytes (Li et al., 2011). It has been suggested that fungal immunomodulatory protein isolated from *Ganoderma australe* may be associated with cancer and a weakened immune system (Muñoz et al., 2014).

3.2.3. Enzymes

It was reported in a study that a 66-kDa laccase isolated from the fruit bodies of Pleurotus cornucopiae inhibited the proliferation of the mouse leukemia cell line L1210 and the human hepatoma cell line HepG2 (Ho Wong et al., 2010). Laccase isolated from *Coprinus comatus* inhibited the growth of HepG2 and MCF7 cells, and its IC_{50} values were determined as 3.46 µM and 4.95 µM, respectively (Zhao et al., 2014). Laccase purified from *Clitocybe maxima* has been reported to inhibit the growth of HepG2 (IC_{50}:12.3 µM) and MCF7 (IC_{50}:3.0 µM) cells (Zhang et al., 2010). It has been reported that laccase isolated from the fruit bodies of *Agrocybe cylindrace* is effective against the same cell lines (Hu et al., 2011). It has been reported that ribonuclease, a protein isolated from *Ganoderma lucidum*, is effective against colorectal carcinoma HCT116 cells and colorectal adenocarcinoma HT29 cells and suppresses the development of the cells by 80% (Dan et al., 2016). It is known that marmorin isolated from the fruit bodies of *Hypsizigus marmoreus* inhibits the development of HepG2 (IC_{50}: 0.15 µM) and MCF-7 (IC_{50}: 5 µM) cells (Wong et al., 2008).

It is thought that marmorin may be an important chemotherapeutic agent in preventing breast cancer progression in both in vitro and in vivo studies (Pan et al., 2013; Ng, 2013).

3.2.3. Other Proteins

It has been reported that a protein isolated from the fruit bodies of the Cordyceps militaris mushroom has cytotoxicity against human breast and bladder cancer cells (Park et al., 2009). A protein derived from *Pleurotus eryngii* suppressed the growth of human and mouse colon cancer (HCT116 and MC38) cells in a dose- and time-dependent manner. It has been reported that this protein affects the cell cycles of cancer cells and thus leads to apoptosis (Yuan et al., 2017). It has been reported that the protein called LP1 isolated from *L. edodes* has significant effects on SGC-7901 and BGC-823 cells, and its IC_{50} values are 31.5 and 40.7 μg/mL, respectively (Batool et al., 2018). Ubiquitin-like peptide isolated from the fruit bodies of *Calvatia caelata* reduced the growth of breast cancer cells by half at a concentration of approximately 100 nM (Lam et al., 2001). A different ubiquitin-like peptide isolated from *Agrocybe cylindracea* has been reported to inhibit the development of leukemia cells (M1) and hepatoma cells (HepG2) (Ngai et al., 2003). In a cytotoxic study conducted with proteins obtained from Cordyceps militaris against breast cancer, bladder cancer and lung cancer cell lines, it was observed that the proteins obtained had a cytotoxic effect against breast cancer and bladder cancer cell lines (Kim et al., 2004). Protein extracts of *Pleurotus nebrodensis* were obtained using various solvents (NaCl, MeOH) and their anti-tumor activities were tested against mice carrying Sarcoma 180 type tumors. As a result, the survival rate was seen in NaCl with 27.06% (Cha et al., 2012).

4. Conclusion

The main recommendations recommended by patients who have received cancer treatment to minimize this situation and avoid cancer are healthy eating habits, weight management, and regular exercise. The importance of dietary nutrients for health is one of the issues that attracts attention day by day. For this reason, interest in the consumption and use of mushrooms for health is increasing, and many clinical studies have been carried out in this sense in recent years (Panda and Lutyen, 2022; Li et al., 2023). Especially, cancer mycotherapy is one of the most promising fields. This increases the focus on

mushroom components and extracts that show antitumor activity. As seen in the literature studies, these components in mushrooms show this effect indirectly by stimulating the immune systems rather than directly attacking cancer cells.

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CHAPTER VI

OCCUPATIONAL CANCERS AND THE HISTOLOGICAL EFFECTS OF CARCINOGENIC FACTORS

GÖKHAN NUR[1]* & ELVAN AK[2] & HACI AHMET DEVECİ[3]

*[1] (Assoc. Prof. Dr.), Iskenderun University, Faculty of Engineering and Natural Sciences, Department of Biomedical Engineering, Hatay, Turkey, *Corresponding author, E-mail: gokhan.nur@iste.edu.tr, ORCID: 0000-0002-5861-8538*

[2] (Nutritionist), Iskenderun State Hospital, Polyclinic of Nutrition and Diet, Hatay, Turkey, E-mail: elvdyt@gmail.com, ORCID: 0000-0003-0470-3427

[3] (Assoc. Prof. Dr.), Gaziantep University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Gaziantep, Turkey, E-mail: h.ahmet_deveci@hotmail.com, ORCID: 0000-0002-3862-1991

1. Introduction

Cancer is a general term for diseases characterized by unchecked cell proliferation and spread to neighbouring or distant organs for a variety of reasons, with different signs and symptoms, clinical course, treatment and approach (Sandal el al., 2017). Various factors, such as alcohol, the use of tobacco products, malnutrition, obesity, viruses, exposure to ionized radiation, occupational diseases, and environmental pollutants, can contribute to the emergence of cancer. Cancer cells survive with little oxygen, little food, resistance to harsh conditions, and turn these conditions into advantages over time. Cancer cells can also change shape over time. Normal cells can grow

and live in a certain place, while cancer cells are able to live and grow and reproduce without being held anywhere (De Martel et al., 2019). Only 5-10% of the cellular changes that cause cancer are explained by hereditary mechanisms, and most of the remaining are related to environmental factors. Occupational cancers are defined as cancers that develop depending on environmental factors and occupational risk factors (Sandal et al., 2017).

There are international criteria for the classification of a disease as an occupational disease: the relationship between exposure and incidence is strong and scientifically proven, the disease occurs in specific occupations or areas of work, the strong relationship between the number of workers exposed and the severity of the risk, and the disease is listed on the national list of occupational diseases in many countries. Occupational diseases have typical characteristics (Berk et al., 2011; Özlü et al., 2017). These are; a disease that can be completely prevented, is caused by one or more harmful factors (nutrition, individual sensitivity, smoking, alcohol, drug use, etc.), the cause of the disease is usually a factor in the workplace, although it is a well-defined disease factor, it is sometimes difficult to differentiate from situations that show similar non-professional characteristics, the legislation provides for periodic environmental measurements and medical examinations, allowing employees to detect and take the necessary precautions without any proximity to the employee and allowing them to identify occupational diseases, to diagnose occupational disease, to facilitate early diagnosis for the worker's close colleagues who has the diagnosis, to create a specific clinical picture, to ensure that the disease may appear between 1 and 30 years after the first contact with the employer and to prevent the progress of occupational illness if it is effectively interrupted.

2. Carcinogenesis

Although both genetic and environmental factors contribute to the development of cancer, environmental factors seem to be the primary risk factor for the majority of cancers (Kumar et al., 2015). Most cancers occur in the advanced stages of life (after age 55). When considering environmental risk factors for cancer, the most common ones include nutrition, infectious agents, alcohol consumption, smoking, obesity, reproductive history, and exposure to environmental carcinogens (such as environmental and occupational exposures) (Langevin and Kelsey, 2014). Small sections of non-coded nucleotide RNAs, or microRNAs, are essential to every biological pathway in multicellular organisms (Garzon et al., 2009). Deregulation of a single or small subset of

miRNA changes the expression of a large number of mRNAs (Jeansome and et al. 2015; Pinatel et al., 2014). In this case, the cells are transformed. For example, miRNAs are abnormally expressed in cancer tissues, and there is a close link between irregular miRNS and the inhibition of tumor-suppressing genes in the cancer (Ben-Hamo and Efroni, 2015; Soturopoulou et al., 2009). MiRNAs have provided a new perspective in the field of cancer diagnosis and treatment. In a compilation study by Dağlıoğlu and Öztürk in 2022, he noted that the distribution of tumor suppressant miRNAs through nanoparticles into the tumor region emerged as a relatively new and attractive option in cancer treatment (Dağlıoğlu and Özturk, 2022).

3. Classification of carcinogens

Essentially, there are two kinds of classification schemes. The primary distinction between the two systems is that while carcinogens in one system are categorized based on the strength of the evidence supporting their carcinogenic effects on humans, in the other system, they are grouped based on their efficacy. The substances categorized as carcinogens are essentially the same, despite differences in classification schemes (Sanner et al., 1996).

Carcinogens that cause the development of cancer in humans are classified by the International Agency for Research in Cancer (IARC, 2016), an independent scientific body within the World Health Organization (WHO). The IARC has grouped carcinogens by evaluating scientific data on carcinogenic substances in its series of 117 monographs, published from 1971 to 2016 (Table 1) (IARC). According to this classification, certain factors that cause cancer in humans are classified in Group 1. As of December 2016, 118 agents have been classified as group 1 carcinogens, according to IARC monographs.

The degree to which these agents display one or more essential traits of carcinogens is one of the critical evaluation criteria for categorizing chemicals as carcinogenic. These provide an organized and methodical framework for integrating, coordinating, and classifying the various molecular and cellular biological processes that underlie chemical carcinogenesis (Reisfeld et al., 2022). The typical characteristics of cancer are shown in Figure 1 (Hanahan and Weinberg, 2011).

Table 1. The grouping of carcinogenic agents according to the IARC.

Group	Explanation	Number of events
Grup 1	Human carcinogen	118
Grup 2A	Possible carcinogenic to humans	81
Grup 2B	It's very low in carcinogenicity to humans	299
Grup 3	Can not be classified as carcinogenic to humans	502
Grup 4	It's probably not carcinogenic to humans.	1

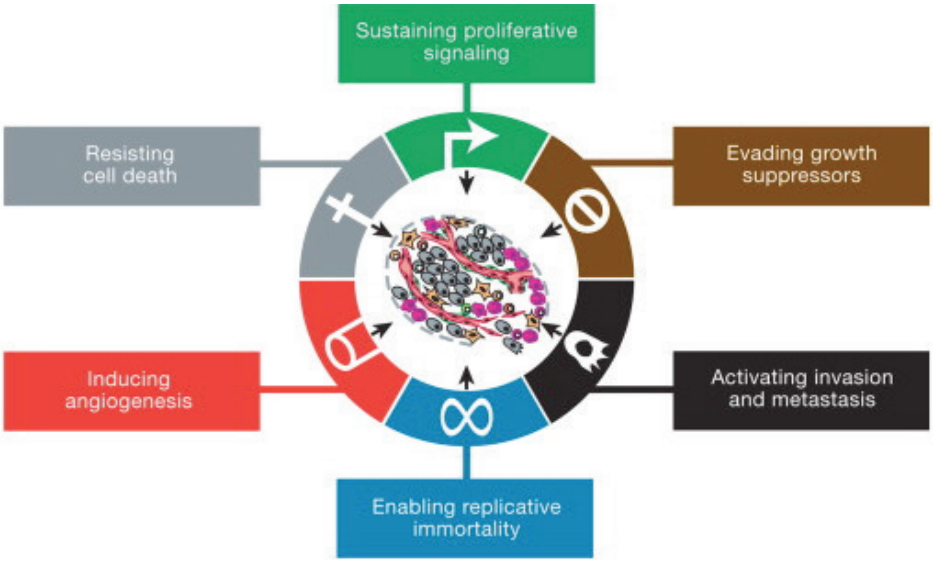

Figure 1. Typical characteristics of cancer (Hanahan and Weinberg, 2011).

4. Occupational Cancer Types

The most common cause of cancer-related mortality as well as the most hazardous type of exposure at work is lung cancer. If we sort it by frequency, it follows breast, colorectal, prostate, skin, liver, and pancreas cancers (Siegel et al., 2017).

Mezotelioma is a malignant tumor of serous membranes such as pleura, pericardium, and peritoneum. It is a rare but poorly seen cancer. Its etiology is largely influenced by occupational and environmental exposures (Boffetta, 2014; Roe and Stella, 2015). Asbestos is one of the leading causes of mesothelioma in occupational cancers. The term "asbestos" refers to a class of mineral fibers that

share qualities like flexibility, high voltage, and resistance to chemicals and heat (Attanoos, 2014). Although they have different types of asbestos fibers, there are no safe limit values for any type of mesothelioma. In our country, asbestos imports of the crocidolite group have been prohibited since 1996, the removal and use of all asphalt fibers of the amphibolite group in 2001 and the use and removal of chrysotyl asphalte in 2010 (Sandal et al., 2017).

Lung cancer, the most dangerous result of occupational exposures, is the most common cause of cancer-related deaths. Although the most frequent risk factor for lung cancer is smoking, lung cancer is the most dangerous occupational exposure (Pavlisko et al., 2014). This has to do with the way that many occupational risk factors are inhaled. In addition, smoking and occupational carcinogens have a synergistic effect that contributes to the development of cancer. Determining the correlation between professional factors and lung cancer, however, may prove challenging due to the potent carcinogenic effect of smoking. Table 2 lists the carcinogens that, based on IARC classifications, have been linked to lung cancer (IARC).

One of the most dominant causes of occupational lung cancer is asbestos. It has a synergistic effect when exposed together with smoking. Other leading occupational lung carcinogens include radon, a major carcinogen for workers in uranium mines and workers exposed to radiation products, chloromethyl ethers used in the production of solvents and pesticides, and polycyclic aromatic hydrocarbons (PAHs). It is a risk factor for those working in coal gasification, asphalt production, arsenic, cadmium, beryllium, chromium, nickel and silica (Fischman and Rugo, 2014).

Table 2. Carcinogens associated with lung cancer.

Agents carcinogenic to humans for which there is adequate evidence	**Factors with limited evidence in humans**
Acheson Process, Related Occupational Exposures	Acid Steams, Strong Inorganic
Arsenic and compounds	Vitray Glass, Glass Containers and Pressed Glass Items (Production)
Beryllium and Berylium Compounds	Emissions from solid fuels (especially wood), domestic fuels
	Roofing Against Asphalt, Oxidized Asphalt and Its Emissions
	Occupational Exposure to Asphalt, Hard Asphalt and Its Emissions
Methyl Ether (Technical Level)	Carbon Electrode Production
Cadmium and compounds	Toluenes
Chromium Compounds	Benzoyl Chloride
Emissions from coal, domestic fuels	Cobalt Metal, Tungsten Carbide
Coal Gasification	Creosote
Coal-Cathrane Zifti	Diazinon
Coal Production	Fibrous Silicon Carbide
Engine Exhaust, Diesel	Emissions from Frying Process, High Temperature
Hematite Mining (Underground)	hydrazine
Iron and Steel Production	Insecticides and their applications
Mopp (Vinkristine – Prednizon – Nitrogen Mushroom – Procarbazine Mixture)	Printing Processes
Nickel Compounds	2,3,7,8-Tetrachlorodibenzoparadioxin
External Environment Air Pollution	Welding Vapors
Coloring	
Particulates in External Environment Air Pollution	
Soot	
Tobacco Smoke, Passive Drinking	
Radon-222 and degradation products	
Silica Powder, Crystal	
Plutonium	
X and gamma rays	
Rubber Manufacturing Industry	
Sulphur Mushroom	

Various treatments for breast cancer have emerged in recent years. Genetic and environmental factors are involved in the heterogeneity of breast cancer. The most common cancer in women is breast cancer. It is estimated that one in eight women will experience breast cancer in their lifetime (Weiderpass and Labreche, 2014). The hypothesis suggested to prove the relationship between varied study and breast cancer is that the circadian rhythm disorder and the disordered circadian gene function of the suprachiasmatic nucleus in the hypothalamus, with the disruption of melatonin synthesis, eliminate the protective effects of Melatonin against cancer. (Hill et al., 2015). While smoking is the most important known risk factor for bladder cancer, occupational risk factors also play an important role in etiology. Aromatic amine exposure to bladder cancer is at risk for the paint and rubber industry (Kogevinas and Garcia-Closas, 2014).

Although historically important for occupational cancers, the most important risk factor for all types of skin cancers is ultraviolet (UV) light from the sun. Occupational risk factors that lead to skin cancer can be divided into two groups, physically and chemically. UV light is also a significant physical risk factor from a professional point of view. Chemical risk factors include arsenic, polycyclic aromatic hydrocarbons and metal washing fluids (Sim et al., 2014).

Lenfo-hemotopoietic cancers include a variety of malignancies, including Hodgkin's lymphoma, non-Hodgkin lymfoma, small lymphocytic lymphatic leukemia, multiple myeloma, acute myeloid leukaemia, and acute lymphacytic leukemia. In 1974, Prof. Dr. Muzaffer Aksoy and his colleagues published the development of leukemia in shoes exposed to benzene and evaluated the results of similar research in the literature in the same year, and the IARC classified benzene as a Group 1 carcinogen. In addition to benzene, studies in the ionizing radiation, 1,3-butadiene, ethylene oxide, formaldehyde, rubber industry have also been shown to be linked to the development of lymph-hemotopoietic cancer (De Roos and Bhatti, 2014).

The most common form of cancer in upper respiratory tract cancers is laryngeal cancer. Laryngeal cancer is more common in men. In developed countries, most larynx cancers are associated with alcohol consumption, smoking, or a combination of both. (Olsen, 2006). The occupational factors associated with larynx cancer are exposure to asbestos and inorganic acid vapour (such as sulfuric acid) (Boffetta, 2014).

While there is no professional risk factor associated with reproductive cancer in males, incubation has been found to be a group 2B carcinogen for prostate and testicular cancers (Richiardi and Zuccolo, 2014). In women,

asbestos exposure was linked to over cancer and tetracholoretylene exposure to cervical cancer (Weiderpass and Labreche, 2014).

Malignant tumors originating from renal parenchyma and kidney pelvis are the cause of kidney cancer in adults. Of the less than 10% of kidney carcinomas with microscopically confirmed cases, nearly all kidney pelvic cancers are of the transitional cell type. Adenocarcinomas occur primarily in the renal parenchym and account for more than 90% of renal carcinomas (Chow et al., 2010). Renal cell carcinoma is the type most frequently occurring in kidney cancer. The professional factor associated with kidney tumors is tricolorethylene. Such areas as dry cleaning with exposure to solvents, pesticides and metals, agriculture and the food industry, the paper/paper/printing industry, petroleum industry, iron/steel industry, and the automotive industry are considered risk areas (Moore et al., 2014).

Liver cancer ranks fifth in the United States and is the most common cause of cancer-related deaths globally, is the only cancer that increases annually with the incidence among the five most lethal cancers (Siegel et al., 2019). Liver disease is more prevalent in developing countries (Starley et al., 2010). According to Center and Jemal (2011), risk factors for alcohol-related cirrhosis include hepatitis B and hepatitis C viruses, fatty liver disease, smoking, diabetes, high iron loads, obesity, and a variety of dietary exposures (Center and Jemal 2011). Hepatocytes are the primary cause of hepatocellular carcinoma (HCC), a common form of cancer that can be influenced by a wide range of recognized risk factors, such as alcoholism, smoking, viruses, and different genetic disorders. The most significant known risk factor for liver angiosarcoma is occupational exposure to vinyl chloride (VC), which is a rare cancer that arises from endothelial cells (Dragani and Zocchetti, 2008).

4.1. New Occupational Factors and Cancer

Mineral wool such as man-made vitreous fibre, which is increasingly used with developing technology and is important in areas such as thermal insulation, sound insulation and philtration, and its high hardness and strength properties, and the increasing use of carbon nano tubes in fields such as nanotechnology, electronics, optics, despite the stated advantages, it also needs to be studied and monitored for the development of occupational diseases (Roe and Stella, 2015). Available literature findings on carbon nano tubes, carbon nano fibers and man-made mineral fibers in relation to the development of occupational cancer are presented in Table 3 (Kuempel et al., 2017; IARC, 2016). Research is still

under way for many of the new agents in these groups, and there is still no clear assessment of their carcinogenic propertie (Roe and Stella, 2015).

Table 3. Carcinogenicity grouping of new occupational factors.

Material Type	Carcinogenicity grouping
Carbon nano tubes	
Multi-walled carbon nanotube	Group 2B
Single-walled carbon nanotubes	Group 3
carbon nanofibers	No carcinogenicity studies
mineral wool Glass wool	Group 2B
glass wool	Group 3
specialized glass wool	Group 2B
Unending glass filament	Group 3
Stone wool	Group 3
particles wool	Group 3
Fibers made of refractory ceramic	Group 2B

5. Occupational Carcinogens

5.1. Arsenic

Arsenic is a silver-white, brittle, crystalline, semi-metallic solid chemical element. One of the most hazardous metals found in the natural world is arsenic (As). Rather than coming from mining or agricultural sources, the contamination of natural geological resources exposes people to arsenic toxicity to a greater extent (pesticides or fertilizers). In other words, the transport of arsenic from geological sediments to resources such as underground and drinking water has the potential to seriously harm the health of living things (Sikdar and Kundu, 2018). Even industrial or less industrialized countries' drinking water may be contaminated with arsenic. Furthermore, due to its extreme toxicity, arsenic is known as the "king of poisons." It is the element that ranks highest on the World Health Organization's 2001 priority list of hazardous substances and disease registries (Shaji et al., 2021). The amount of As can be found in soil, water and living organisms in concentrations ranging from ppm to ppb. While the As concentration in seawater is around 2 µg/L, in soil this concentration is generally between 1-40 µg/g. The chemical form and dosage of arsenic determine its toxicity in natural waters. Since 1993, there has been a decrease in the allowable

level of arsenic in drinking water from 50 µg/L to 10 µg/L (Shaji et al., 2021). With the decision taken by the Environmental Protection Agency in 2001, the level of arsenic allowed in drinking water in the USA was reduced from 50 ppb to 10 ppb (Ratnaike, 2003). In Turkey, the amount of arsenic in drinking water was determined as <10 µg/L with the standard numbered TSE 266 prepared by the Ministry of Health (Anonymous, 2005). The most dangerous and frightening feature of arsenic is that it has been shown by IARC to be carcinogenic (IARC, 2012), and it also has mutagenic and teratogenic properties. (Ratnaike, 2003). It is known that long-term exposure of living beings to these metals can cause damage such as DNA mutations, cell cycle disorders, neurological problems, liver and kidney damage, endocrine system disruption, cardiovascular dysfunction and even apoptosis (Genchi et al., 2020; Jadoon and Malik, 2017). Arsenic occurs naturally in trace amounts in almost all environmental conditions. Three categories-elemental, inorganic, and organic-are used to categorize arsenic and compounds containing arsenic in terms of public health. The types of chemicals, the dose, and the length of exposure all affect how toxic the effects are (Wu et al., 1989).

Chronic exposure affects hundreds of millions of people globally, mostly in Bangladesh, Chile, India, Mexico, Taiwan, the United States, and Uruguay. The World Health Organization's recommended limits of 10 µg/L for arsenic are exceeded in these groundwater sources (Wei et al., 2018; Ravenscroft, 2009). Numerous organ systems are affected by arsenic's toxicity, including the neurological, hepatic, respiratory, cardiovascular, hematological, and renal systems. Additionally, numerous cancers, including those of the skin, lungs, liver, and bladder, have been linked to it (Tchounwou et al., 2019).

Rana et al. observed that arsenic applied to rats at a dose of 20 ppm for 12 weeks caused an increase in erythrocyte nitric oxide and malondialdehyde levels and a decrease in superoxide dismutase and glutathione peroxidase activity. They revealed that ascorbic acid given as a preservative brought the analyzed parameters closer to control values (Rana et al., 2010). Liver damage is brought on by exposure to environmental toxins or by high blood sugar. The potential impact of their combined exposure on liver function is unclear, though. Because of this, in their 2018 study, Souza and colleagues assessed the morphological and functional hepatic parameters in diabetic mice exposed to arsenic. They found that arsenate exposure reduced antioxidant enzyme activities such as GST in healthy mice and also increased the animal hepatic inflammatory process characterized by a high mast cell count and TNF-α production exposed to arsenate (Souza et al., 2018). In a research where rats were given sodium

arsenite, hyperemia in brain parenchymal vessels, focal glial cell infiltration, and vacuolization in neurons were reported. Thickening of the interstitial areas, hyperemia in the vessels and edema in the alveoli were detected in the lung sections. Areas of necrosis and hyaline degeneration were observed in cardiac myocardial cells. Hyperemia, vacuolar degeneration and binucleated hepatocyte formations in the central vein were reported in liver sections. In the kidney tissue, widening of the Bowman space and vacuolization of the glomerular capillary ball were observed (Boduroglu, 2022).

5.2. Asbestos

Asbestos is a silicate mineral of high tension resistance, resistant to heat, friction and alkaline environments, fibrous, flexible (easy to bend) and of commercial importance. Humans use asbestos, a mineral consisting of long, thin, heat-resistant fibers, for a variety of commercial and industrial purposes, including fire protection, insulation, and cement composite products. An IARC group 1 contains naturally formed mineral silicate fibers, which are human carcinogens and are extensively utilized in commercial and industrial settings for things like insulation, roof coating, and asbestos, by means of mechanisms that induce genotoxicity, inflammation and oxidative stress. Although asbestos is prohibited in many nations, occupational exposure is still possible in situations like ship demolition (Wu et al., 2015; IARC, 2012). It is widely used in the building and construction industries. In addition to being practical and affordable, this material is extremely toxic to humans and may have good fire resistance and insulating qualities (Castleman, 2015).

5.3. Benzene

The smallest and most stable aromatic hydrocarbon is benzene. In 1987, the International Agency for Research on Cancer (IARC) came to the conclusion that there is enough evidence for benzene exposure to cause cancer in both humans and animals (McMichael, 1988). This study provides compelling evidence that exposure to benzene results in acute non-lymphocytic leukemia and acute myeloid leukemia. Additionally, there is a correlation between exposure to benzene and non-Hodgkin lymphoma, myeloma, and acute/chronic lymphocytic leukemia that is positive (IARC, 2012). Benzene needs to break down into reactive intermediates in order to become toxic. According to recent research, various metabolites that impact various cell targets mediate the toxicity of benzene (Bolcsak and Nerland, 1983; Goldstein, 1989). Commercial use of benzene, dating back to the late nineteenth century, is one of the oldest proven

industrial chemicals that affects the health of many workers. (Snyder, 2012). Analyses of benzene's health effects have shown that it causes anemia, respiratory allergies, skin rashes, kidney disease, diabetes, and urinary disorders (Gist and Burg 1997). Chronic exposure to benzene at high concentrations, especially through respiration, can affect the functioning of the human immune system.

Concern over the harmful health effects of benzene pollution in the workplace and environment is growing. It harms many essential physiological functions as well as a variety of human tissues, including the ovarian and bone marrow tissues. A study by Ben Dhia and his colleagues in 2021 to assess the potential effectiveness of kefir in improving benzen toxicity in rats has identified alterations in bone marrow cells and haematological. Kefir therapy has been found to alleviate benzene-related weight loss and increase the number of full blood cells in peripheral blood and nucleic cells on bone marrow. Animals exposed to benzene have shown a significant decrease in anemia and white blood cell levels (Ben Dhia et al., 2021). In their study, Yoon and Ark. that benzene is associated with all lymphoma carcinogenesis (Yoon et al., 2018). According to Sauer et al. (2018), a study conducted on gas station employees revealed that immunity avoidance promotion and decreased p53 gene expression may be important factors in the mechanisms underlying benzene toxicity and carcinogenicity (Sauer et al., 2018). Exposure to a mixture of benzen and chromium has been to decrease thyme mass and thymoid count. It has also been observed that exposure to both chemicals in the thyroid and lymphatic nodes causes lymphoretic hyperplasia and plasma cell-macrophage transformation, as well as apoptosis in thyrocytes and the lymphocytes in the T regions of the vein. These effects were similarly observed in groups where benzen and chromium were applied separately (Karaulov et al., 2022).

5.4. Cadmium

Due to its high rates of transition from soil to plant, cadmium is a pollutant that is present in the majority of human foods. For non-smoking and non-professional populations, this means that cadmium exposure is the main cause of exposure (Clemens, 2006; Franz et al., 2008; McLaughlin et al., 2006). Cadmium is an environmentally and professionally important heavy metal and is classified as carcinogenic to humans. Exposure to cadmium results primarily in prostate and kidney cancer, including lung cancer (Waalkes, 2003).

Liver damage can result from exposure to bullet (Pb) and cadmium (Cd). On the other hand, it is unclear how combined exposure to Pb and Cd affects liver function. In 2020, Zou and associates studied the effects of lead and

cadmium exposure on mice's liver function. The findings after the exposure revealed a significant build-up of lead and cadmium in the liver, a reduction in liver weight, and a loss of liver structure and function. It has been demonstrated that hepatocytes' levels of autophagy and oxidative stress are impacted by Cd and Pb (Zou et al., 2020).

In research done by Deveci and his colleagues, cadmium was experimentally applied to rats. In the histopathological examination of liver tissue in rats, degenerative areas with central and portal vein conjunction and focal necrosis were detected in group cuts with cadmium (Figure 2) (Deveci et al., 2023).

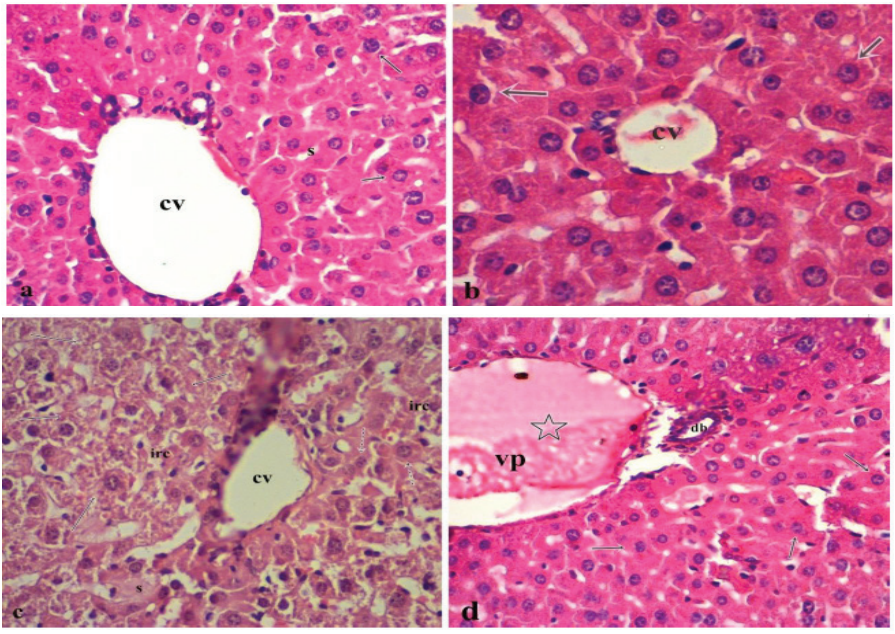

Figure 2. a. Liver tissue from animals in the control group. Hepatocytes and sinozoidal structure in typical appearance (cv: vena centralis, s: sinuzoid, arrows: hepatosit). b. 10 µM/kg of liver tissue obtained from animals in the CAPE group. Hepatocytes and sinozoidal structure in typical appearance (cv: vena centralis, s: sinuzoid, arrows: hepatosit). c. liver tissue obtained from animals in the group administered cadmium at a dose of 1 mg/kg (examples: focal apoptotic regions, dashed arrows: hepatocytes, cv: vena centralis, irc: irregular remark cordons, s: sinuzoidal conjunction). d. liver tissue obtained from animals in the group administered 1 mg/kg cadmium and 10 µM/kg CAPE (star: vascular conjunction, db: ductus biliferi, arrows: hepatocytes, vp: portal vein). H&E (Deveci et al., 2023).

5.5. Lead Acetate

A biotoxic industrial and environmental contaminant, lead acetate builds up in nearly every bodily tissue, including the immune system, liver, lungs, bones, kidneys, and reproductive organs. (Gagan et al., 2012). Oxidative stress is the mechanism underlying lead nephrotoxicity, and the generation of reactive oxygen species (ROS) in the kidneys is out of balance with antioxidants' ability to purify (Patrick, 2006; Hussein et al., 2014). In one study, severe necrotic changes in hepatocytes, mononuclear cell infiltrations, hydrophic degenerations, copper cell proliferation and severe hyperemia in the veins were in the livers of the group administered lead acetate (Coşkun, 2019).

In rat kidney tissue, Sudjarwo and his colleagues examined the protective role of piperine against renal damage brought on by lead acetate. They came to the conclusion that piperine might be a strong natural herbal remedy with nephroprotective properties that can shield rats from the harmful effects of lead acetate (Figure 3) (Sudjarwo et al., 2017).

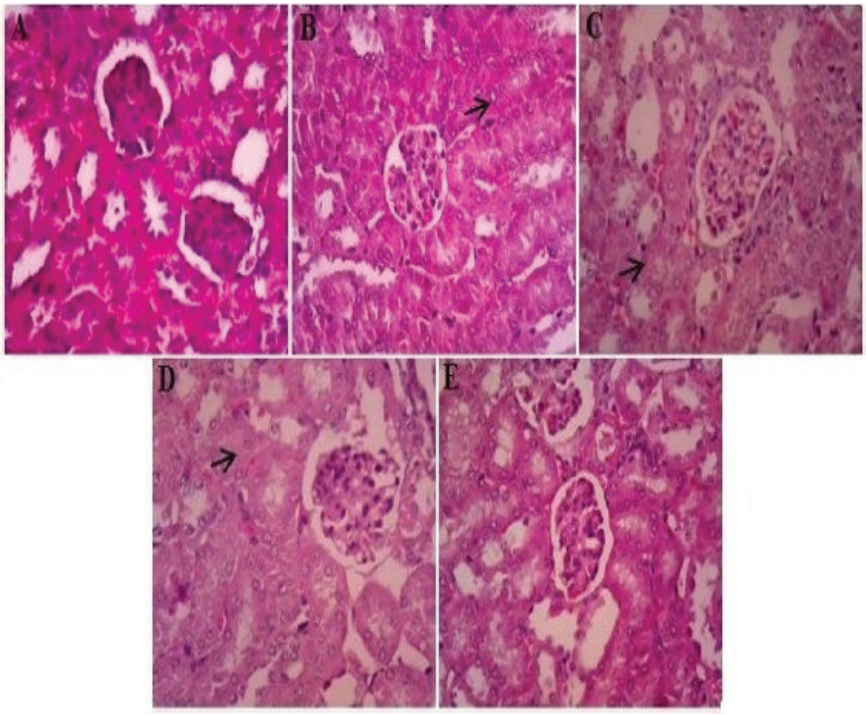

Figure 3. Histological study of the pre-treatment with piperine on the nephrotoxicity caused by lead acetate. Normal morphology of the (A) microscopic images of kidneys in the negative control group. Tubular necrosis

(arrows) was detected in the lead acetate applied group (B). In addition to lead acetate, necrotic changes were observed in rats given 50 mg kg^{-1} and 100 mg kg^{-1} BW of piperine (C and D). In rats administered 200 mg kg^{-1} of piperine in addition to lead acetate, regeneration (E) was observed in tubular epithelial cells. ×400 (Sudjarwo et al., 2017).

Reactive oxygen species (ROS) generation appears to be linked to the toxicity of lead in the liver. Propolis from honey bees contains a compound called caffeic acid phenethyl ester (CAPE), which is similar to a flavonoid and has been shown to be an antioxidant and free radical remover. Happy and associates observed degeneration and necrosis as a consequence of lead exposure when researching the protective effects of CAPE against hepatotoxicities brought on by lead acetate in rats (Figure 4) (Mutlu et al., 2011).

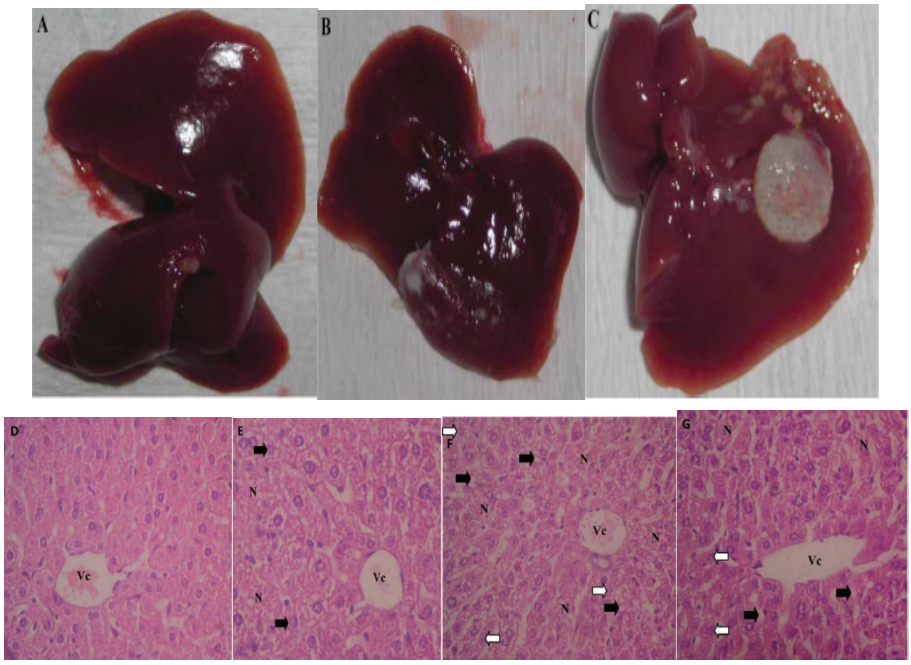

Figure 4. Macroscopic and microscopical imaging of liver tissue A. Control group, B. Lead+CAPE group, C. Lead group, D. Hepatocytes and sinusoidal structures under a microscope in the liver tissue of animals receiving CAPE, E. Hydrophic degeneration (arrows) and focal necrosis (N) areas in liver tissue of animals administered ethanol, F. microscopic image of liver tissue taken from animals given lead. Common areas of necrosis (N) vacuum degeneration (black arrows) and hydropic (white arrows),, G. The microscopic appearance

of the liver tissue of animals that were infused with CAPE. Focal necrosis (N) regions, hydropic (white arrows) and vacuole degeneration (black arrows), Vc: Vena sentralis, hematoxylin-eosin staining, x40.

Lead primarily affects hormonal imbalances and male reproductive functions through testicular tissue damage in terms of morphology, along in addition to notable modifications in the oxidative markers and sperm profile. Because of its polyphenol content and antioxidant qualities, Olaniyan and his colleagues' investigation into the impact of Cocos nucifera L. oil on the reproductive toxicity of lead acetate in male rats revealed that the oil reduces the negative effects of lead acetate (Olaniyan et al., 2021).

5.6. Pesticides

The term "pesticide" refers to a group of various chemicals used to control insects, bugs, and plants. Everyone is exposed to pesticides because they are widely used in residential, commercial, and agricultural settings. According to Barr et al. (2010), the National Health and Nutrition Survey, most Americans had detectable levels of different pesticide metabolites in their urine (Barr et al., 2010). To get rid of pests that endanger people, animals, and plants, pesticides are used. Their actions contaminate food and water systems, and they can harm people's health through ingestion of contaminated food or water or occupational exposure (El-nahhal and El-nahhal, 2021). Malathion is the best-selling wide-spectrum pesticide and is taken up by the body via the skin, respiratory and gastrointestinal system, accumulating at high density in the liver and kidneys. Toxic symptoms of malathion in humans include respiratory problems, nausea, headaches, and dizziness. In a study, Deveci and colleagues examined how malathion affected liver enzymes and bioenzymes under oxidative stress, as well as how caffeic acid phenethyl ester preserved against malathione. Malathione significantly altered the oxidative stress bio-boosters, as evidenced by a decrease in plasma PON activity and HDL levels and an increase in AST-ALT activity, MDA, and NO levels. However, CAPE was able to stop these changes and provided protection against malathion toxicity (Deveci et al., 2021).

The insecticide alpha-sipermethrin (α-CYP) belongs to the class of synthetic pyrethroid pesticides. In a study in 2023, Nur et al. evaluated the subacute histopathological and biochemical consequences of rats on kidney tissue. Increased frequency and severity of glomerulary lobulation and bleeding as a result of exposure, Bowman capsule enlargement, glomerular atrophy, and proximal and distal tubule degeneration were observed (Figure 5) (Nur et al., 2023).

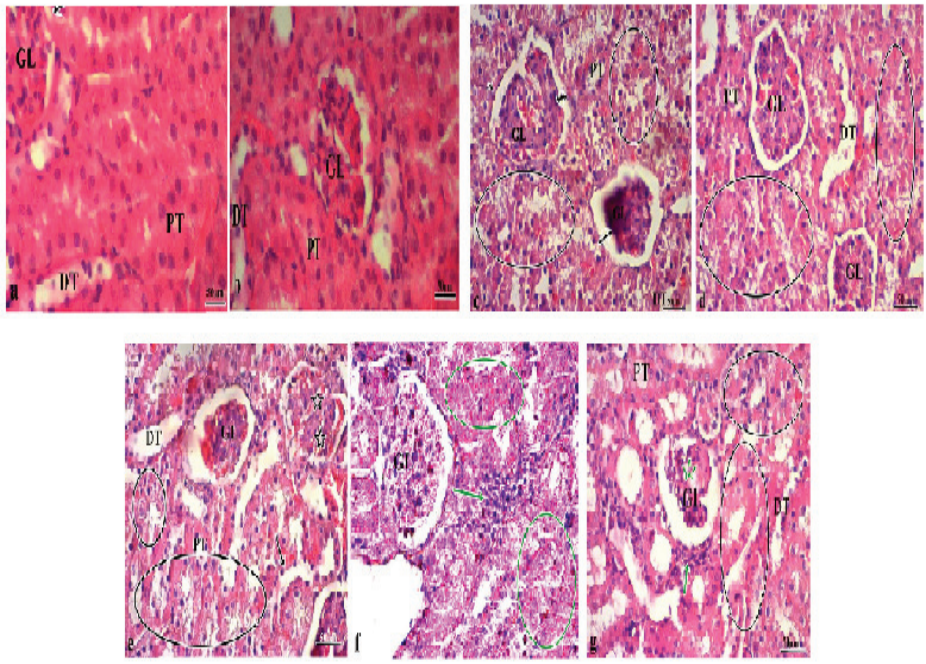

Figure 5. (a and b) kidney tissue taken from the CAPE and control groups of mice. Tubules and the glomerular structure looked normal. (c) Kidney tissue in the 10 mg kg^{-1} α-CYP group. (d) Kidney tissue in the 10 mg kg^{-1} α-CYP + 10 μmol kg^{-1} CAPE group. (e and f) Kidney tissue in the 20 mg kg^{-1} α-CYP group, (g) Kidney tissue in the 20 mg kg^{-1} α-CYP + 10 μmol kg^{-1} CAPE group (GL: glomeruli, PT: proximal tubule, DT: distal tubule, diffuse tubular degeneration [circle], glomerular atrophy [green star], Bowman capsule [arrowhead], enlargement of Bowman's capsule and glomerular atrophy [double-sided arrowhead], separation of the basal lamina [arrows], glomerular lobulation [asterisk], inflammatory cell infiltration [green arrow], extensive areas of necrosis [green circle]). Bar: 50 microns (Nur et al., 2023).

In research done by Nur et al., they investigated the possible toxic effects of dichlorvos applications, a class of organophosphates utilized in agricultural operations as a broad-spectrum insecticide, moving up the food chain. In addition, the goal was to ascertain whether vitamin E provided any defense against this toxicity. As a result of dichlorvos administration, histological examination of the kidney tissue revealed enlargement of the Bowman capsule, glomerular atrophy, inflammatory cell infiltration, vascular occlusion and areas of tubular degeneration. Inflammatory cell infiltration, glomerular lobulation, tubular

degeneration and separation of the basal lamina were noted in the sections of the kidney obtained from the group receiving vit-E together with dichlorvos. It has been stated that the combination of Vit-E with dichlorvos reduces the severity of lesions. Due to dichlorvos exposure, it was determined that vitamin E supplementation would be advantageous in addition to standard medical treatment (Figure 6) (Nur et al., 2021).

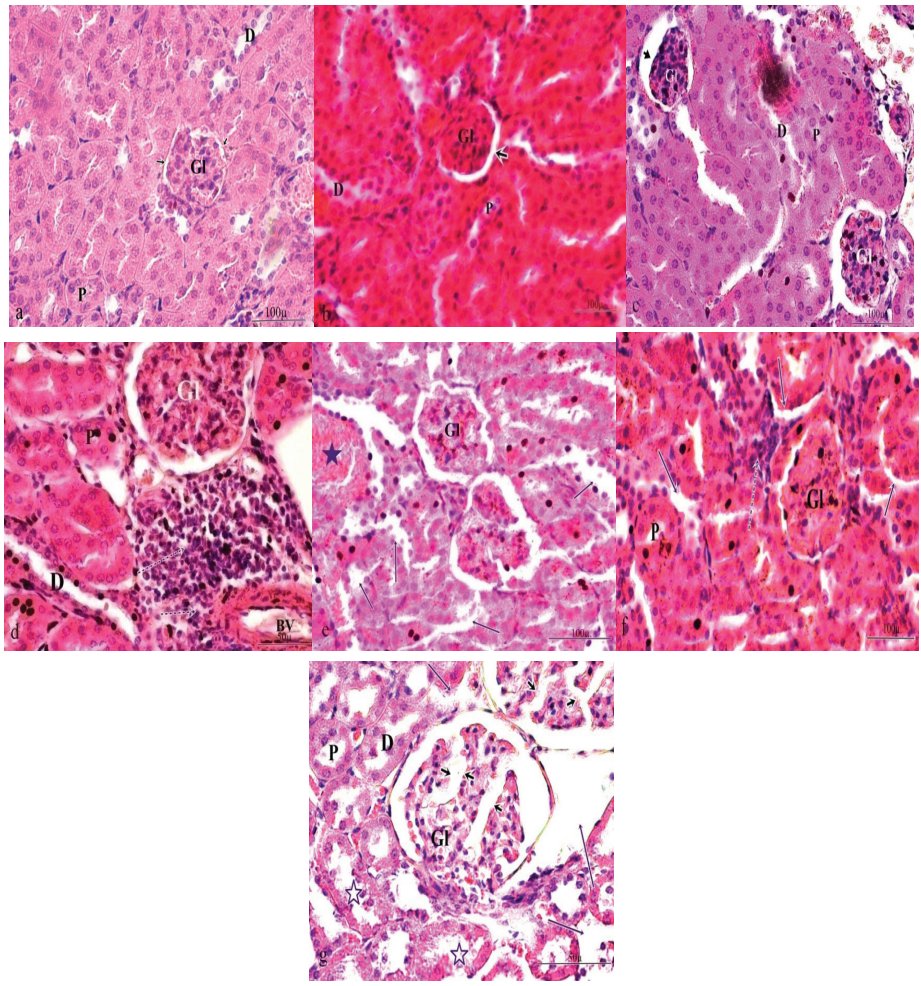

Figure 6. Microscopic images of kidney sections of the groups (hematoxylin-eosin staining) a. Control group, b. vit-E group, c, d, e. Dichlorvos group, f, g. Dichlorvos+vit-E group [GI: glomeruli, P: proximal tubule, D: distal tubule, bowman capsule (arrowhead), inflammatory cell infiltration (split arrows), enlargement of bowman's capsule and glomerular atrophy (arrowhead), separation of the basal lamina (arrows), inflammatory cell infiltration (split

arrows), BV: blood vessel, vascular occlusion (asterisks), tubular degeneration (arrows), tubular degeneration (asterisks), glomerular lobulation (arrowhead)], x400, x600, (bar: 50 μ, 100 μ) (Nur et al., 2021).

5.7. Polycyclic Aromatic Hydrocarbons (PAH)

The broad class of organic compounds known as polycyclic aromatic hydrocarbons (PAHs) is made up of two or more dense aromatic rings. Polycyclic aromatic hydrocarbons are organic pollutants and consist mainly of the aromatic circuit of two or more molten solid compounds containing carbon and hydrogen atoms are colorless, white, or shimmering yellow (Abdel-Shafy and Mansour, 2016). Some PAHs cause mutagenicity and cancer. Their concentrations in air, water and soil need to be constantly monitored. (Jakovljevic and Zuzul, 2011). It has been discovered that PAH pollutants are extremely toxic, immunotoxicogenic, teratogenic, mutagenic, and carcinogenic to a variety of life forms. Lung cancer is the most common cause of death for both men and women, with the second-highest incidence. Despite ongoing research, lung cancer still has the highest death rate of all cancers, in part due to the lack of accessible and reliable screening techniques (Wong et al., 2017).

Tobacco use is linked to lung cancer in about 90% of cases, but outdoor air pollution, passive exposure to cigarette smoke, inhalation of coal and insufficient burning of wood indoors/outdoors can also contribute to lung cancer (Reid et al., 2017; Moorthy et al., 2015). Certain occupations that expose workers to polycyclic aromatic hydrocarbons, which are present in different kinds of smoke, may be linked to certain diseases. Mixings comprising PAH can harm the human body in a variety of ways, such as mutagenicity and predisposition to developing cancer (Bernardo et al., 2016). They often biologically accumulate in the soft tissues of living things. It's interesting that many have synergistic effects rather than being directly carcinogenic. The capacity of PAH to bind to DNA and thus have a variety of harmful effects, including the potential for tumor initiation, is what makes it carcinogenic (Ifegwu and Anyakora, 2015). It has been determined that 3-Methylcholanthrene, which is also in the PAH group, increases the number of micronucleated polychromatic erythrocytes and also shows cytotoxic effects on bone marrow cells. In addition, it has been shown to reduce mitotic activity (Aksu et al., 2013).

Little research has been done on whether exposure to muscle strength and mass are correlated with polycyclic aromatic hydrocarbons. In a 2022 study, Sun and colleagues investigated the gender-specific negative relationship of PAHs to rat skeletal muscle mass and strength, as well as potential explanations. In male

subjects, the metabolites of PAH in urine showed a negative correlation between muscle mass and perception strength, while in females, there was no meaningful connection between the metabolites of PAHs in the urine and muscle massa or perception force. This study shows that the link between PAH and muscle atrophy may be gender specific (Sun et al., 2022).

5.8. Silica

Inhaled crystalline silicon dioxide, or silica, can cause silicosis, a potentially fatal worldwide occupational disease characterized by a progressive impairment of lung function and non-reversible fibrosis (Zhu et al., 2013). In a 2022 study by Gaun and colleagues, it was that pulmonary tissue edema was caused through the development of silicotic nodules and widespread lung fibrosis and collagen fibers after exposure to silica, resulting in an increase in the weight of the lung parenchyma. The lungs of mice exposed to silica showed interstitial fibrosis along with a significant accumulation of collagen, and the pathological alterations were more noticeable after extended exposure to the silica (Gaun et al., 2022).

In addition to being used extensively in paints, food, cosmetics, and automobiles, silica nanoparticles are also used in biomedical purposes (Mebert et al., 2017). During the cutting of artificial or processed stones, inhalable crystalline silica (RCS) is formed, and silicosis outbreaks can be observed when they are inhaled for long periods of time due to occupational exposure. As a case report in 2020, Turner and his colleagues studied the relationship between silica exposure and connective tissue degradation in three workers working in the artificial stone processing industry: the worker in the first case is 45 years old and has been working in artificial stones for 15 years. He said he did not use the necessary protective equipment when working on the stone. He was hospitalized for stress dyspnea, arthropathy, sclerodactylia, and Raynaud's phenomenon, and was diagnosed with progressive massive fibrous silicosis. The patient died shortly after the lung transplant was considered inappropriate. The worker in the second case is 47 years old and has been working artificial stone for 23 years. In addition to being diagnosed with silicosis and pulmonary fibrosis, he was admitted to the hospital with progressive stress dyspnea and arthropathy and is currently waiting for a lung transplant. The third worker is 53 years old and has been working dry cutting and artificial stone for 23 years. The mask was working, but the stone cutting was carried out without using exhaust ventilation or water suppression. The artificial stone worker was admitted to hospital with symptoms of shortness of breath and sickle, was diagnosed with

silicosis along with pulmonary hypertension and expanding massive fibrosis, and had a successful double-sided single-sequence lung transplant (Figure 7) (Turner et al., 2020).

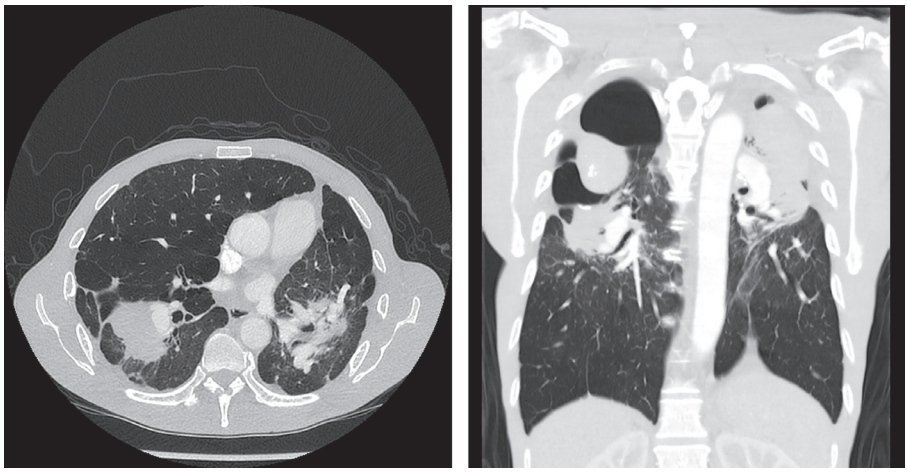

Figure 7. High-resolution chest computed tomography showing progressive massive fibrotic silicosis complicated by pneumothorax.

5.9. Vinyl Chloride

Vinyl chloride (VC) monomer is a colourless and volatile organochlorine primarily used in the manufacture of pipe, wire coating, construction materials and other consumer products (Zelko et al., 2021). At room temperature, vinyl chloride monomer (VCM) is a colorless gas. Vinyl chloride polymerized into polyvinyl chloride (PVC) is a common plastic industry material. Since VCM does not exist naturally, PVC manufacturing facilities are the main locations where it is found. Cigarette smoke also contains VCM, the amount of which varies according to the tobacco's chloride content. Vinyl chloride is known to cause cancer in both humans and animals (Vainio, 1978). Vinyl chloride has been associated with tumors in the liver, brain, lungs, and the hematolenophopoietic system (IARC, 1979). In 1975, exposure to vinyl chloride used in the manufacture of polyvinylchloride (PVC) the development of liver angiosarcoma (Creech and Johnson, 1974). When mice, rats, and hamsters were given vinyl chloride orally or through inhalation, it resulted in tumors in the skin, lungs, mammary glands, and glands, as well as angiosarcoma in the liver (Drew et al., 1983; Suzuki, 1983). Vinyl chloride forms covalent bonds with isolated DNA when a metabolic system is present. In rats exposed to vinyl chloride in vivo, chromosomal abnormalities, sister chromatid changes, and micronucleus formation have been

observed; however, lethal mutations are not caused by vinyl chloride. It caused DNA alkylation in a variety of tissues from rats and mice exposed in vivo. In vitro, vinyl chloride altered the brother chromatid in human lymphocytes. It transformed BALB/c 3T3 cells, the virus-infected Syrian hamster cell, and resulted in mutations in Chinese hamster cells and unforeseen DNA synthesis in in vitro rat hepatocytes. In a different study, workers exposed to vinyl chloride at concentrations of 5-500 ppm had chromosomal abnormalities in their peripheral blood lymphocytes (IARC, 1987).

5.10. Other Factors

Its toxicity in cancer treatment is low, but high efficacy in drug development studies attach great importance to specific and successful pharmacotherapy. Aflatoxins, nitrozo compounds, aromatic amins, and unsaturated polycyclic aromatic hydrocarbons are among the chemicals that cause tumors. It is crucial to comprehend the biology of tumors and create preventative and therapeutic strategies. The native scientists and archaeologists examined the preventive effects of systemine on mice's fibrosarcoma caused by 3-methylcolantrene (3-MC). Research has shown that systamine has a protective effect against fibrosarcoma induced by 3-methylcolantrene in mice (Born et al., 2013). In a different study, they studied the effects of the combination of cystamine, putresin, and cysteamine-putresin on fibrosarcoma induced by 3-methylcolanthrene in mice. As a result of the research, it has been indicated that the fibrosarcoma induced by 3-MC already has a protective effect of purrhine, cysteamine+purezine and systamine (Nasrin et al., 2016). Teflon and other consumer and industrial goods are used to make perflorooctanoic acid. Studies on animals have demonstrated that PFOA-induced toxicity can target the liver. Receptor-α activation and peroxysome proliferator-triggered cytotoxicity are two possible mechanisms for carcinogenesis (Steenland et al., 2010; IARC, 2017).

A study that resulted in liver damage as a result of subchronic formaldehyde poisoning in rats found apoptotic cells in the liver tissue sections of the toxic group, altered hepatocytes, increased number of kupfer cells, and histopathological damage occurred (Çay, 2012).

6. Conclusion

Cancer, called the disease group of similarly structured, uncontrolled and rapidly growing cells with abnormal structure, is now the second disorder that causes death after deaths from the circulatory system. Cancer can develop as

a result of exposure to personal carasterial characteristics, dietary patterns, certain drug groups, environmental agents, and some agents in the workplace professionally. When we look at the mortality rates from both good and malignant tumors, 30 per cent occurred from throat, trachea, lung cancers, 10 per cent from colon cancer, and about 10 percent from stomach cancers (Blackadar, 2016). Only 5-10% of the cellular changes that cause cancer are explained by hereditary mechanisms, and most of the remaining are related to environmental factors. Occupational cancers are defined as cancers that develop depending on environmental factors and occupational risk factors. It is well known that occupational exposure to pollutants and carcinogens leads to the development of cancer in exposed workers. When considering environmental risk factors in cancer, the most common ones are diet, infectious agents, alcohol consumption, smoking, obesity, reproductive history, and exposure to environmental carcinogens (such as environmental and occupational exposures). Merely a minor portion of the chemicals are considered to be occupational carcinogens, with each factor associated with a specific occupational activity increasing the risk of developing cancer. International Cancer Research (IARC) is developing specific criteria to provide a competent study on this and to identify the causality of exposure-sickness relationships. Occupational carcinogens rose from 28 in 2004 to 47 in 2017. The most frequent cause of cancer-related deaths from exposure at work is lung cancer. The most common occupational cancers today are lung, skin, bladder cancer and leukemia. In addition, breast, colorectal, prostate, liver, and pancreatic cancers are commonly found, depending on the nature of the agent exposed in the professional arm. While an average of 5% of cancer cases are occupational exposure, this rate can reach up to 15% in lung and bladder cancers. When the incidence of occupational cancers is studied, it is most commonly found to be lung cancer. The most prevalent cancer-causing agent at occupational for lung cancer is asbestos, followed by arsenic, cadmium, silica and many more. Many experiments have been conducted on animals to investigate how carcinogens work on tissue. Looking at the results of these experimental studies, it is suggested that occupational cancers resulting from exposure can be prevented. Occupational tumor tissue histology in developing cancer phenomena is the same as in normal cancer cases. The rate of death due to developing diagnostic methods and person-specific treatments, cancer cases, has declined in recent years, but unfortunately in occupational cancer cases the situation is the opposite. Improving working conditions, providing employees with the necessary training, taking measures to minimize interaction with

carcinogens and using the necessary protective equipment are essential to reduce mortality in this preventable cancer group. Nowadays, among the preventable occupational cancers, lung and skin cancers are the primary pathways of exposure when we think of respiratory and skin contact. These include breast, colorectal, prostate, bladder, liver, and pancreatic cancers, according to the characteristics of the exposed factors, depending on the professional workforce. While significant progress has been made in the identification of occupational carcinogens, new research is needed due to insufficient epidemiological evidence and lack of quantitative exposure data.

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CHAPTER VII

SOME STUDIES IN THE FIELD OF STOMACH CANCER AND MACHINE LEARNING

NISA KAYA[1] & SEVCAN AYTAÇ[2]

[1]Fırat University, Deparment of Biotechnology, Elazığ
E-mail: nisakaya2823@gmail.com
ORCID: 0009-0004-1967-9903

[2]Fırat University, Deparment of Electronics and Automation, Elazığ
E-mail: saytac@firat.edu.tr
ORCID: 0000-0001-6689-2337

1. Introduction

The stomach is one of the important organs of digestion. It is recorded that the organ belongs to the stomach. The main cause of this disease is a type of cancer called stomach cancer. Stomach cancer is one of the most dangerous types of cancer that causes cancer deaths after lung cancer. Although stomach cancer rates are decreasing, it is still a common type of cancer (Yaşar, 2018; Esmeray, 2020). When ranked according to cancer types, it ranks 4th with 7.8% of stomach cancers and 2nd with 9.7% of deaths caused by cancer (Parkin et al., 2001; Esmeray, 2020). The average age at which stomach cancer is diagnosed after a treatment is performed is 57 years old and the male-to-female ratio is 1/2 (Alacalı, 2012; Esmeray, 2020).

With the introduction of video endoscopes in the 1990s, a completely new era began in endoscopic examinations. Endoscopy devices currently in use provide the opportunity to take direct images and biopsies by reaching almost every point of the digestive system, especially thanks to their bending and forward movement features. This situation can also be tolerated more easily by patients (Ferlengez et al., 2012; Esmeray, 2020).

Vinod et al. (1992) determined the components that can create noise by providing grayscale pictorial information to the neural network. In this study about hand radiograms, histograms of gray-scale results were obtained. Jianzhong et al. (2002) conducted a study that increased the accuracy of radiologists' decisions in lung nodule detection. As a result of the study, they argued that more nodules could be detected than the number of nodules that radiologists could detect only with their own interpretation. Edis et al. (2007) started from a study calculating the cost of lung cancer in our country. They argued that in addition to early and accurate diagnosis of nodules that cause lung cancer, the cost of treatment of lung cancer would also decrease with the use of computer-aided diagnosis systems. Sasaki et al. (2010) studied a computer-aided system that predicts risk factors for stomach cancer. The system they worked on was studied on endoscopy images of patients with Helicobacterium Pylori (HP) bacteria, and among the 15 parameters used, 3 parameters were used to classify the gastric mucosa in the background. The data found as a result of this classification were processed with Bayes theory and the results were obtained. This study helps identify patients who are at high risk of cancer and need to undergo endoscopy.

In their study, Ural et al. (2016) studied a system that identifies cancerous areas for stomach images taken with computerized tomography (CT). In the study, the coordinates of the cancerous areas in the stomach region are determined on the color plane, and these areas containing the mass are detected with high accuracy and the system provides feedback to the user. Thanks to this process, a system emerged as a result of multiple image processing methods. In their study, Aubry et al. (2006) examined the status of the computer-aided diagnosis system on the nodule detection performance of radiologists. It has been determined that the sensitivity of nodule detection increases in the system used by radiologists. In their study, Hirose et al. (2008) found that the sensitivity of nodule detection increased by using a computer-aided diagnosis system to detect nodules from lung images taken with a multiple detector.

In their study, Lee et al. (2009) used the bayesian classifier, naive bayesian classifier and support vector machine classifier to make a comparison between each of them. They found that the support vector machines classifier gave the best results. Ahmadzadeh et al. (2013) developed a gastric cancer diagnosis system using the local pattern algorithm and vector-assisted machine method. The system has created a system that can accurately diagnose cancer at an early stage by performing noise reduction, feature extraction, feature identification

and classification processes on the image. From 55 randomly selected patients, 91.8% correct diagnostic results were obtained. Penedo et al. (1998) developed a computer-aided diagnosis system using local image curvatures provided by a two-level artificial neural network (ANN) method. 288 simulated nodules, 90 in real number, were classified by ANN, and the sensitivity was 89%-96% and the number of false positives per image was 5-7.

Retico et al. (2008) developed a computer-aided detection (CAD) system for the identification of small pulmonary nodules in low-dose and thin-slice CT scans. The CAD system was trained to be sensitive to small internal and subpleural pulmonary nodules collected in the database of CT scans. System performance was evaluated on 39 CT datasets containing 75 internal and 27 subpleural nodules. In this data set, high sensitivity values of 80-85% for lung nodules and an acceptable number of false positive findings of 10-13 per patient were detected. Magesh et al. (2011) proposed the CAD system for the detection of lung cancer in CT images. Therefore, it is necessary to overcome many problems in order to produce a successful CAD system. In order to identify cancer nodules in the lung, the segmentation process that will contribute to the formation of the sample region is an element that should be taken into consideration. They applied segmentation algorithms to detect cancer nodules from the extracted lung image. In their study, Kakar et al. (2009) presented a fully automatic tissue-based segmentation and recognition system for the lungs with CT. They extracted the texture features related to the segmentation part by filtering the images of Gabor and did this using Fuzzy C Means (FCM) clustering and Genetic Algorithm. They extracted statistical features as well as shape-based features using a cortex-like structure for the recognition phase. As a result, they confirmed that the lesion detection rate was 89.04%. Additionally, the average sensitivity rate of the classifier was found to be 89.48%.

Farag et al. (2004) advocated an algorithm for nodule identification using 2- and 3-dimensional templates that can vary between similar nodules. The proposed algorithm is the identification algorithm, which uses a genetic algorithm, which is a type of algorithm, and mixes cross-correlation pattern matching and Bayesian post-classification processes. Thus, 107 of 130 nodules were correctly classified with the detection algorithm.

Bae et al. (2005) developed an automatic pulmonary nodule detection program using three-dimensional volumetric data. They detected nodules using computed tomography images of 20 patients with pulmonary disease. They identified 164 nodules and used the detected nodules to evaluate a computer-

aided detection (CAD) program. As a result of their studies, the general sensitivity for nodule detection is 95.1%. This ratio shows that 156 of 164 nodules were calculated correctly.

In parallel with the rapid development of medical imaging devices and information technologies, many cancers such as brain, lung and breast cancer have recently begun to be diagnosed. In addition, computer-aided diagnosis systems regarding the stage of cancer have begun to increase (Dandıl et al., 2015; Esmeray, 2020). Thanks to advances in screening technologies, the diagnosis rate of stomach cancer is increasing. After the detection of stomach cancer, Computer Aided Diagnosis (CBT) Systems were used to help users who use this system. There are two important points in computer-aided diagnosis systems. These are the identification and classification of nodules in the lung. Thus, it is aimed to make both faster and more accurate diagnoses (Kaya et al., 2018; Esmeray, 2020).

2. Conclusion

Many studies have been conducted on stomach cancer to date (Korkmaz and Binol, 2018; Korkmaz et al., 2017; Korkmaz et al., 2017; Korkmaz, 2021; Korkmaz and Esmeray, 2018). Early detection and diagnosis of cancer conditions is a very important step. At this stage, the death rate due to cancer will decrease. When diagnosed and detected early, cancer treatment options have also been positive. In this case, although the organ is preserved, bile cancer treatment gives positive results and regeneration of the cancer occurs. In addition to the development of imaging technologies, sequential computer-aided diagnosis (CBT) systems also play an effective role and are protected by early recognition. Processing of image symbols is very important in CIS systems. Image processing, in general terms, is a process that allows data consisting of images to be brought together and, after evaluation, converted into a form that can be read on a different device or transferred from one digital environment to a different digital area (Gonzalez and Woods, 2008; Esmeray, 2020). Image processing is mostly used to process, modify, differentiate or improve images on existing recorded images. Additionally, classifying the processed image for cancer diagnosis is important for CBT systems. The most commonly used method in the diagnosis of stomach cancer worldwide is endoscopy. This method is a very specific and sensitive method. High-resolution endoscopy provides the opportunity to detect slight color changes, swelling and structural changes of the mucosa surface.

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CHAPTER VIII

BREAST CANCER AND TREATMENT APPROACHES

HACI AHMET DEVECİ[1] & GIZEM AŞASIN[2] & AYLA DEVECİ[3]

[1](Assoc. Prof. Dr) Department of Nutrition and Dietetic,
Faculty of Health Science, Gaziantep University, Gaziantep,
E-mail: h.ahmet_deveci@hotmail.com,
ORCID: 0000-0002-3862-1991

[2](Master Student) Department of Biology,
Institute of Science, Kafkas University, Kars,
E-mail: gizem.asasin@hotmail.com,
ORCID: 0000-0002-1879-8597

[3](Lecturer) Technical Sciences Vocational School,
Kilis 7 Aralik University, Kilis,
E-mail: ayladeveci@kilis.edu.tr,
ORCID: 0000-0003-2574-0251

1. Introduction

Cancer is a disease that is characterized by uncontrolled cell proliferation, tumor formation and spread to surrounding tissues, and also develops depending on genetic and environmental conditions. Metastasis, known as spread to surrounding tissues, is one of the important causes of death from cancer (Hanahan and Weinberg, 2011). Cancer ranks second among all causes of death in the world, and approximately one in every 6 deaths in the world occurs from cancer (Deniz, 2022). The most common types of cancer in the world are lung, breast, colon, prostate and stomach cancer. Cancer incidence, mortality and survival rates vary between populations, and these differences may depend on a variety of factors such as environment, genetic factors, lifestyle and population (Sung et al., 2021; Kaya et al., 2022). Table 1 and Table 2 list the types of cancer frequently seen in men and women.

Table 1: The most common types of cancer in men, according to the International Agency for Research on Cancer (IARC) (2020) (Sung et al., 2021).

Turkey	World	West Asia	Central-Eastern Europe	USA
Lung	Lung	Lung	Lung	Prostate
Prostate	Prostate	Prostate	Prostate	Lung
Colon	Colon	Colon	Colon	Colon
Bladder	Stomach	Bladder	Stomach	Bladder
Stomach	Liver	Stomach	Bladder	Skin Melanoma

Table 2: The most common types of cancer in men, according to the International Agency for Research on Cancer (IARC) (2020) (IARC) (2020) (Sung et al., 2021).

Turkey	World	West Asia	Central- Eastern Europe	USA
Breast	Breast	Breast	Breast	Breast
Thyroid	Colon	Thyroid	Colon	Lung
Colon	Lung	Colon	Uterine Corpus	Colon
Lung	Uterine Cervix	Lung	Lung	Uterine Corpus
Uterine Corpus	Thyroid	Uterine Corpus	Uterine Cervix	Skin Melanoma

2. Breast Cancer

Breast cancer ranks first among the cancer types seen among women. Breast cancer constitutes approximately 25% of cancers in women and 15% of cancer deaths, and it is known that mortality is mostly due to metastases and it is stated that breast cancer metastasizes to distant organs such as bone, liver, lung and brain (Sung et al., 2021; Momenimovahed and Salehiniya, 2019). At the same time, thanks to advances in early diagnosis and treatment of the disease, a decrease in mortality rates has been observed over the years (DeSantis et al., 2016).

2.1. Breast Cancer Etiology and Risk Factors

It is stated that the etiology of breast cancer is not known exactly, but many factors such as gender, age, genetic, environmental, psychological or

biochemical play a role in the development of breast cancer (Howell et al., 2014; Sun et al., 2017). Gender, which ranks first among the risk factors, represents a 100-fold increased risk, and the incidence of breast cancer also increases rapidly in older women (Çakır et al., 2016). Factors such as radiation, viral infections, hormone replacement therapy, nutrition, alcohol use, smoking, and dense breast tissue constitute environmental factors (Koçak et al., 2011). Environmental factors have an important place in the development of breast cancer. Chemicals help tumor development by causing damage to DNA, or they can increase sensitivity by changing the pattern of development in the mammary glands, thus contributing to the development of breast cancer. For example, what attracts attention in breast cancer are chemicals that mimic estrogen. Cadmium, one of these chemicals, is an important environmental factor that mimics the effects of estradiol in estrogen-sensitive breast cancer cells (Brody and Rudel, 2003; Coyle, 2004).

Viral factors such as Human Papilloma Virus (HPV) and Epstein-Barr Virus (EBV) are thought to play an important role in the development of breast cancer. In a study, it was reported that various subtypes of HPV were detected in the tumor tissues of cervical and breast cancer patients, and in another similar study, EBV was detected in the tumor tissues of breast cancer patients. It has also been stated that the risk of breast cancer increases in women with infectious mononucleosis disease caused by EBV (Hennig et al., 1999; Bonnet et al., 1999; Yasui et al., 2001). One of the most prominent environmental factors in breast cancer is ionizing radiation. Studies have reported that breast cancer rates increase in experimental animals and humans exposed to high ionizing radiation (Ronckers et al., 2005).

Nicotine found in cigarettes has been associated with many different types of cancer because it increases cell division and angiogenesis and also suppresses apoptosis (Bıyık, 2009). There are more than 60 substances in cigarette smoke that are carcinogenic to humans and experimental animals. These include N-Nitrosamines, polyaromatic hydrocarbons (PAH), heterocyclic aromatic amines (HAA), various organic and inorganic compounds, and some of them have been observed to trigger mammary tumor formation in experimental animals. There are also studies showing that P53 gene mutations are higher in the breast tissues of women who actively smoke compared to non-smokers (Hoffmann et al., 2001; Li et al., 2002; Rundle et al., 2002). It has been shown that high alcohol consumption causes an increase in estradiol levels before and after menopause, and the amount and duration of consumption are positively

associated with breast cancer (Liu et al., 2015; Shield et al., 2016). One of the important risk factors in the development of breast cancer is obesity. The effects of obesity on breast cancer are greatly affected by the menopause process. While high body mass index (BMI) has a protective effect against the risk of breast cancer in the premenopausal period, it increases the risk of breast cancer in the postmenopausal period (Schoemaker et al., 2020). In some studies, it has been observed that foods with high fat and sugar content increase the risk of breast cancer, and at the same time, considering the phytochemical content of diets such as fruits and vegetables, they reduce the risk of breast cancer with anticarcinogenic effects (Edefonti et al., 2009; Heath et al., 2020). As a result of the studies, it is emphasized that hormone replacement therapy, which is used to alleviate the effects of menopause, increases the risk of breast cancer by 34% when used for a long time. At the same time, it has been reported in some studies that its use has not been found to be associated with cancer, and it is stated that this difference occurs due to the change in the oral contraceptive content over time (Casey et al., 2008; Kaminska et al., 2015). Another of the most important risk factors in breast cancer is genetic structure and family history, and these factors are also among the risk factors that cannot be changed. The risk of breast cancer in those with a family history of breast cancer is 2-5 times higher than normal (Smeltzer and Bare, 2005). Some changes in proto-oncogenes and tumor suppressor genes play an important role in the occurrence of breast cancer. While proto-oncogenes show gain-of-function mutations, suppressing apoptosis and stimulating cell proliferation effects, tumor suppressor genes encode proteins that help the DNA repair process, control the cell cycle and suppress cell proliferation, and mutations in these genes help the carcinogenesis process by causing tumor formation (Baudet et al., 2004; Kopnin, 2000). The most common mutations associated with breast cancer are mutations in the tumor suppressor genes BRCA1 and BRCA2. While mutations in the BRCA1 and BRCA2 genes are important genes that are effective in a large proportion of hereditary breast cancer and also increase the susceptibility to ovarian cancer, mutations in the TP53 and PTEN genes are also responsible for the development of hereditary breast cancer (Concolino et al., 2018; Coignard et al., 2021; Angeli et al., 2020). It is emphasized that BRCA1, BRCA2 and TP53 gene mutations account for approximately half of early breast cancers and cause predisposition to various tumors. It is stated that BRCA 1 and BRCA2 genes interact with proteins involved in cell proliferation control, DNA repair and cell cycle checkpoints, and it is also known that the BRCA1 gene has a mechanism to control estrogen,

which causes cell proliferation in breast tissue (Mitrunen and Hirvonen, 2003). The TP53 gene is a tumor suppressor gene that regulates cell proliferation, provides DNA repair and induces apoptosis, and is active in hereditary and non-hereditary (sporadic) cancers. A loss of function in this gene causes an increase in tumor proliferation, leading to cancer development. TP53 mutation is said to be associated with familial breast cancer and also causes Li-Fraumeni syndrome (Kerbel, 2008; Kim et al., 2016).

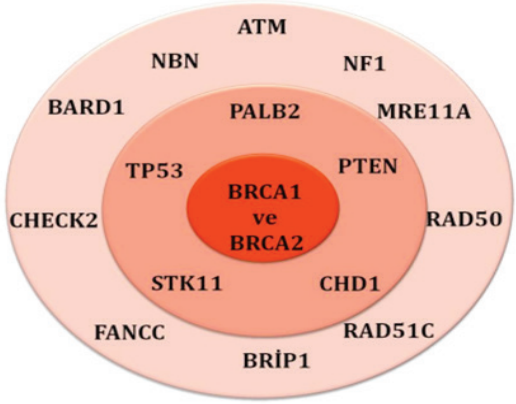

Figure 1. Genes associated with breast cancer (Tokdemir et al., 2019).

2.2. Clinical Symptoms in Breast Cancer

The most important thing to consider in preventing breast cancer and cancer-related deaths is early diagnosis and treatment. Breast cancers that occur in the early stages often do not show symptoms, that is, they are asymptomatic. Recognizing breast cancer before symptoms begin and allowing appropriate treatments emphasize the purpose of early diagnosis. For this reason, it is of great importance for women to have their own breast examinations and screening tests (Harris et al., 1992; Salp, 2003).

Among the symptoms seen in breast cancer, the first finding that is usually noticed is a mass in the breast, but it is emphasized that not every mass and change detected is cancer. The mass usually has a painless, hard and immobile structure. They are especially easily distinguished from fibroadenomas because they are a mass that does not move with the breast tissue. The mass is mostly blurred and particulate in nature. In 10% of breast cancer cases, there is discharge from a single milk duct at the nipple. This discharge may be serous or bloody. Tumors in the breast may create an imbalance in lymph flow, allowing local edema to

occur. At the same time, tumors in the breast quadrants pull the nipple towards their location, causing asymmetrical images on the nipples and dimpling of the nipple (Salp, 2003; Kalaycı and Özmen, 2002; Kalaycı et al., 2002). Apart from these findings, it is stated that symptoms such as dimpling in the breast skin, ulcerations and orange peel appearance are generally seen in advanced stage breast cancers. When tumors in the breast interact with the Cooper ligaments that provide support to the breast tissue, they cause the ligaments to shorten, pulling the skin towards the tumor and causing the skin to become hollow. It is also known that tumor cells block the skin lymphatics, causing skin nutrition to deteriorate, thus developing skin erythema and initiating ulceration. In addition, tumor cells in the breast are carried to the breast skin lymphatics, causing a narrowing of the lymphatic vessels. In this case, the slowdown in lymphatic flow causes excessive nutrition of the subcutaneous tissue, resulting in skin thickening and hair follicles being pulled inwards, giving the skin an orange peel appearance (Parlar et al., 2005).

2.3. Breast Cancer Stages

TNM staging is used in breast cancer staging. T is defined as primary tumor size, N is defined as lymph node status, and M is defined as the presence or absence of metastasis. Although TNM staging is the most widely used staging system, it is divided into certain stages. At the same time, knowing the stage of cancer directs the treatment (Ding et al., 2019; Lukasiewicz et al., 2021).

T: Tumor Size

TX: primary tumor cannot be detected

T0: no evidence of primary tumor

T1: tumor size 2 cm or less

T2: tumor size between 2cm and 5cm

T3: tumor size more than 5 cm

T4: tumor of any size has spread to the chest wall or skin

N: Lymph Nodes

NX: lymph nodes cannot be detected.

N0: no lymph node metastasis

N1: metastasis to lateral lymph nodes (axilla lymph nodes)

N2: metastases to lateral lymph nodes and other structures in the axilla

N3: metastasis to lateral breast lymph nodes or supraclavicular lymph nodes

M: Metastasis

MX: distant metastasis could not be evaluated

M0: no distant metastasis

M1: there is distant metastasis to other organs (Edge and Compton, 2010).

2.4. Breast Cancer Molecular Subtypes

Molecular subtypes of breast cancer are defined according to growth factor and hormone response. The main known receptors in this regard include estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Breast cancer subtypes; luminal A, luminal B, HER2 overexpression and triple negative (basal type) subtypes (Moasser, 2007; Howlader et al., 2014).

The most common types of breast cancer are luminal tumors, and luminal type A covers 40% of breast cancer patients. In this type, estrogen and progesterone receptor expression is positive, HER2 expression is negative, and low proliferation is observed. Tumor growth is slow and they have a good prognosis, and they receive antihormonal therapy as treatment. Luminal B is a subtype of breast cancer that tends to be ER and/or PR positive, HER2 negative or positive, and has high proliferation. It shows faster growth and worse prognosis compared to Luminal A, and chemotherapy is also used in addition to hormone therapy (Feng et al., 2018).

ER, PR and HER2 expression is negative in triple negative (basal type) breast cancer, which constitutes almost 20% of all breast cancers. It is usually seen in women with mutations in the BRCA1 gene and in women of early age. This type has a worse prognosis than other types of breast cancer and histologically they consist of ductal carcinoma and medullary carcinoma. Additionally, the metastasis pattern distinguishes this type from other types of breast cancer (Dai et al., 2015). In the HER2 overexpression type, which constitutes 10-15% of breast cancer, ER and PR are negative, HER2 is positive and shows high expression. Although the prognosis in this type of breast cancer is worse than luminal types, successful treatment is provided with agents that affect the HER2 protein (Dass et al., 2021).

The majority of malignant breast tumors consist of adenocarcinomas and are known to originate from the terminal ductal lobular region of the breast. In histology classification, breast cancer is divided into two: in situ and invasive carcinoma (Malhotra et al., 2010). In situ carcinoma is divided into ductal and lobular types, and tumor cells are localized cancers that are limited to the breast

ducts and lobules. Invasive breast carcinoma is characterized by metastasis to surrounding breast tissues. It is divided into various subtypes such as in situ carcinoma. These subtypes; invasive ductal, invasive lobular, mucinous, tubular, cribiform, papillary, medullary and micropapillary carcinomas (Makki, 2015; Deshmukh et al., 2019).

2.5. Treatment Approaches in Breast Cancer

Breast cancer treatment is carried out in the form of chemotherapy, immunotherapy, endocrine treatments and personalized treatments, taking into account certain features such as surgical removal of the part of the tumor cell, tumor size and presence or absence of lymph node involvement, radiation application, and the status of receptor expressions. Chemotherapy aims to kill cancer cells or stop their proliferation. Treatment may vary depending on the medication and doses used. Various cytotoxic drug treatments such as alkylating agents, antimetabolites and tubulin inhibitors are used in breast cancer chemotherapy. Radiation therapy is applied to all or part of the breast after surgical removal of the tumor. Radiotherapy is also used together with personalized endocrine therapy (Burguin et al., 2021).

As a result of developments in cancer, more specific and targeted treatments have begun to be used (Harmankaya et al., 2014; Harmankaya and Harmankaya, 2023). Personalized treatments aim to prevent cell proliferation through these pathways by detecting mutations occurring in the cancer cell (Montemurro et al., 2020; He et al., 2018). Approximately two-thirds of breast cancers are hormone receptor positive (estrogen receptors, progesterone receptors or both) and endocrine treatments such as tamoxifen and aromatase inhibitors are used. Estrogen receptor modulators act as receptor antagonists, binding to the sites where estrogen binds to stop the proliferation of cancer cells. Tamoxifen and toremifene are the most common receptor modulators (Peart, 2015).

Another treatment method used is immunotherapy. In immunotherapy, treatment methods such as breast cancer vaccines, monoclonal antibodies, and immune checkpoint inhibitors are applied. Breast cancer vaccines are used as an alternative to the disadvantages of drug resistance and low survival after chemotherapy and radiotherapy. DNA vaccines, tumor-associated protein vaccines and recombinant viral and bacterial vectors are the most well-known vaccine approaches in cancer. In gene-based vaccine treatments, therapeutic proteins are produced by delivering a functional gene to the target cell. As a result, the given gene performs functions such as suppressing the proliferation

and development of metastasizing tumor cells, stimulating apoptosis, and preventing vascular formation. Transport of the gene to be delivered to the target cell is carried out by agents such as viruses and bacteria. Bacterial vectors are frequently used in breast cancer vaccine treatment because they are not resistant to chemotherapeutic agents and can reach distant metastasis tumors (Santos-Carballal et al., 2018; Brentville et al., 2018). One of the vaccines used in breast cancer is the E75 peptide vaccine. This vaccine is one of the vaccines created from the extracellular components of the HER2 protein and induces the CD8 + T cell response (Milani et al., 2014). Monoclonal antibodies respond to surface antigens found on the tumor cell. It is known that the trastuzumab agent binds to HER2 receptors and stops cell division in the G1 phase in breast cancer patients. In some cases, resistance to this agent may develop, and in this case, the use of the treatment in combination with another inhibitor agent that blocks the resistance mechanism can reverse the treatment (Daniele and Sapino, 2009). Tumor cells use multiple regulatory mechanisms to inhibit immune responses. These mechanisms refer to immune checkpoints. PD-1 is one of the control receptors expressed on T cells, B cells, and dendritic cells in humans and mice, and the expression of its downstream ligands, PD-L1 and PD-L2, increases when cancerous cells are challenged by immune cells. PD-L1 overexpression in breast cancer has been associated with high proliferative index and increased tumor size. Blocking the interaction between the receptor and the ligand to enhance the immune response against cancer cells is one of the treatments for breast cancer (Nallasamy et al., 2018; Zhang et al., 2018). It is known that nanoparticles that target therapeutic genes in cancerous cells and carry drugs without damaging normal cells are effective against drug resistance, have few side effects, remain in the blood circulation in the long term, and help transport low-level drugs thanks to their water solubility. Liposomes, polymers, and gold nanoparticles are used as drug carrier systems in cancer treatments (Singh et al., 2017).

In addition to all treatment methods, it is known that there are many studies in the literature that can help breast cancer treatment and its effects. Scientific studies say that the use of additional vitamins in breast cancer reduces the risk of cancer and that vitamin A also reduces proliferation and differentiation in breast epithelial cells. At the same time, in vitro studies have observed that vitamin D and its synthetic analogs kill breast cancer cells (Narvaez et al., 2014; Welsh, 2021). A study shows that serum vitamin D levels of breast cancer patients are 55,8% lower than the control group, and the risk of developing breast cancer increases significantly in individuals with low vitamin D levels (Shaukat et

al., 2017). Ponatinib, a tyrosine kinase inhibitor, is reportedly being evaluated in the treatment of breast cancer and various other types of cancer. In a study investigating the apoptotic, antiproliferative and cell cycle effects of ponatinib in breast cancer cell lines, it was observed that its use in combination with various plant extracts induced apoptosis and its anti-proliferative effect in breast cancer cell lines increased significantly (Kayabaşı et al., 2022). In a different study, the antiproliferative effects of quercetin and curcumin, which have anti-inflammatory, antiproliferative, anticancer, antitumor and antioxidant activities, were used alone and in combination on metastatic and non-metastatic breast cancer cell lines. It has been stated that they inhibit cell viability individually and their combined use can be used in non-metastatic breast cancer (Altundağ et al., 2020). In a study conducted on MCF-7 breast cancer cells, it was reported that the combination of Alpha lipoic acid (ALA) and cisplatin (Cisp) could be an effective treatment method in the treatment of breast cancer (Nur et al., 2017). In another study, it was reported that the antitumor effect of 5-Fluorouracil (5-FU), which is known to have an antitumor effect on MCF-7 breast cancer cells, could not be increased by Hypericum perforatum (HPer) treatment (Deveci et al., 2018).

3. Conclusion

Breast cancer is one of the most common cancers in women. The primary goal of breast cancer treatment is to minimize or eliminate disease recurrence, drug resistance and toxic effects, while also ensuring that patient individuals have a good quality of life. Its heterogeneous structure is one of the most important problems encountered in therapeutic approaches in breast cancer, so the responses received from treatment vary greatly among breast cancer subtypes. The existence of subtypes brings with it various treatment options. Considering reasons such as drug resistance and tumor recurrence, it is necessary to develop new targeted agents in addition to new approaches that target only tumor tissues without harming healthy cells. At the same time, early diagnosis and screening are of great importance in increasing awareness of breast cancer (Ozturkkan and Aksu-Kılıcle, 2022; Akyolcu et al., 2019).

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CHAPTER IX

MECHANISM OF FERROPTOSIS AND RELATIONSHIP WITH CANCER

UTKU DURAN[1] & RECAİ ACİ[2]

[1](PhD.,) Zonguldak Bülent Ecevit Üniversity, Çaycuma Food and Agriculture Vocational School, Veterinary Health Program, E-mail: utku.duran@beun.edu.tr, ORCID: 0000-0002-0002-8893

[2](PhD.), Samsun University.Faculty of Medicine, Department of Biochemistry, E-mail: recaiaci35@gmail.com, ORCID: 0000-0002-1517-3356

1. Introduction

Reactive oxygen species formed as a result of various biochemical events within the cell can lead to dysfunction and/or cell death if not eliminated, causing oxidation of crucial cellular structures (Görlach et al., 2015). In order to avoid this ROS byproducts are transformed into substances thanks to the action of an antioxidant called glutathione that exists within the cell. This process safeguards the cells membrane lipids and other molecules from oxidation. Consequently the presence of glutathione plays a role, in sustaining viability (Ortega et al., 2011). Cells require the presence of certain amino acids to synthesize glutathione. One of these, cysteine, needs to be acquired from outside the cell as it may not be present in sufficient levels internally. The xCT system facilitates this transport. The xCT system consists of two transmembrane proteins: SLC7A11 and SLC3A2. The data obtained from the experiments show that the ferroptosis inducer erastin directly inhibits SLC7A11 and as a result, the cell goes into ferroptosis due to decreased glutathione production (Dixon & Stockwell, 2014). When glutathione is not present the levels of ROS molecules increase resulting in the oxidation of lipid compounds and the formation of lipid peroxides. If there is iron (Fe^{+2}), in the surroundings these accumulated

lipid peroxides undergo the Fenton reaction generating harmful free radical derivatives. In the end this process leads to death in cells, which will be further explained on (Feng & Stockwell, 2018).

In conclusion, unraveling the intricacies of the mechanism of ferroptosis and its association with cancer provides valuable insights into potential therapeutic strategies. The intricate equilibrium between reactive oxygen species (ROS) and cellular antioxidant defenses, notably glutathione, accentuates the imperative of comprehending these mechanisms at a molecular level. The pivotal function of the xCT system in facilitating the transport of cysteine, a crucial precursor for glutathione synthesis, introduces an additional stratum of intricacy to the orchestration of cellular redox homeostasis. In essence, elucidating these processes becomes paramount for a comprehensive understanding of the intricate molecular dynamics governing cellular oxidative stress responses. The identification of erastin as a ferroptosis inducer through direct inhibition of SLC7A11 highlights a potential target for therapeutic interventions. As we delve deeper into the molecular pathways governing ferroptosis, there emerges a promising avenue for developing targeted therapies in the realm of cancer treatment. Future research endeavors will undoubtedly build upon this foundation, opening new possibilities to modulate ferroptosis for the benefit of human health.

2. Ferroptosis and Cancer

Cell death plays a role not, in regular development and maintaining balance but also in preventing diseases characterized by excessive cell growth, like cancer. Throughout the years scientists have categorized the processes of cell death into two types; programmed and non programmed (Conrad et al., 2016). Scientists have held the belief for a time that apoptosis responsible, for almost all programmed cell deaths in mammalian cells. However recent findings have shown that certain types of cell death previously thought to be accidental are actually, under regulation.

Ferroptosis, an identified type of demise sets itself apart, from conventional mechanisms of cell death like apoptosis and necrosis. It relies on the presence of iron and transpires when lipid peroxides accumulate. The term “ferroptosis” was initially coined in a study carried out by Stockwell and colleagues back, in 2012 (Yu et al., 2017). Apoptosis, which is the form of programmed and regulated cell death has been extensively studied at the level. However there is still much to uncover about types of regulated cell death that occur in physiological processes.

The specific mechanisms and conditions surrounding these occurrences remain largely unknown.

Cell deaths such as ferroptosis and apoptosis in cells can be distinguished morphologically and biochemically (Table 1). In cells experiencing ferroptosis there are no characteristics linked to apoptosis such, as the condensation of chromosomes and the formation of bodies. A study utilizing inhibitors has effectively showcased the nature of the process when compared to apoptosis (Figure 1) (Masaldan et al., 2019)

Table 1. Patterns of cell death and their characteristics

Cell Death	Genes	Regulator pathways	Activators	Inhibitors	Biochemical features
Apoptosis	*Caspase, P53-, Bcl-2, Bax, Fas*	Regulation of p53-, Bcl-2- and Caspase-dependent signaling pathway	FASL, DCC, UNC5B	XIAP, non-specific caspase inhibitor Z-VADFMK, NAIP, ML-IAP/ livin, c-IAP2, ILP-2, c-IAPI	Activation of caspases, formation of oligonucleosomal DNA fragments
Ferroptosis	*GPX4, LSH, Nrf2, xCT, TFR1*	Xc-system, the pathways of GPX4, MVA, HSF1-HSPB1, p62-Keap1-Nrf2, and LSH signal pathways	Erastin, DPI2, BSO, SAS, lanperisone, SRS, RSL3, DPI7, DPI10, FIN56, sorafenib, artemisinin	Desferoxamine, vitamin E, U0126, ferrostatin-1, SRS, CA-1, cycloheximide, aminooxyacetic acid Liproxstatin-1 HCl	Xc-system inhibition and glutamate reduction, GPX4 inhibition. Iron accumulation and lipid peroxidation
Autophagy	*DRAM3, TFEB, ATG5, ATG7*	PI3K-AKT-mTOR, MAPK-ERK1/2-mTOR Signal pathways	Rapamycin, lithium, sodium, valproate, carbamazepine, C2-ceramide, rapamycin	3-ME, LY294002, wortmannin, PIKIII, compound 31, SAR 405, Vps34-In1,MRT68921, Spautin-1, Bafilomycin A1, hydrochloroquin	Increased lysosomal activity
Necrosis	*RIP1, RIP3, LEF1*	TNFα, TNFR1, TLR3, TRAIL, FasL, ROS, PKCMAPK-AP-1-Dependent signaling pathways	TNFα, zVADfmk, PAMPS	Nec-1, NSA, Kongensin- A	Decrease in ATP levels and activation of *RIP1, RIP3, MLKL*

There are variations when it comes to the properties, physical attributes, crucial genes, regulatory pathways, triggers and blockers of death processes (Mou et al., 2019).

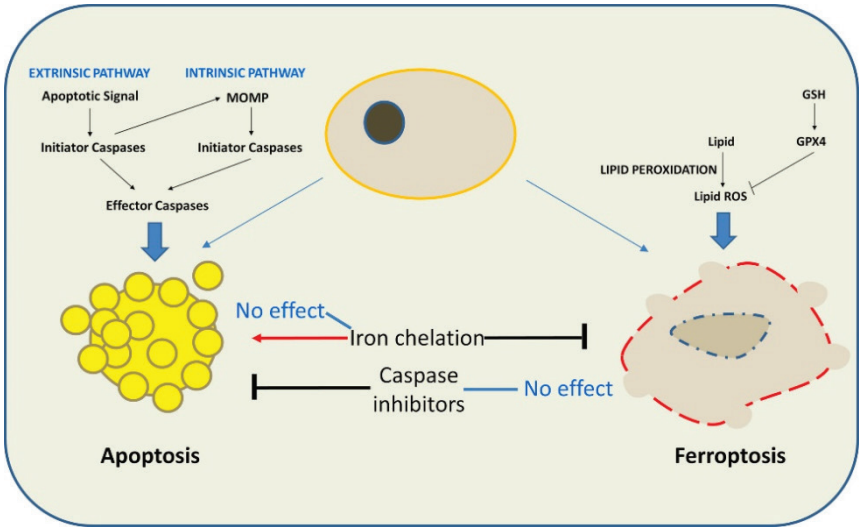

Figure 1. The Effect of Apoptosis and Ferroptosis Inhibitors

Iron chelators have no impact, on apoptosis. They do hinder ferroptosis. Likewise caspase inhibitors impede apoptosis without interfering with the occurrence of ferroptosis (Masaldan et al., 2019).

3. Mechanism of Ferroptosis

Ferroptotic cell death, a type of cell death regulated by lipid repair systems, like glutathione and GPX4 is controlled through a series of reactions that also include the production of fatty acids (PUFA). This form of cell death can be activated through pathways (Cao & Dixon, 2016). Ferroptosis is initiated by loss of GPX4 activity mediated by two different mechanisms (Figure 2). One approach is to block the GPX4 enzyme by inhibiting the Xc system, such, as through treatment, with erastin. The Xc system, which is found in the cell membrane consists of a chain subunit called xCT (SLC7A11). A heavy chain subunit known as CD98hc (SLC3A2). This inhibition occurs indirectly by targeting the Na+ dependent cystine antiporter (Figure 3). It removes glutamate from the cell and transports cysteine from the extracellular space into the cell (Bridges et al., 2012). Once the cell absorbs cystine it is then transformed back into cysteine, which's essential, for synthesizing glutathione (GSH).

Glutathione is produced through the combination of cysteine with glutamate and glycine. The process of taking up cystine and converting it into cysteine plays a role in the synthesis of glutathione, which's crucial for safeguarding cells, against oxidative stress induced harm (Yu et al., 2017). Glutathione plays a role in the functioning of the lipid repair enzyme called glutathione peroxidase 4 (GPX4). This enzyme helps prevent the build up of oxygen species (ROS) derived from lipids, lipid hydroperoxides. By utilizing glutathione as a cofactor GPX4 catalyzes the reduction of lipid peroxides. Contributes to its phospholipid peroxidase activity (Lu et al., 2018). Erastin inhibits glutamate/cystine antiport system Xc^- (Fearnhead et al., 2017).

Erastin suppresses this system, leading to a decrease in cellular glutathione levels and an increased susceptibility of the cell to oxidative damage. Inhibiting γ-glutamylcysteine synthetase (γ-GCS), the enzyme that limits the speed of glutathione synthesis, with buthionine sulfoximine (BSO) is also sufficient to initiate ferroptosis (Yang et al., 2014).

GPX4 converts glutathione into its oxidized form, glutathione disulfide (GSSG), to transform lipid hydroperoxides into alcohols and free hydrogen peroxides into water (Liang et al., 2009).

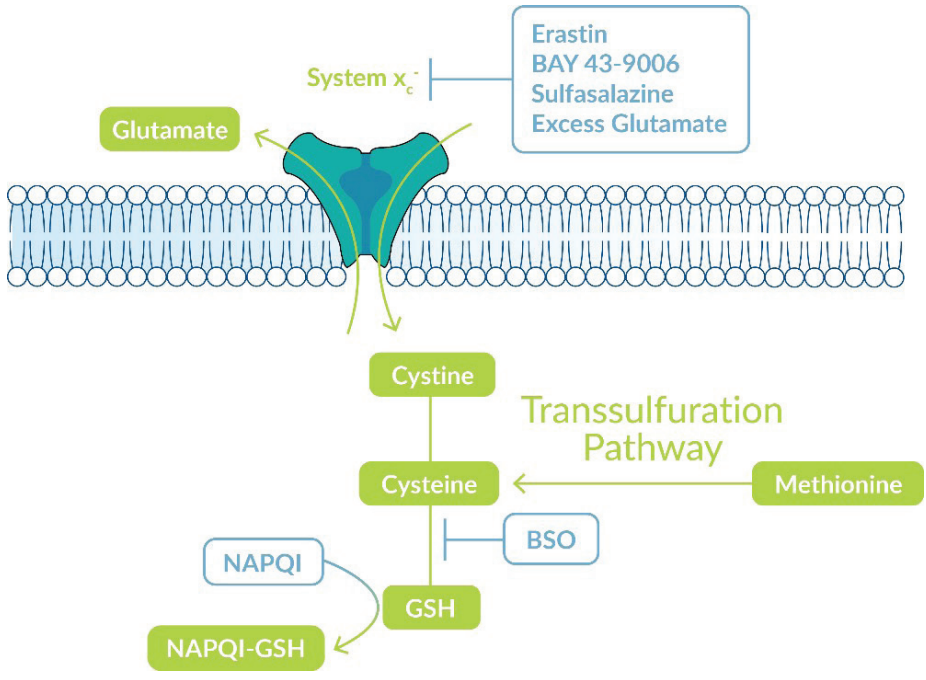

Figure 2. System Xc – function (Cayman, 2020).

The Xc system is a transporter found in the cell membrane that relies on sodium to move cystine and glutamate. It moves glutamate from, inside the cell to the outside while bringing in cystine from outside into the cell. The cell then converts the taken up cystine into cysteine, which is used to synthesize glutathione (GSH). When combined with glycine and glutamate cysteine forms glutathione. Glutathione plays a role in enabling the lipid repair enzyme known as glutathione peroxidase 4 (GPX4). GPX4 transforms glutathione into its oxidized form called glutathione disulfide (GSSG) which helps convert lipid hydroperoxides, into alcohols and free hydrogen peroxides into water.

Another way that ferroptosis occurs is, through the blocking of GPX4 activity. The known inducer of ferroptosis (1S, 3R) RSL3 has the ability to permanently hinder the function of GPX4 by specifically targeting the selenocysteine found within the active site of the GPX4 enzyme (Figure 3.)(Yang et al., 2014). Furthermore using siRNA to block GPX4 has proven effective in causing the buildup of lipid reactive oxygen species (ROS) which're crucial, for triggering cell death. To summarize GPX4 plays a role, in governing the processes involved in ferroptosis (Yang et al., 2014).

4. Iron Metabolism in Ferroptosis Mechanism

The iron that exists in the form of Fe^{2+} within the cell has the ability to directly initiate a chemical reaction known as the Fenton reaction. This reaction can produce radicals, which in turn promote the formation of lipid peroxides. If these lipid peroxides are not efficiently eliminated from, within the cell they can accumulate by transforming into lipid alkoxyl (RO•) radicals. This accumulation ultimately leads to a process called ferroptosis. One interesting aspect of this pathway is that it specifically occurs due, to irons presence than being influenced by metal ions (Lu et al., 2018). 'Iron response element binding protein 2 (IREB2)', iron metabolism's main regulator, has been identified as a key gene for erastin-induced ferroptosis in HT-1080 and Calu-1 cells (Dixon et al., 2012). IREB2, also called IRP2, plays a crucial role in intracellular iron homeostasis by controlling post-transcriptional iron metabolism genes (FTH1, FTL, TF, TfR1, Fpn and DMT1).(Bogdan et al., 2016) Ferritin is an intracellular iron storage protein. Autophagic degradation of ferritin by NCOA4, called ferritinophagy, increases ferroptosis sensitivity because it releases free iron into the cell (Figure 3) (Hou et al., 2016).

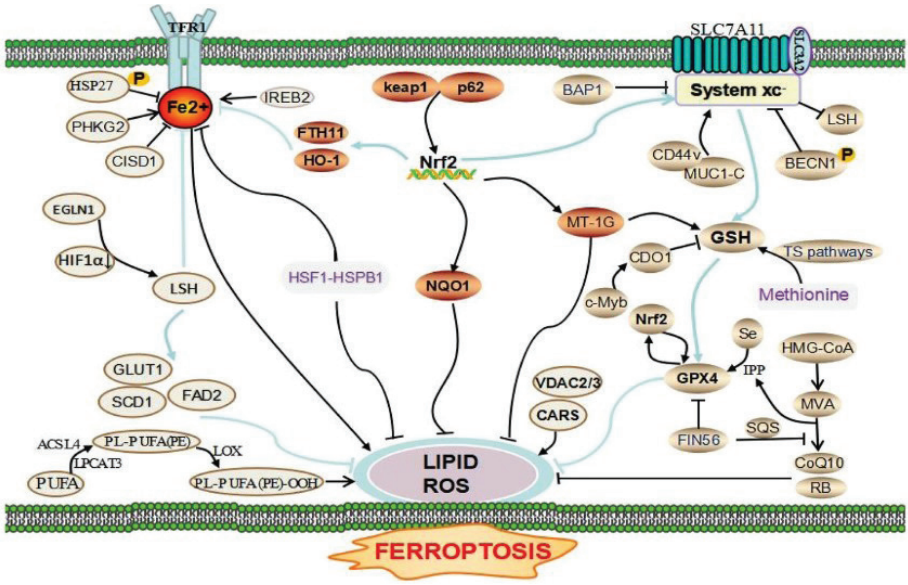

Figure 3. The regulatory network of ferroptosis (Mou et al., 2019).

Iron is transported into the cell by endocytosis via transferrin receptor 1 (TfR1), bound to transferrin (Tf) protein (Bogdan et al., 2016). Studies have shown that Tf is an important component for the induction of ferroptotic cell death (Gao et al., 2015). To safeguard the cell, from stress it is crucial to regulate the amount of iron, within the cell. However if the concentration of iron exceeds its limits it can lead to significant oxidative harm to the cell (Bertrand, 2017).

5. Lipid Metabolism in Ferroptosis Mechanism

Lipid metabolism also plays a role in determining cellular sensitivity to ferroptosis. Polyunsaturated fatty acids (PUFAs), which contain easily removable hydrogen atoms, are prone to lipid peroxidation and are essential for the execution of ferroptosis (Latunde-Dada, 2017). Therefore, the abundance and localization of polyunsaturated fatty acids determine the extent of lipid peroxidation that can occur in cells and, consequently, how effective ferroptosis is (Kagan et al., 2016). Two enzymes, ACSL4 and LPCAT3 play a role, in the synthesis and modification of fatty acids within the cells membrane. These genes are responsible, for producing an amount of PUFA lipids in the membrane, which helps facilitate ferroptosis by promoting lipid peroxidation and the formation of oxygen species (ROS) (Figure 3) (Dixon et al., 2015). In situations when there are no abnormalities fatty acid hydroperoxides are typically transformed into

fatty acid alcohols with the help of GPX4. However in the case of ferroptosis this transformation is hindered because the GPX4 enzyme becomes inactive. As a result the accumulated fatty acid hydroperoxides undergo iron mediated Fenton reactions. Get converted into peroxide radicals. This process ultimately has an impact, on cells (Lu et al., 2018).

Ferroptosis initiates when system Xc -is inhibited by erastin or when GPX4 activity is directly blocked by RSL3 leading to cell death. The process of death is driven by oxygen species (ROS) derived from lipids. While the peroxidation of fatty acids (PUFAs), in the cell membrane plays a role in the pathway of ferroptosis the accumulation of excess iron within the cell is essential for the buildup of lipid ROS and consequently triggers the mechanism, behind ferroptosis.

6. Activators and Inhibitors in the Ferroptosis Mechanism

The identification of feroptosis activators preceded the discovery of feroptosis in 2003. The first feroptotic substance identified was erastin.(Xie et al., 2016) Erastin and its more potent analogues such as imidazole ketone erastin (IKE) and piperazine erastin (PE), as well as the FDA-approved drugs sulfasalazine and sorafenib, small molecule class 1 ferroptoses inducers (Table 2)(Feng & Stockwell, 2018).

Class 2 ferroptosis inducers function by inhibiting GPX4. These substances form a chemical bond, with the selenocysteine located in the site of GPX4, which hampers its enzyme activity. As a result GPX4 is unable to repair lipids leading to the buildup of lipid peroxides and ultimately resulting in cell demise.

Certain compounds, such, as ferroptosis inducer 56 (FIN56) and caspase independent lethal 56 (CIL56) belong to a class of activators known as Class 3 ferroptosis activators. These compounds work by triggering the breakdown of GPX4 protein. Also affect coenzyme Q10 (CoQ10) which's a natural lipid based antioxidant found within cells. CoQ10 is well known for its role, in the electron transport chain (Feng & Stockwell, 2018).

FINO2 stands out as the recognized ferroptosis activator, in class 4. This particular compound, known as an endoperoxide triggers ferroptosis by oxidizing iron and indirectly disabling GPX4. Substances that induce the ferroptosis mechanism are shown in the table (Feng & Stockwell, 2018).

Table 2. Ferroptosis inducers

Class	Class Characteristics	Impact on Ferroptosis	Compound Examples	Suitable for in vivo use
Class 1	Inhibition of system x_c^-	Prevents cystine import, causes GSH depletion and loss of GPX4 activity	Erastin, PE, IKE, other erastin analogs, sulfasalazine, sorafenib, glutamate	PE, IKE, sorafenib
Class 2	Direct inhibition of GPX4	Covalently interacts with GPX4 and inhibits its enzymatic activity	RSL3, ML162, DPI compounds 7,10,12,13,17,18,19	No
Class 3	Depletion of GPX4 protein and CoQ_{10}	Depletes GPX4 protein and simultaneously causes depletion of CoQ_{10} via SQS-mevalonate pathway	FIN56 and CIL56	Unknown
Class 4	Induction of lipid peroxidation	Oxidizes iron, drives lipid peroxidation and indirect inactivation of GPX4	$FINO_2$	Unknown
Others: BSO, DPI2, cisplatin, cysteinase, statins, ferric ammonium citrate, trigonelline, CCl_4, silica-based nanoparticles, nonthermal plasma				

Abbreviations: BSO, buthionine sulfoximine; CCl_4, carbon tetrachloride; CIL56, caspase-independent lethal 56; CoQ_{10}, coenzyme Q_{10}; DPI, diverse pharmacological inhibitor; FIN56, ferroptosis inducer 56; $FINO_2$, ferroptosis inducer endoperoxide; GPX4, glutathione peroxidase 4; GSH, glutathione; IKE, imidazole ketone erastin; ML162, Molecular Libraries 162; PE, piperazine erastin; RSL3, RAS-selective lethal 3; SQS, squalene synthase

Studies have identified various ferroptosis inhibitors that can be used in the treatment of certain diseases (Table 3)(Dixon et al., 2012). Class 1 inhibitors, known as iron chelators work by stopping the start of peroxidation through the inhibition of lipoxygenases (LOXs). Additionally they also hinder the propagation of peroxidation by suppressing the Fenton reaction. On the contrary class 2 inhibitors, called antioxidants, function by capturing and neutralizing compounds to prevent lipid peroxidation.

Table 3. Ferroptosis inhibitors (Feng & Stockwell, 2018)

Class	Class Characteristics	Impact on Ferroptosis	Compound Examples	Suitable for In Vivo Use
Class 1	Iron chelators	Deplete iron and prevent iron-dependent lipid peroxidation	Deferoxamine, cyclipirox, deferiprone	Yes
Class 2	Lipophilic antioxidants	Prevent lipid peroxidation	Vitamin E, BHT, Fer-1, liproxstatin-1, XJB-5-131, CoQ_{10}	XJB-5-131
Class 3	D-PUFAs	Prevents initiation and propagation of lipid peroxidation	D_4-arachidonic acid, D_{10}-docosahexaenoic acid	Yes
Class 4	LOX inhibitors	Inactivate LOX and block LOX-induced lipid peroxidation	CDC, baicalein, PD-146176, AA-861, zileuton	Not sufficiently selective in most cases
Others: glutaminolysis inhibitors, cycloheximide, beta-mercaptoethanol, dopamine, selenium, vildagliptin.				

Abbreviations: AA, arachidonic acid; BHT, butylated hydroxytoluene; CoQ_{10}, coenzyme Q_{10}; D-PUFA, deuterated polyunsaturated fatty acid; Fer-1, ferrostatin-1; LOX, lipoxygenase

7. Genes Associated with Ferroptosis

To date, many genes that regulate ferroptosis or are considered as ferroptosis markers have been identified and the number of these genes is increasing day by day. Some of the genes associated with ferroptosis are summarised in Table 4.

Table 4. Genes Associated with Ferroptosis

Gene	Function
ACSL4 (Acyl-CoA synthetase longchain family member 4)	Convert free fatty acids into acyl-coA esters transforms it. Plays a key role in lipid biosynthesis. Required for ferroptosis
ALOX (Lipoxygenases)	Involved in peroxidation of polyunsaturated fatty acids in ferroptosis
CISD1 (CDGSH iron sulphur domain 1)	An integral membrane protein located in the outer mitochondrial membrane whose function may be to transport iron into the mitochondria
DPP4 (Dipeptidyl-dipeptidase-4)	*DPP4* plays an important role in glucose metabolism. It is responsible for the degradation of incretins such as *GLP-1*.
GCLC/GCLM (Glutamate-cysteine ligase)	*GCL* initiates and limits the synthesis of cellular antioxidant glutathione (GSH) by catalyzing the ATP-dependent condensation of cysteine and glutamate to form gamma-glutamylcysteine (γ-GC).
GLS2 (Glutaminase 2)	The protein encoded by this gene is a mitochondrial phosphate-activated glutaminase that catalyzes the hydrolysis of glutamine to glutamate and ammonia in stoichiometric amounts.
GPX4 (Glutathione peroxidase 4)	*GPX4* catalyzes the reduction of hydrogen peroxide, organic hydroperoxides, and lipid peroxides in reduced glutathione, functioning to protect cells against oxidative stress.
GSS (Glutathione synthetase)	The second enzyme in the glutathione (GSH) biosynthesis pathway.
PTGS2 (Prostoglandine-endoperoxide synthase 2)	*PTGS2* (*COX-2*), converts arachidonic acid (AA) to prostaglandin endoperoxide H2.
SAT1 (Spermidine/spermine N1-acetyltransferase)	It functions as a rate-limiting enzyme in the catabolic pathway of polyamine metabolism.
SLC7A11 (Solute carrier family 7 member 11)	The SLC7A11/GPX4 pathway functions as the fundamental defense against ferroptosis, supporting the synthesis of intracellular glutathione (GSH) and mitigating lipid peroxidation.
TFRC (Transferrin receptor)	*TFRC* is essential for importing iron into cells and is regulated in response to the intracellular iron concentration.

Cancer cells have a need, for iron, than cells. To meet this demand tumor cells increase the levels of transferrin receptors (TfR) to bring iron into the cell while reducing the expression of ferroportin1 (FPN1) receptors that transport iron out of the cell (Torti & Torti, 2013). When the cell receives iron through transferrin it is, in a form called iron (Fe^{+3}). Inside the cell an enzyme called STEAP3 converts it into iron (Fe^{+2}). This converted iron is then directed to the iron pool using DMT1, where it remains until it is needed for activities. Any excess iron is either stored as Fe^{+3} in ferritin. Eliminated from the cell through ferroportin1 (FPN1) (Battaglia et al., 2020). Due, to the iron levels observed in cancer cells the body can harness this abundance of iron by employing ferroptosis as a mechanism, against cancer. Consequently it is believed that a type of cell death may ocur (Dixon, 2017). IREB2, also known as the Iron Responsive Element Binding Protein is a molecule that has been found to have an impact, on regulating ferroptosis. Its activity is influenced by the levels of iron present within cells. When the levels of iron are low IREB2 becomes activated. Controls the translation process of specific mRNAs involved in iron metabolism. As a result this leads to an increase in the amount of iron, within cells.

8. Studies Related to Ferroptosis

After the discovery of ferroptosis many researchers have been exploring its connection, with diseases. Suggesting that triggering ferroptotic pathways especially in cancer cases could present a promising new therapeutic approach. Scientists have investigated whether various anticancer compounds exhibit effects. For instance sorafenib, a kinase inhibitor has demonstrated the ability to induce glutathione deficiency and subsequent ferroptosis by inhibiting the xCT system, in lung, kidney and other types of cancer (Lachaier et al., 2014). In a research study scientists investigated the impact of two substances, siramesin and lapatinib on types of breast cancer cells (MDA MB 231, MCF 7, ZR 75, SKBr3). Siramesin disrupts the stability of lysosomes while lapatinib works as a tyrosine kinase inhibitor. The experiments showed that the cytotoxic effect of siramesin was not reduced by the caspase inhibitor. However when they applied ferrostatin 1 a ferroptosis inhibitor it effectively inhibited the effects of both siramesin and lapatinib. Further experimentation, on pathways revealed that when lapatinib and siramesin were combined they synergistically induced ferroptosis leading to a powerful anticancer effect, in breast cancer cells (Ma et al., 2016). Furthermore scientists have conducted research on the impact of sulfasalazine, which hinders the xCT system and displays properties in head and neck cancer cells that’re resistant, to chemotherapy. Based on findings obtained

from both laboratory tests and animal studies it has been observed that by inhibiting the xCT system and activating pathways the resistance of these cells to cisplatin can be overcome. As a result it is proposed that inducing pathways, in chemotherapy cancer cells holds great promise as a potential therapeutic strategy (Roh et al., 2016).

9. Conclusion

In exploring the intricate interplay between ferroptosis and cancer, this review has systematically examined the multifaceted facets of ferroptosis, elucidating its underlying mechanisms and its correlations with oncogenic processes. As we conclude, it becomes apparent that ferroptosis occupies a critical nexus within complex molecular pathways, exerting a pivotal influence within the landscape of cancer biology. The insights gleaned from this review not only deepen our understanding of the regulatory mechanisms dictating ferroptosis but also pave the way for formulating innovative strategies in the realm of cancer therapy. The evolving terrain of ferroptosis research holds considerable promise for the formulation and refinement of targeted and efficacious therapeutic interventions, thereby designating it as an intellectually compelling domain necessitating further exploration and advancement within the field of cancer biology.

Consequently, there is a compelling and urgent need to identify and develop more precise activators of ferroptosis. Furthermore, the utilization of natural compounds or nanoparticles as ferroptosis inducers may represent a safe and efficacious avenue for cancer treatment, given their inherent properties and minimal side effects. Importantly, the amalgamation of ferroptosis inducers with existing anticancer therapies presents a novel perspective for cancer treatment.

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CHAPTER X

LUNG CANCER STUDIES RELATED TO ARTIFICIAL INTELLIGENCE

GÖKTUĞ AYTAÇ & SEVCAN AYTAÇ[2]

[1]*Fırat University, Deparment of Ecobilisim, Elazığ*
E-mail: goktugaytac23@gmail.com
ORCID: 0009-0002-7192-9696

[2]*(Assoc. Prof. Dr.), F*ırat University, Deparment of Electronics an*d Automation, Elazığ*
E-mail: saytac@firat.edu.tr
ORCID: 0000-0001-6689-2337

1. Introduction

Diseases occur in the lungs which are one of the most important organs of the respiratory system. It is known that cancer is most common in the lungs. Lung cancer is the leading cause of death from other types of cancer, as well as in women. (Korkmaz and Esmeray, 2020). With the advancement of technology, new systems are needed to help the person making this diagnosis by using various systems in cancer diagnosis. There is also a need to use these systems in the diagnosis of lung cancer.

Correct recognition of diseases is very important in planning their treatment. However, with the rapid advancement of medical imaging technology, full surveillance imaging (WSI) is It has become a routine procedure in the clinic. Recently, artificial intelligence, especially deep learning, has demonstrated high-performance such as analyzing the distributions of tumors, prognosis predictions, metastasis detection, and lung skin imaging analysis. When the literature is searched, it is possible to come across a considerable number of procedures to improve the rapid detection of lung cancer. A new CNN-based model for malignant and non-malignant is proposed (Wang et al., 2018). In the model described, whole-slide imaging (WSI) uses much smaller size image

fragments recorded from WSI, typically on the megapixel level, as they are typically input. In 2018, by recording 300x300 pixel image patches from lung adenocarcinoma WSIs, an 89.8% success rate was achieved in the proposed model. Another influential person (Šarić et al., 2019) A fully automatic method was proposed to identify cardiac death samples in full murder images; In this procedure, the ruler is the remaining part at the image patch level through the convolutional neural network (CNN). GoogleNet base network, a deep neural network, which is a classified CNN as having a different demonstration by (Sajja et al,. 2019). To minimize computational cost and avoid excessive degradation in the network, the dense coverage structures of the proposed network are distributed such that 60% of all outputs are spoken to the dropout layers. The performance of the demonstrated network is validated through an isolation on the enhanced CT scan image data view and then compared with the dataset and existing classified AlexNet, GoogleNet, and ResNet50. (Fang, 2018) proposed a fast, accurate and collective lung cancer diagnosis system based on new deep learning techniques. This method, which involves analyzing distortion with a convolutional neural network (CNN) structure similar to GoogLeNet, is used to combine multi-image features of three-dimensional computed tomography (CT) scans. This method is called Median Intensity Projection (MIP) recording. MIPs enable the proposed systems to learn the features of malicious and benign lung nodules in an integrated manner and achieve high accuracy through training and testing programs on healthy clusters. In (Mohite, 2021), the Transfer learning structure of DenseNet-201, MobileNet, ResNet-101 VGG16 and VGG19 was summarized and compared in classifying a dataset of 1100 lung CT scans. Among them, DenseNet-201 showed the best performance. In (Jayaraj and Sathiamoorthy, 2019), a computer-aided model consisting of a series of processes was applied to detect lung cancer in CT images. After pre-processing the input, the splitting process will be performed, resulting in a split image in binary format. A collection is then created from the important parts of the subdivided sections. The resulting random forest classifier model then classifies the records, classifying the proportions as ‘normal’ or ‘abnormal’, with a maximum accuracy of 89.90%. In (Zhang and Li, 2020), a part of ResNet was introduced for gross target volume segments in computed tomography images of non-small cell lung patients. Then increase their wiring to provide normalization to shrink the differences between the images and further enrich the training set. To efficiently extract deep features from computed tomography images, two separate evolutions of fused ResNet are used, where all features emerge as a single feature. This simple design creates a view of shallow

views with deep meaningful persistence to produce pixel-dense output. This model was significantly more accurate, despite errors in U-Net compatibility and device operating characteristics. (Xie, 2018) listed a new method (Fuse-TSD) to select malignant and benign lung nodules using result-oriented texture, shape, and features. deep knowledge learned through the model. Gray plane co-occurrence matrix-based texture identification uses a Fourier shape descriptor to expand the heterogeneity of nodules and a deep convolutional neural network to automatically learn cross-sectional features of nodules on it. It trains the backpropagation neural network using AdaBoosted for each feature propagation and combines the decisions made by the three classifiers to separate the nodes. This software evaluated LIDC-IDRI's data distribution according to three. The maximum accuracy rate reached 89.53%. (Tekade and Rajeswari, 2018) detected lung nodules using Computed Tomography (CT) scan images of lung patients and separated these sections using U-Net architecture to determine their malignancy. from these nodules. The study proposes a 3D multipath VGG-like network by evaluating labeled 3D cubes from datasets. Predictions from U-Net and 3D multipath VGG-like networks are combined for the final results. With this architecture, lung nodules can be classified on a daily basis and successful results can be determined at the level of malignancy.

2. Scientific Studies on Lung Cancer and Artificial İntelligence

Many studies have been conducted to date using machine learning techniques on cancer. (Korkmaz et al., 2017; Korkmaz and Binol, 2018; Korkmaz and Esmeray, 2018; Korkmaz and Esmeray, 2020 ; Korkmaz, 2021). Ginger et al. (1994), using various computer imaging techniques and information about the morphological features of polynular nodules, they subjected the features within the lung borders to gray level thresholding. As a result of this study, they concluded that developing an automatic system for detecting pulmonary nodules in CT scans would help radiologists. Xu et al. (1997) detected nodules using forward-looking artificial neural networks. This study is one of the first studies due to the method used. Qian et al. (2002) conducted a study that increased the accuracy of the decisions made by radiologists in nodule detection. As a result of the study, they argued that more nodules could be detected than the number of nodules that radiologists could detect only with their own interpretation. Edis and Karlıkaya (2007) started from a study that calculated the cost of lung cancer to Turkey. They argued that with the application of computer-aided diagnosis, the cost of treatment of lung cancer will decrease, as well as the early and accurate detection of nodules that cause lung cancer. A shape-based matching

system was used to detect nodules. As a result of this study, 90% detection was achieved (Dehmeshki et al. 2007).

Akiranet et al. (2003) observed that the sensitivity of nodule detection increased by using a computer-aided diagnosis system to detect nodules from lung images taken with multiple detectors. Beigelman-Aubry et al. (2007) examined the status of the computer-aided diagnosis system on the nodule detection performance of radiologists. It has been determined that the sensitivity of nodule detection increases in the system used by radiologists. Lee et al. (2009) made comparisons using the bayesian classifier, pure bayesian classifier and support vector machine classifier methods. They found that the support vector machines classifier gave the best results. Penedo et al. (1998) developed a computer-aided diagnosis system using local image curvatures provided by a two-level artificial neural network (ANN) method. 288 simulated nodules, 90 in real number, were classified by ANN, and the sensitivity was 89%-96% and the number of false positives per image was 5-7.

The computer-aided detection (CAD) system will be trained to be sensitive to small internal and subpleural pulmonary nodules in the database of CT scans. System performance was evaluated on 39 CT datasets containing 75 internal and 27 subpleural nodules. The FROC curve obtained in this data set recorded an acceptable level of false positive findings with 10-13 counts per patient and a high rate of 80-85% for lung nodules (Retico et al., 2008). Lee et al. (2010) argued that an automatic lung nodule detection system can help detect lung abnormalities in CT lung images and that this detection can be achieved using listed, segmentation-based and selected-based molecular. The method of combining cells by clustering (CAC) method was proposed to increase automatic lung nodule detection. They used these random forest implementations. 32 lung scans were performed, including 5721 images with nodule locations marked by expert radiologists. Overall, the highest membership for the proposed system was recorded as 98.33% and specificity as 97.11%.

Coppini et al. (2003), Their work uses biologically inspired filters for different image features. They used artificial neural networks to effectively exploit the shape and background structure information of the nodules. In the study, 247 images were used to establish and test the system. They observed that study results were between 60% and 75%, false alarms per image were between 4-10, and accuracy was between 95.7% and 98.0%. Kakar and Olsen (2009) presented a fully automatic tissue-based segmentation and recognition system for the lungs with CT. Segmentation texture features were extracted by filtering Gabor images and this was done using Fuzzy C Means (FCM) clustering and

Genetic Algorithm. For the recognition phase, features are extracted as well as shape-based features using a cortex-like structure. As a result, the detection of lesions was confirmed at 89.04%. Additionally, the average gender ratio of the classifier changes to 89.48%.

An option for nodule definitions using 2D and 3D templates that can vary between similar nodules is suggested by Farag et al. (2004). The assisted support is a detection study that blends the processes of a genetic promoter, a type of distributor, and crossover pattern matching and Bayesian aftermath. Thus, 107 of 130 nodules were correctly classified with detection matching. By Bae et al. (2005), an automatic pulmonary nodule detection program was developed using three-dimensional volumetric data. They detected nodules using computed tomography images of 20 patients with lung disease. They identified 164 nodules and used a computer-aided detection (CAD) program to examine the detected nodules. Their results revealed that the overall sensitivity in detecting nodules was 95.1%. This ratio shows that 156 out of 164 nodules were calculated correctly.

Armato et al. (2002) evaluated the suitability of a fully automated computerized method for detecting lung nodules with computed tomography scans. A database of 38 low-dose computed tomography scans containing 50 lung nodules obtained from a cancer diagnostic program was created. They implemented a computer detection method using gray level thresholding techniques. An automatic classifier programs presented a rule-based scheme and presented a series of nodule candidates, separating parts of normal anatomy from real nodules. The recordings they used achieved detection characteristics of 80%, with an average false positive detection rate of 1.0, that is, sensitivity to correctly detect 40 out of 50 nodules. Hardie et al. (2008) used a new computer-assisted detection system to detect lung nodules on chest radiographs. For this, they presented a performance analysis using a publicly available database. They used an independent data set containing 167 chest radiographs containing a total of 181 lung nodules. The publicly available test set was generated by the standard digital image database. Calculated for 114 feature sets each day. As a result, the system detected 78.1% of nodules in the test and an average of 4.0 false positives per image.

3. Conclusion

As a result, according to the literature reviews, computer-assisted automatic diagnosis methods are being developed to reduce time and cost in preventing or curing lung cancer. It is predicted that artificial intelligence will be able to perform better day by day for precise imaging and destruction of lung cancer and tumors.

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