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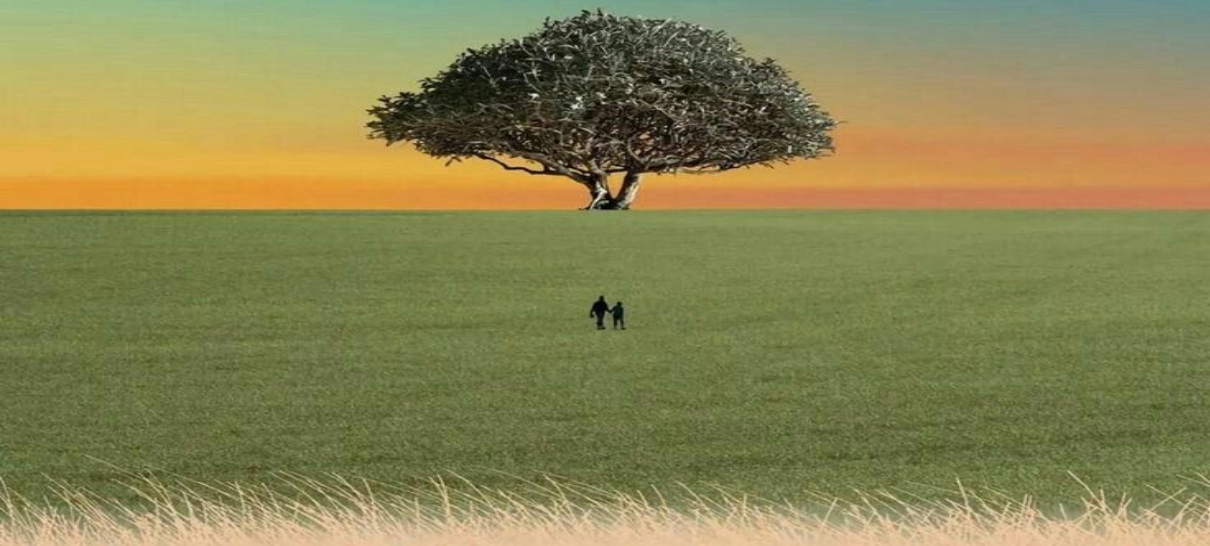

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İÇİNDEKİLER

HEDGEHOG SİNYAL YOLAĞI'NIN KANSER KÖK HÜCRELERİNDEKİ ROLÜ

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1. GİRİŞ

Kanser kavramının kabul edilmesinden bu yana tarih boyunca kanserin neden oluştuğu ve nasıl tedavi edileceği konusunda pek çok araştırma yapılmıştır. Mevcut tedavilerin kanseri önleme ve ortadan kaldırma konusundaki başarısızlığı yeni teorilerin ortaya çıkmasına neden olmuştur. Kanser kök hücre (KKH) kavramı, kanser kitlesinin tamamen yok olmasının engellenmesinden ve kendini yenilemesinden sorumlu olan bir üreme merkezi fikrinden doğmuştur. Bunu kanıtlamak için son 30 yılda yapılan araştırmaların sonuçları da bu teoriyi desteklemektedir. Bu bölümde kanser kök hücrelerin de Hedgehog sinyal yolağının önemi, mevcut araştırmalar ve bu araştırmaların faydaları tartışılmaktadır. Hedgehog sinyal yolağı, hücre farklılaşmasının, doku polaritesinin ve hücre çoğalmasının önemli bir düzenleyicisidir. Çalışmalar bu sinyal yolağının bazal hücreli karsinomların ve cilt dışı kanserlerin %30'una kadar aşırı aktif olduğunu göstermiştir. Hedgehog sinyallemesinin seçici

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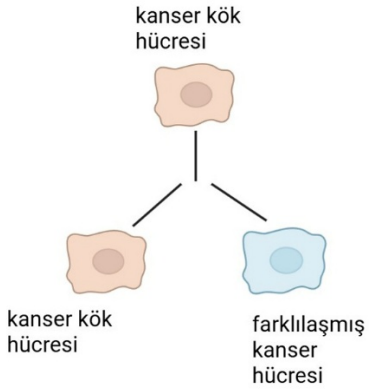
inhibisyonunun birçok tümör tipinin tedavisinde etkili olabileceği görülmektedir

2. KANSER KÖK HÜCRE TANIMI

Kanser, hücrelerin kontrolsüz çoğalması ile ortaya çıkan önemli bir hastalıktır. Dünya çapında 6 ölümden birine neden olan, ikinci önde gelen ölüm nedenidir(Cordani, Dando, Ambrosini, & González-Menéndez, 2024). Normal bir hücrenin tümör hücresine dönüşmesinin, mutasyonların ve epigenetik değişikliklerin birikimi sonucu ortaya çıkan çok aşamalı bir süreç olduğu genel olarak kabul edilmektedir(Schwartz, 2024). Günümüzde, kanser tedavisi için geliştirilen cerrahi rezeksiyon, kemoterapi, radyoterapi, hedefli tedavi veya immünoterapi gibi geleneksel tedavi stratejilerinin, büyük ölçüde metastaz ve kanserlerin lokal tekrarlaması nedeniyle sınırlı klinik etkileri vardır(Walcher et al., 2020). Tedavi başarısızlığının bir diğer nedeni ise direncin ana nedeni olan kanser kök hücreleridir (KKH). Kanser kök hücreleri ilk kez 1994 yılında lösemide tanımlandı(Zeng et al., 2023). Bu hücreler tümörün başlatılmasını yönlendirebilen, direncine ve nüksetmesine neden olabilen tümör hücrelerinin küçük bir alt popülasyonudur. KKH'ler, asimetrik bölünme yoluyla hem kendilerini yenileyebilmekte hem de farklılaşmış tümör hücreleri oluşturabilmektedir (Şekil 1). Tümörlerin çoğu heterojen bir hücre popülasyonundan oluşur. ek bir hücreden köken almalarına rağmen hepsi klonal değildir. Bu, kök hücrelerin farklı şekiller oluşturma davranışıyla tutarlıdır(Zhou, Tan, Liu, & Guan, 2023). Tümörlerin bu klonojenik ve heterojen doğası, kanser kök hücreleri gibi davranan nadir bir hücre popülasyonunun tümör büyümesi ve metastazından sorumlu olduğunu düşündürmektedir. Kök hücreler, kendi kendini yenileme kapasitesi ve çeşitli özel hücre tiplerine farklılaşma yeteneği ile karakterize edilir. Bu kavram,

embriyonik kök hücrelerden (ESC'ler) ve yetişkin kök hücrelerden KKH ve indüklenmiş pluripotent kök hücrelere (IPS) kadar genişletilmiştir(Yu, Pestell, Lisanti, & Pestell, 2012). KKH'ın edindiği ek mutasyonlar ve kendini yenileme yeteneği, kötü huylu tümörlerin gelişmesine neden olabilir (Şekil 1).



Şekil 1. KKH oluşumun da asimetrik bölünme. Tek bir kök hücrenin iki yavru hücre ürettiği bir süreçtir: biri kök hücre kimliğini korurken diğeri özelleşerek kök hücre özelliklerini kaybeder.

KKH'ler oldukça heterojendir. Meme KKH'lerinin CD44+, CD24−, SP ve ALDH+ gibi yüzey biyobelirteçlerinin farklı ifade kalıpları vardır(Ginestier et al., 2007). CD271− veya CD271+ melanom kök hücreleri SCID farelerinde tümör oluşturabilir(Quintana et al., 2010). KKK'lerin heterojenliği glioblastoma, prostat kanseri ve akciğer kanseri dahil olmak üzere diğer kanserlerde de bulunmuştur(Singh et al., 2004; van den Hoogen et al., 2010; Zhang et al., 2012).

Solid tümörlerde kanser kök hücrelerinin saptanmasına ilişkin çalışmalar bulunmaktadır. Al-Hajj ve meslektaşları meme kanseri hücrelerini farelere uygulayarak insan hasta örneklerine benzer şekilde heterojen birincil ve ikincil tümörler üretebilen bir alt popülasyon (CD44+, CD24-/düşük) belirleyerek, tümörijenik

hücrelerin, tümör hücreleri olarak kendini yenilemeyi ve büyümeyi başlatabildiğini ve tümör dışı kanser hücreleri oluşturabildiğini göstermişlerdir(Al-Hajj, Wicha, Benito-Hernandez, Morrison, & Clarke, 2003). Meme kanseri kök hücrelerinin varlığını destekleyen diğer bazı çalışmalar, tümör başlatan hücrelerin kökenini analiz etmiş ve metastatik meme kanseri hastalarının kemik iliğinde kanser kök hücrelerinin klinik önemini araştırmıştır(Dick, 2003; Pecora et al., 2002). Solid KKH varlığına bir başka örnek ise, pediatrik beyin tümörleri üzerine yapılan bir çalışmadır. Hemmati ve ark., nöral kök hücrelere benzer özelliklere sahip tümörojenik hücreleri izole ederek, bu hücrelerin multipotent olduğu, kendi kendini yenileyebildiği ve nöronlara ve glia'ya farklılaşma yeteneğini koruyan, çoğalan nörosferler üretebildiği gösterilmiş. Bu tümör kaynaklı nörosferlerin gen ekspresyonu normal nörosferlerden farklı değildi. Nöral kök hücreden türetilen nörosferlerin aksine, bu hücreler daha uzun süre hayatta kaldığı ve anormal ikiz fenotipli hücrelerin ortaya çıkmasına neden olduğu görülmüş(Hemmati et al., 2003).

Normal kök hücrelerin hayatta kalma, çoğalma, kendini yenileme ve farklılaşma özelliklerine katkıda bulunan birçok sinyal yolağı, tümör oluşumunda veya KKH'lerinde anormal şekilde aktive edilir veya baskılanır. Bmi-1, Notch, Wnt ve Sonic hedgehog gibi yolakların, tümör baskılayıcı genlerin ve onkogenlerin hem normal hem de kanser kök hücrelerinin kendini yenilemesinin düzenlenmesinde rol oynadığı tespit edilmiştir(Yang et al., 2020).

3. HEDGEHOG SİNYAL YOLAĞI

Hedgehog (Hh) sinyal yolağı ilk olarak Drosophila'da tanımlanmıştır. Hedgehog familyasının salgılanan sinyal proteinleri, birçok doku ve organların morfogenezini

düzenleyerek Drosophila'dan insanlara kadar çeşitli hayvan soylarının gelişiminde önemli bir rol oynar(McMahon, Ingham, & Tabin, 2003). Drosophila'da Hh sinyal yolağının, keşfinden bu yana, Drosophila'da Hh sinyallemesinde yer alan birçok bileşen tanımlanmış ve karakterize edilmiştir(Taipale & Beachy, 2001). Ancak omurgalılardaki Hh sinyal yolu hâlâ bazı sürprizler sunabilir. Hh sinyali aynı zamanda yetişkin dokularda kök hücre çoğalmasının düzenlenmesinde de rol oynar. Hh yolunun anormal aktivasyonu ise, melanoma, medulloblastoma, pankreas, meme, kolon, yumurtalık ve küçük hücreli akciğer karsinomları gibi birçok kanserlerle ilişkilidir(Evyapan, Luleyap, Kaplan, & Kara, 2022).

3.1. Hedgehog Salgılanması ve İşlenmesi

Hh, birçok türde çok çeşitli dokuların modellenmesinde rol oynar. Bu nedenle, farklı Hh izoformlarının ifadesi çok karmaşıktır ve farklı transkripsiyonel güçlendiriciler tarafından önemle düzenlenir(Sagai, Hosoya, Mizushina, Tamura, & Shiroishi, 2005). Hh işleme ve salgılama mekanizmaları evrimsel olarak korunmuştur ve memeli Hh proteinleri Sonic (Shh), Desert (Dhh) ve Indian (Ihh) dahil olmak üzere tüm Hh izoformları için geçerlidir(Ingham & McMahon, 2001). İnsanlarda, Hh yolağının birkaç ana bileşeni vardır: 1) üç Hh homologu, 2) Patched1 (insanlarda PTCH1, farelerde Ptch1 ve Drosophila'da Ptc), 3) G-protein-bağlantılı reseptör (GPCR) benzeri reseptör Smoothened (insanlarda SMO ve farelerde/Drosophila'da Smo) ve 4) GLI1 ve glioma korelasyonundan adlandırılan üç transkripsiyon faktörü (GLI1, GLI2 ve GLI3) (Şekil 2).

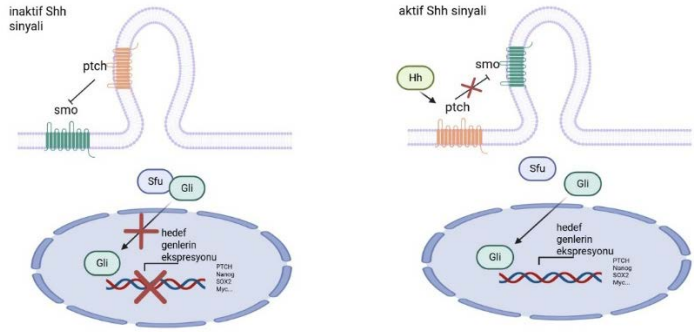
3.2. Hh Proteinleri

Hh proteinleri birkaç işlem adımından geçer. İlk olarak sinyal dizisi ikiye bölünür. Hh polipeptidinin C-terminal alanı daha sonra C-terminal kolesterolle modifiye edilmiş N-terminal Hh sinyalleme alanının (HhN) oluşumuna yol açan bir molekül

içi kolesterol transfer reaksiyonunu katalize eder. Kolesterol modifikasyonu, Hh'nin membranlara bağlanmasına yol açar ve transmembran asiltransferaz skinny hedgehog (Ski) (Chamoun) tarafından HhN'nin N terminaline bir palmitoil kısmının eklendiği son işlem adımını kolaylaştırır. Tamamen aktif, lipid ile değiştirilmiş HhN üretilir(Cochrane, Szczepny, Watkins, & Cain, 2015).

3.3. Hh'nin Dokular Yoluyla Salınması ve Taşınması

Sıkı membran ilişkisine rağmen Hh, uzun vadeli, zamana ve konsantrasyona bağlı bir şekilde doğrudan etki ederek distal dokuların farklılaşmasını etkileyebilir. Salgı hücreleri tarafından Hh aktivitesinin bir gradyanının oluşturulması, Hh salınımını, taşınmasını ve depolanmasını düzenleyen çeşitli makromoleküller tarafından kolaylaştırılır(Stamataki, Ulloa, Tsoni, Mynett, & Briscoe, 2005). Hh, salgı hücresinden transmembran taşıyıcılara sekans benzerliği olan Dispatched'ı (Disp) serbest bırakır. Hh'nin dokular yoluyla daha sonra taşınması, heparan sülfat gerektirmektedir. Hh'nin kolesterol modifikasyonu ayrıca palmitoilasyonunu, stabilitesini, difüzyonunu ve/veya taşınmasını etkileyerek Hh etki aralığını da düzenler(The, Bellaiche, & Perrimon, 1999).

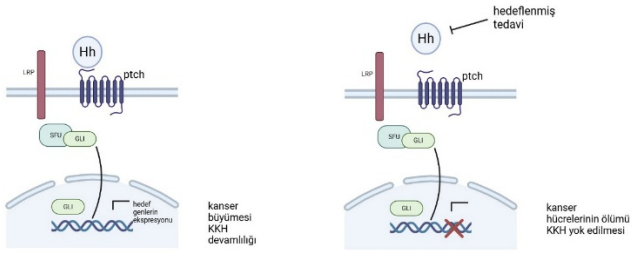


Şekil 2. Hedgehog Sinyal Yolağı. İnaktif sinyalleme de, Hh ligandlarının yokluğunda meydana gelir, burada PTCH SMO'yu inhibe eder ve bu ise SuFu tarafından sitoplazmada Gli'nin çekirdek içerisine girişi engellenmiş olur ve hedef genlerin ekspresyonu yapılamaz, Hh varlığında ise, PTCH'nin SMO baskılaması ortadan kalkar ve Gli hücre içerisine girererek hedef genlerin aktivasyonuna neden olur.

4. HEDGEHOG SİNYAL YOLAĞI VE KANSER KÖK HÜCRESİ

Kontrollü bir Hh sinyal yolağı, normal doku ve organların embriyonik gelişiminin yanı sıra doku onarımı ve doku homeostazisi boyunca çok sayıda biyolojik sürece katkıda bulunurken, bu yolağın kontrolsüz aktivasyonu tümörigenezi teşvik edebilir. Dahası, artan kanıtlar Hedgehog sinyalinin çeşitli kanserlerde KKH'lerin özelliklerinde rol oynadığını göstermektedir. Örneğin, PTCH1, GLI1 ve GLI2 gibi Hedgehog sinyal bileşenlerinin ifadesi mammosferlerin normal insan meme kök/progenitör hücrelerinde yukarı doğru düzenlenir ve Hh sinyal yolağı insan meme KKH'sinde indüklenir. Ayrıca Hh yolağı, miyeloid lösemideki KKH'lerin yanı sıra melanom ve pankreas kanseri gibi solid kanserlerde de dahil olmak üzere hematopoetik sistemin normal ve neoplastik kök hücrelerinin idamesi ve

kendini yenilemesi için kritik öneme sahiptir(Santini et al., 2012; Zhao et al., 2009). İlginç bir şekilde, genetik ve/veya epigenetik değişikliklerle aşırı aktive edildiğinde, Hh yolağı birçok dokuda tümör oluşumunu teşvik etmektedir(Barakat, Humke, & Scott, 2010). Hh yolağının kritik tümör oluşturma rolü, kanser kök hücreleri üzerindeki aktivitesiyle, kanserde aşırı ifade edilen kök hücre belirleyici genlerin (Oct3/4, Sox2, Nanog) düzenlenmesinin bozulmasıyla vurgulanmaktadır.



Şekil 3. Hedgehog Sinyal Yolağının Hedeflenmesi

Kanser, bilimsel anlayışımızdaki önemli ilerlemelere ve klinik bakımın iyileştirilmesine rağmen, kanser tedavileri önemli bir karşılanmamış ihtiyaç olmaya devam etmektedir. Burada, tümörler içinde kendini yenileme, farklılaşma ve tümör oluşumu gösteren küçük bir hücre alt popülasyonu olarak var olan KKH kavramının önemi açığa çıkmaktadır. Dikkat çekici bir şekilde, KKH'leri geleneksel kemoterapi ve radyasyon tedavisinden kaçmaktadır ve bu da KkH'lerin kanser metastazının kökeni olabileceğini düşündürmektedir. KKH özelliklerinin devamlılığı ve aktivasyonunda rol oynayan fenotipik belirteçler ve sinyal yolaklarının hedeflenmesi kanser tedavilerinde umut vadetmektedir. KKH'ler yönlü moleküler ipuçlarına ve köklülükleri ile ilgili özellikle sinyal yolaklarına sahip olduklarından, bu yolakların doğrudan manipüle edilmesi,

proliferatif fenotiplerini ortadan kaldırabilir ve böylece KKH aracılı nüksetmeyi önleyebilir(Merchant & Matsui, 2010).

5. SONUÇ

Özetle, yapılan çalışmalar hedgehog sinyal yolağının çeşitli kanserlerin gelişiminde ve ilerlemesinde çoklu rollerinin olduğunu ortaya koymaktadır. KKH'ler, çeşitli tümörlerde tümör oluşumu, ilaç direnci, kemoterapi ve radyoterapi gibi geleneksel tedavilere direnç için kritik öneme sahip gibi görünmektedir. Hh'un son yıllardaki çalışmalarla beraber karanlık bilinmeyen taraflarınında ortaya çıktığını açık bir şekilde görmekteyiz. Bu nedenle, Hh sinyal yolağının kanser gelişimindeki rolünün daha fazla araştırılması ve anlaşılması, yeni tedavi yöntemlerinin geliştirilmesine ışık tutacaktır.

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TIM-3: FROM REGULATION OF THE IMMUNE SYSTEM TO ITS ROLE IN CANCERS

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1. INTRODUCTION

The human immune system has a dynamic nature to maintain homeostasis by balancing immune cell proliferation (Wu and Wu, 2012). Cells of the immune system overcome foreign antigens; however, they should be inactivated to prevent the attack of the immune cells on organisms' own healthy tissues, and due to uncontrolled proliferation, they can become cancerous (Pathania et al., 2022). Understanding the relationship between cancer and the immune system as well as immune checkpoints brings scientists to develop new therapies (Pardoll, 2015). Targeting the blockage of the immune system, primarily CTLA-4 and PD-1, results prolonged survival rates (Buchbinder and Desai, 2016). Inhibition of immune checkpoint regulator molecules could vary among cancers, such as hematologic malignancies, solid cancers, and myeloid neoplasms, due to the nature of the difference between tissues and features of cancer cell (Zeidan et al., 2021).

Over the past few decades, it was investigated whether these regulator molecules could be targets for cancer immunotherapy. In 2018, James Allison and Tasuku Honjo

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gained the Nobel prize for their work on the effect of PD-1 and CTLA-4 on cancer (Guo, 2018). This could point out the importance of these molecules, and studies on immune checkpoint regulators have increased. T-cell immunoglobulin and mucin-containing domain (Tim-3) is one of the most important immune checkpoint regulator molecules expressed by several lymphocyte populations (Monney et al., 2002). Now, its role in cancer and cancer immunotherapy is starting to be understood more clearly, which has prompted the development of therapeutic agents to target this molecule.

2. STRUCTURE OF TIM-3 AND ITS LIGANDS

Tim-3, situated on human chromosome 5 and comprising 302 amino acids and belongs to the type-I cell-surface glycoprotein TIM family (Qin et al, 2020; Tang et al, 2019). The TIM gene family modulates immunological responses in autoimmune diseases, infectious diseases, and tumor immunosurveillance (Tang et al., 2019). Figure 1 illustrates that the TIM-3 molecule comprises a conserved extracellular immunoglobulin domain (IgV) featuring a mucin domain with O-linked glycosylation sites, a transmembrane region, N-linked glycosylation sites, and a cytoplasmic tail comprising five tyrosines (Gorman and Colgan, 2014).

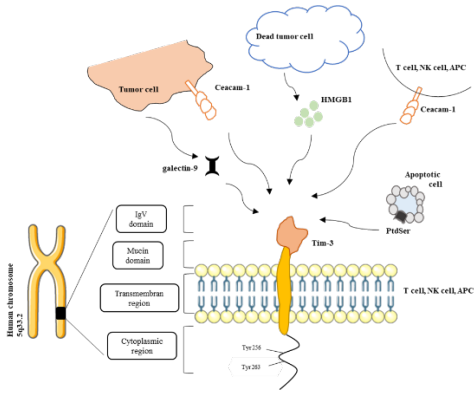

Figure 1. Structural overview of the Tim-3 molecule and its ligands. This diagram illustrates the key structural components of Tim-3, including its extracellular immunoglobulin-like domain, transmembrane region, and cytoplasmic tail. The figure also highlights the ligands that interact with Tim-3, such as galectin-9, which play crucial roles in regulating immune responses.

The IgV domain binds ligands and includes two anti-parallel chains and four cysteines that form two disulfide bonds (Hattori et al., 2022). These unique disulfide bonds create a ligand binding cleft for galectin-9. Binding of Tim-3/Gal-9 causes apoptosis of the helper T cells which causes the immune escape of tumor cells (Zhao et al., 2020). It was reported that Ceacam-1 and phosphotidylserine (PtdSer) bind near this cleft (Huang et al., 2015).

Tim-3's cytoplasmic tail has five tyrosine residues that may be useful phosphorylation sites for the pathway. The Src family kinase Fyn and PI3K component p85 bind to these residues, while Itk phosphorylates Tyr 265 (Lee et al., 2011). Moreover, HLA-B-associated transcript 3 (Bat3) represses Tim-3 function by binding the cytoplasmic tail (Rangachari et al, 2012). The soluble Tim-3 form (sTim-3) does not consist of mucin and transmembrane domains, and this type is also important in tumor growth (Geng et al., 2006).

An S type galectin called Gal-9 is one of the most studied Tim-3 ligands. Gal-9 recognizes the sugar chain of the IgV domain. Galectin-1, 3 or 4 can also interact with Tim-3, but galectin-9 has the highest affinity to bind (Blenda et al., 2022). The binding of Gal-9 and Tim-3 induces the influx of calcium into the cell, leading to apoptosis (Figure 2). Consequently, the Gal-9/TIM-3 pathway is crucial for the organism's homeostasis (Lhuillier et al, 2015). PtdSer is a Tim-3 ligand, and the interaction between PtdSer and Tim-3 causes phagocytosis of

apoptotic bodies and cross-presentation by dendritic cells. PtdSer is in the inner part of the cell membrane and in apoptotic cells move to the outer membrane (Zhao et al., 2021). This movement is an important apoptotic signal that attracts phagocytic cells such as macrophages. Apart from macrophages, T cells and dendritic cells express Tim-3 on their surfaces can recognize apoptotic signals. Therefore, PtdSer-Tim-3 interaction led to the clearance of the apoptotic cells (Wang et al., 2022). High mobility group B1 (HMGB1) is a chromatin factor that plays an important role in the promotion of transcription. In cell stress, HMGB1 is secreted out of the cell and plays roles in inflammation (Tang and Lotze, 2012). It was shown that the interaction of Tim-3 molecule with carcinoembryonic antigen cell adhesion molecule-1 (Ceacam-1) and inhibit T-cell function (De Sousa et al., 2020). In colorectal cancer patients, it was shown that blockage of CAECAM1 and Tim-3 causes an enhanced antitumor immune response (Zhao et al., 2021).

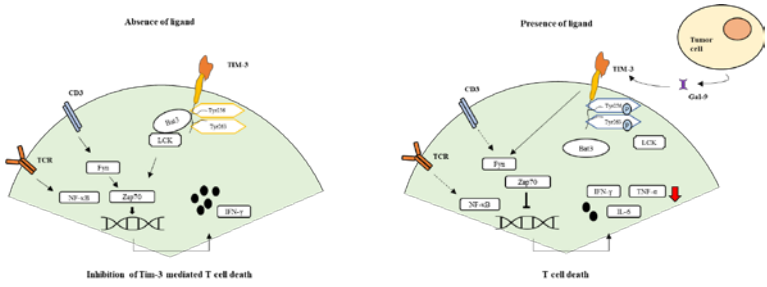

Figure 2. Schematic representation of events occurring in the presence and absence of TIM-3 ligand. In the presence of the TIM-3 ligand, immune cell activation, modulation of T-cell responses, and potential immune evasion mechanisms are highlighted. In contrast, the absence of the TIM-3 ligand leads to altered immune responses, potentially resulting in either reduced tumor control or heightened immune activity. These differential effects highlight the significance of TIM-3 ligand interaction in immune regulation and cancer progression.

TIM-3's structure and its ligands have provided valuable insights into its regulatory roles within the immune system. However, its significance has grown immensely in recent years due to its emerging role in various cancers. The increasing body of research suggests that TIM-3 plays a critical part in modulating immune responses within the tumor microenvironment. As we continue to unravel its mechanisms, the potential of TIM-3 as a target for cancer therapy becomes more evident. Subsequent sections will examine the specific functions of TIM-3 across many cancer types, underscoring its increasing significance in cancer immunology.

3. BREAST CANCER

The role of the TIM-3 and other immune checkpoint inhibitor molecules are investigated in breast cancer. Chemotherapy continues to be a fundamental treatment for numerous cases of breast cancer while immunotherapies, such as checkpoint inhibitors and cancer vaccines, are emerging as promising alternatives. The integration of these therapies, based on the cancer subtype and patient profile, represents a multidimensional approach to improving treatment outcomes in breast cancer (Figure 3) (Lukasiewicz et al., 2021). Upregulation of CTLA-4, PD-1, TIM-3 and LAG3 were indicated in both TNBC and non-TNBC cell lines (Saleh et al., 2019). In a study, cocultured MCF-7 cells with ALL-derived CD8+ T cells and indicated that galectin-9 expression protects breast tumor cells against the cytotoxic effects of T cells. Moreover, when they compared gal-9 expression levels between breast tissue cells and breast cancer cells, they noticed lower expression of gal-9 in healthy breast tissue cells than in cancer cells (Yasinska et al., 2019). Pulido et al focused on intratumoral dendritic cells in murine breast cancer models and showed high Tim-3 expression

levels instead of T cells. Therefore, they concluded that dendritic cells may be one of the important mediators in combinational chemotherapeutic and anti-Tim-3 mediated therapy (Pulido et al., 2018). Cheng et al. found a correlation between Tim-3 overexpression, tumor metastasis, migration and invasion of breast cancer cells and the inhibition of apoptosis (Cheng et al., 2018). The main mechanism of Tim-3 expression on breast cancer cells is obscure; therefore, understanding the role of Tim-3 in tumor progression can be helpful for developing new targets for therapies (Burugu et al., 2018).

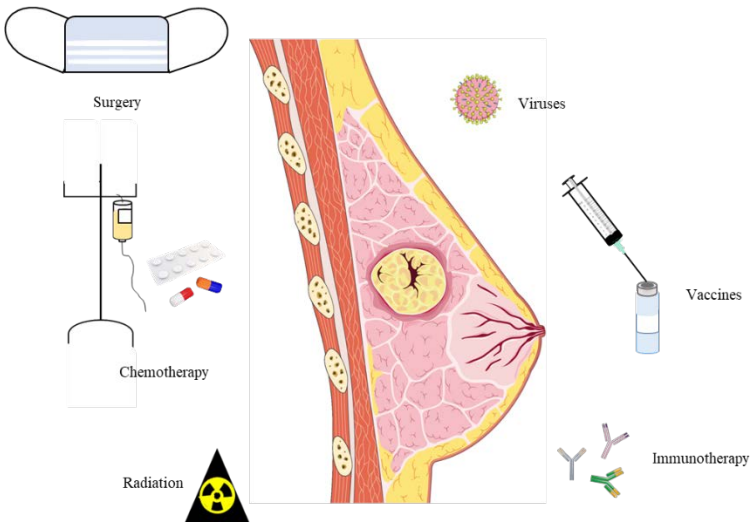

Figure 3. Overview of treatment options for breast cancer. This schematic illustrates the various therapeutic strategies available, including traditional chemotherapy, immunotherapy, and novel vaccine-based approaches.

4. LUNG CANCER

Lung cancer exhibits a high death rate due to challenges in detection and fast progression (Zhuang et al., 2012). Eighty-five percent of lung cancers consist of non-small cell lung cancer

(NSCLC), while 15% consist of small cell lung cancer (Schabath and Cote, 2019). Preclinical cancer models and cell culture studies demonstrated that Tim-3 inhibition, particularly in conjunction with PD-1 inhibition, enhances the antitumor efficacy of Tim-3 (Acharya et al., 2020). However, increased Tim-3 levels associated with resistance to PD-1 blockade in lung cancer patients (Koyama et al., 2016). It was the first time that Datar et al. demonstrated that Tim-3 is expressed mainly in innate immune cells in lung cancer rather than in cytotoxic CD8+ T cells (Datar et al., 2019). Moreover, Gao et al. targeted the exosomal expression of Tim-3 in the plasma of NSCLC patients and found a significant relationship between tumor metastasis, advanced stages of disease (Gao et al., 2018).

Expression of Tim-3 on circulating T cells could be an early marker for NSCLC patients who were treated with nivolumab, a monoclonal antibody targeting PD-1. For this reason, the idea that Tim-3 can be used as an immunotherapy target is gaining increasing support. Moreover, Tim-3 blockage with PD-1 inhibitors such as nivolumab has demonstrated promising preclinical results, suggesting that multiple immune checkpoints may affect anti-tumor immunity. Given its role in regulating immune responses, Tim-3 holds potential as a therapeutic target, either as a monotherapy or in combination with other immune checkpoint inhibitors, offering new avenues for improving patient outcomes in NSCLC (Limagne et al., 2019).

5. BLADDER AND KIDNEY CANCER

Bladder cancer can be a muscle-invasive form (MIBC) or non-muscle invasive and metastatic form. MIBC is one of the most mutational cancers. Monoclonal antibodies against PDL-1 were approved for MIBC therapy (Tran et al., 2021). However, in some patients, these inhibitors are not effective, and new

checkpoint targets are needed. Attala et al. demonstrated the upregulation of the Tim-3 molecule in bladder cancer patients, which specifically inhibits NK and T cells in the tumor environment. They evaluated Tim-3 as both a predictive marker in bladder cancer and a target for immunotherapy (Attalla et al., 2022). The effector functions of NK cells are diminished during cancer progression by Tim-3 upregulation (Zhang et al., 2018). Therefore, the idea of TIM-3 blockage can enhance the functions of NK cells and was shown in a preliminary study in bladder cancer patients (Farkas et al., 2018).

Renal cell carcinoma is the predominant form of kidney cancer characterized as an immunogenic tumor (Couto-Cunha et al., 2022). As in most tumor environments, T-cell activation is the most critical process that affects the treatment of cancer. Zhang et al. compared Tim-3 expression on renal cell carcinoma cells and evaluated a higher detection rate in primary tumors than in metastatic tumors (Zhang et al., 2019). Yuan et al analyzed Tim-3 expression levels in another common kidney cancer called clear cell renal cell carcinoma (ccRCC) and investigated higher Tim-3 expression in ccRCC cells when compared to normal renal tissue (Yuan et al., 2014). The use of anti-Tim-3 antibodies in the therapy of RCC is still being investigated in combination with conventional therapies. The FDA approved anti-PD1 blockade in the treatment of advanced RCC patients; however, only a few of the patients benefit from immune checkpoint inhibitor (ICI) therapy. By obstructing the inhibitory signals of PD-1/PD-L1 interactions, immune checkpoint inhibitors (ICIs) facilitate the reactivation of T-cell (Figure 4). Therefore, preclinical research has focused on new ICI therapy targets, such as Tim-3 (Diaz-Montero et al., 2020).

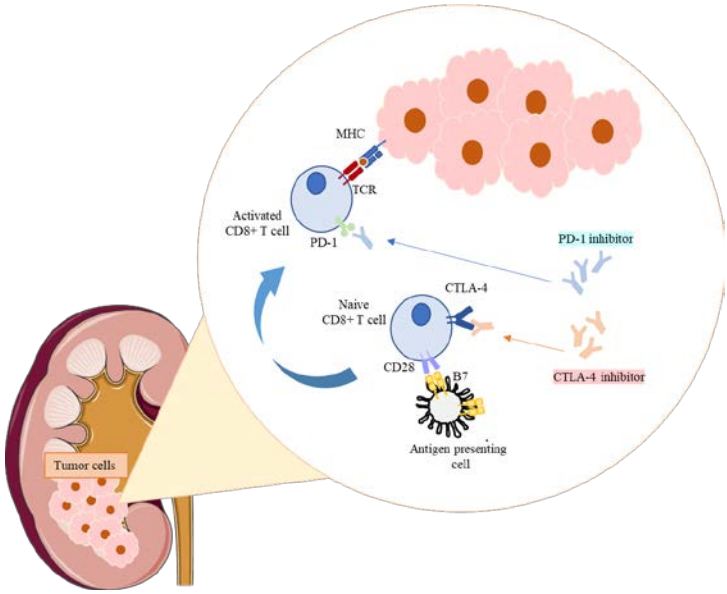

Figure 4. The mechanism of action of PD-1/PD-L1 inhibitors, in promoting immune responses against RCC.

6. LIVER CANCER

As hepatitis B and C viruses can be a risk factor for liver cancer, alcohol-related cirrhosis, smoking, obesity, and diabetes are the other common risk factors (Anwanwan et al., 2020). Liver cancer can mainly be divided into two types: primary liver cancer, which starts in liver cells, and secondary liver cancer, which is caused by metastasis of cancer cells from other parts of the body. Primary liver cancer includes intrahepatic cholangiocarcinoma (ICC), hepatocellular carcinoma (HCC), and combined HCC-cholangiocarcinoma (cHCC-CC) (Gao et al., 2020). High levels of Tim-3 expression were found on tumor-associated macrophages, which accelerates tumor cell growth (Liu et al., 2018). In addition to high expression levels of Tim-3, IFN-γ promotes Gal-9 expression in Kupffer cells, which further damages liver cells and negatively affects liver cancer (Knght et

al., 2007). Tim-3 expression was also observed in HBV-associated HCC tumor tissues, especially in lymphocyte-infiltrated livers. Overexpression of Tim-3 molecules on antigen presenting cells and T cells cause tumor proliferation, migration, and invasion of the cells (Li et al., 2012). The importance of Tim-3 expression in HCC leads to the idea of focusing on developing therapeutics that inhibit Tim-3 (Ganjalikhani et al., 2020). After investigation of CTLA-4 and PD-1 immunotherapy to obtain better survival rates in different cancer patients who do not respond to classic therapies, Tim-3 immunotherapy can be a good option for both HCC and other types of cancers (Mellero and Lasarte, 2015; Le Mercier et al., 2015).

7. PANCREATIC CANCER

Pancreatic cancer is one of the most malignant cancer types, and the incidence is increasing worldwide (Krishnamoorthy et al., 2020). Failure of early diagnosis, a high level of mortality after operation, low treatment rates, and poor prognosis cause the 5-year survival rate of pancreatic cancer to be 5% (Leinward and Miller, 2020). Conventional chemotherapy is the current standard of treatment and provide only a few months survival (Ho et al., 2020). Prognostic subtypes of pancreatic cancer can be defined by tumor-specific antigens (Topalian et al., 2012). This identification can also have an impact on the response to immune checkpoint blockade. Knudsen et al. focused-on gene expression levels of immune checkpoint regulators as PDL1 and CTLA-4 and showed negative correlation with survival times (Knudsen et al., 2017). Regrettably, research indicates that pancreatic ductal adenocarcinoma, a highly aggressive kind of pancreatic cancer, is unresponsive to immune checkpoint inhibitors such as ipilimumab (an anti-cytotoxic T lymphocyte antigen 4) or atezolizumab (an anti-programmed cell death

receptor ligand 1) (Royal et al., 2010). Only pembrolizumab (anti-PD1) was approved for immunotherapy for pancreas cancer (Marabelle et al., 2020).

Peng et al. examined TIM-3 expression in pancreatic cancer against normal pancreatic tissues and found significantly elevated TIM-3 levels in pancreatic cancer tissues ($p<.01$). They proposed that TIM-3 expression in pancreatic cancer may serve as a significant marker for monitoring the invasion and dissemination of cancer cells. They also observed that TIM-3 expression was not associated with the patients' gender, age, tumor differentiation, or location. (Peng et al., 2017).

8. LEUKEMIA

Acute myeloid leukemia cells can escape from immune response by preventing the process of immune attack of the cells (Teague and Kline, 2013). Studies have shown that the Tim-3/Gal-9 interaction induces the β-catenin pathway, which plays a role in cell survival (Kikushige, 2016; Goncalves et al., 2016). Increasing levels of Tim-3 in AML-LSC are related to poor prognosis and immune system dysregulation (Wu et al., 2023). The exhibition of anti-leukemic activity both in vitro and in vivo has been demonstrated by chimeric TIM-3 antigen therapy (Lee et al., 2021). Combinational therapy of CAR-Tim-3 with cancer vaccines, inhibitors of other checkpoint regulator molecules such as PD-1 or conventional therapies such as chemotherapy and radiotherapy can enhance the effect on AML cells (Astor, 2022).

9. HODGKIN'S (HL) AND NON-HODGKIN'S LYMPHOMA (NHL)

Lymphoma is a common hematological malignancy that arises in lymphoid systems and is caused by NK cells, T and B

lymphocytes (Sapkota and Shaikh, 2022). HL and NHLnon-Hodgkin's lymphoma are the two classes of disease, with more than 30 subtypes consisting of NHL (Huang et al., 2022). Immunohistochemistry and flow cytometric analysis of the Tim-3 expression detected rarely in T-cell lymphomas, together with LAG-3 and VISTA. In most solid organ tumors, high levels of Tim-3 were demonstrated, while in Hodgin's lymphoma, low or no Tim-3 expression was found on T cells. Therefore, the role of Tim-3 in T-cell lymphomas is still not clear (Murga-Zamalloa et al., 2020). On the other hand, high levels of Tim-3 expression were shown in diffuse large B-cell lymphoma (DLBCL), which is an aggressive non-Hodgkin lymphoma (NHL) (Chen et al., 2019). It was reported that the expression of immune checkpoint regulators LAG-3 and TIM-3 in Classical Hodgkin Lymphoma (HL). While PD-1/PD-L1 blockade has shown significant efficacy in treating relapsed/refractory HL, the roles of LAG-3 and TIM-3 were explored as potential therapeutic targets. Immunohistochemical analysis of 57 biopsy samples revealed that TIM-3 was expressed in 36% of Hodgkin and Reed/Sternberg (HRS) cells, while LAG-3 exhibited minimal expression. Both regulators were significantly present in the tumor microenvironment, suggesting their potential as therapeutic targets with PD-1 inhibitors (Halabi et al., 2021).

10. GASTRIC CANCER

Gastric cancer is the fifth most prevalent cancer worldwide and the third leading cause of cancer-related deaths, based on incidence and fatality rates (Zhuang et al., 2020). Targeting Tim-3 expression in gastric cancer patients can be an effective method to inhibit and control gastric cancer because of the role of Tim-3 in the T-cell response (Yang et al., 2021). It was demonstrated that antitumor function of T cells was reduced in

gastric cancer patients, resulting in escape from immune surveillance (Takano et al., 2016). Cheng et al. investigated higher Tim-3 expression levels in both CD4+ and CD8+ T cells of gastric cancer tissues when compared with gastric tissues, suggesting that the cancer microenvironment and pathogenesis could regulate immune system cells. Moreover, they noticed that tumor size, metastasis and depth of tumor invasion affect Tim-3 expression on CD4+ T cells, indicating that Tim-3 expression can be used as a marker for gastric cancer progression (Cheng et al., 2015). The correlation between increasing levels of Tim-3 expression and decrease in IL-2 plays an important role in T-cell activation and shows inhibition of antitumor immunity (He et al., 2018). This suggests that increased Tim-3 levels corelate with impaired immune responses, which may contribute to tumor evasion. Wang et al reported notably higher TIM-3 expression in gastric cancer tissues compared to normal tissues, particularly in cytokeratin-positive (CK+) regions (Wang et al., 2024). A meta-analysis indicated that elevated TIM-3 levels are associated with worse overall survival (HR: 1.17) and advanced T and N stages in upper gastrointestinal cancers (Yan et al., 2023). The presence of TIM-3+ cells may identify an immunoevasive subtype of gastric cancer, suggesting a need for targeted immunotherapy strategies (Chen et al., 2022).

Due to these studies, it was understood that optimization of T cells by targeting Tim-3 could be a route for T-cell immunotherapy (Yu et al., 2017). Moreover, Tim-3 polymorphisms located at promoter region of Tim-3 gene, -574G/T, -882C/T, -1516G/T, was screened and suggested that these three polymorphisms might be associated with gastric cancer risk in Chinese population. In another study, however, the +4259T/G polymorphism was identified as a risk factor for different types of cancer, and no association was observed with gastric cancer (Cao et al., 2010).

11. COLORECTAL CARCINOMA

Colorectal carcinoma (CRC) affecting the colon or rectum, is a common cancer worldwide, particularly in individuals over 50 [86]. Increasing levels of tumor-infiltrating CD8+ T cells expressing Tim-3 and PD-1 on their surface cause a poor prognosis in CRC (Mofhtari et al., 2023).

Yu et al. showed high expression levels in CRC tissues, and Tim-3 knockdown could inhibit cell proliferation and migration. As a result, they offered Tim-3 as a target for CRC therapy (Yu et al., 2017). Moreover, Mokhtari et al. indicated high PD-1 and Tim-3 expression levels in their study consisting of 136 CRC patients. They also pointed out that Tim-3 is a more effective target for immunotherapy than PD-1 (Mokhtari et al., 2023). Khalaf et al. compared Tim-3 expression on T cells in the tumor microenvironment and peripheral blood T cells and noticed that the tumor microenvironment influences Tim-3 expression on T cells. Moreover, they concluded an association between disease stages and Tim-3 expression (Khalaf et al., 2020).

12. OVARIAN CANCER

Ovarian cancer is one of the most common cancer types among women. Diagnosis of the disease can mainly be maintained at the last stages (III-IV) because of the difficulty in detecting symptoms (Weimer et al., 2022). Standard therapy includes chemotherapy and surgery; however, the majority of patient's relapse (Kozlowski et al., 2022). In recent years, therapies targeting immune checkpoint regulator molecules such as PD-1 have been shown to be novel therapy in ovarian cancer (Blanc et al., 2021). Due to the complexity of the tumor microenvironment, many patients do not benefit from PD-1 inhibitors (Sharma et al., 2017). As a result, immunoregulatory factors beyond PD-1 have become increasingly important. In this

context, Radestad et al. evaluated the increased expression levels of LAG-3 and Tim-3 in ovarian cancer patients, highlighting the potential significance of these immune checkpoint inhibitors. The co-expression of these molecules supports the need for multi-targeted therapies focused on immune checkpoint molecules (Radestad et al., 2019). Similarly, Wu et al. observed the same results regarding Tim-3 expression on both CD4+ and CD8+ T cells (Wu et al., 2013). However, it was also shown that targeting Tim-3 did not yield the same effect as PD-1 and CTLA-4 inhibitors. Based on these findings, the authors suggested that further studies are needed to determine whether Tim-3 blockade should be considered as a viable therapeutic approach (Wei et al., 2013).

13. PROSTATE CANCER

In metastatic prostate cancer, neither chemotherapy nor surgery has proven to be successful treatment options, unlike in other types of cancer. As a result, discovering predictive biomarkers for disease progression becomes increasingly valuable (Zheng et al., 2015). Imaging techniques alone are often insufficient to detect prostate cancer, and although biopsy samples are commonly used as an indicator of the disease, they come with numerous disadvantages for the patients (Drost et al., 2019). In this context, Japp et al. reported a correlation between increased Tim-3 expression and poor prognosis in prostate cancer patients (Japp et al., 2015). Similarly, Lan et al. demonstrated higher Tim-3 mRNA levels in prostate cancer tissues compared to hyperplastic prostate tissues, showing a positive correlation between Tim-3 expression and the clinical stage of the disease (Lan et al., 2017). Furthermore, Piao et al. revealed increased levels of Tim-3 expression on both CD4+ and CD8+ T cells in prostate cancer patients, further evaluating the correlation

between Tim-3 expression and prostate cancer pathogenesis (Piao and Jin, 2017). These findings collectively highlight the potential role of Tim-3 as a biomarker for prostate cancer progression and prognosis. The increased expression of Tim-3 in both prostate cancer tissues and T cells suggests that it may play a significant role in immune evasion and tumor progression. Given the limited effectiveness of traditional treatment options such as chemotherapy and surgery, targeting Tim-3 could represent a promising therapeutic strategy. However, further studies are required to fully understand the mechanisms underlying Tim-3's role in prostate cancer and to evaluate its potential as a target for immunotherapy. Additionally, the development of more reliable and non-invasive biomarkers could significantly improve early detection and personalized treatment approaches in prostate cancer.

14. CONCLUSION

The growing understanding of the complex interplay between cancer cells and immune response elements has paved the way for the development of innovative therapeutic strategies aimed at inducing tumor cell death. These strategies, including oncolytic viruses, cancer vaccines, and immunotherapies, target various stages of tumor progression and the immune response. Among these, cancer immunotherapy has emerged as a promising therapeutic approach, complementing traditional treatment modalities such as surgery, chemotherapy, and radiotherapy. In this context, the role of Tim-3 in cancer biology has become increasingly significant, particularly as research reveals its involvement in immune evasion mechanisms that contribute to tumor progression. Given its dual expression as both membrane-bound and soluble forms, Tim-3 presents an intriguing target for therapeutic intervention. The development of agents that

specifically inhibit Tim-3 could offer an effective addition to existing immune checkpoint therapies, such as those targeting CTLA-4 and PD-1. As such, Tim-3 represents a valuable candidate for cancer immunotherapy, with the potential to enhance the efficacy of current treatment regimens. However, the clinical application of Tim-3 inhibitors requires further investigation. Comprehensive clinical studies are essential to fully elucidate the role of Tim-3 in cancer immunity, its impact on patient outcomes, and the most effective ways to target it in combination with other immunotherapeutic strategies.

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THE ROLE OF TUMOR MICROENVIRONMENT IN PANCREATIC CANCER

CHAPTER I

IMPLICATIONS FOR CHEMORESISTANCE

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1. INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal malignancies, with an overall five-year survival rate of less than 12.8% according to the (2014-2020) National Cancer Institute statistics [1, 2]. Currently, it ranks as the third leading cause of cancer-related mortality worldwide and is projected to become the second by 2030 [2]. According to a statistical study in Turkey, between 2009 and 2013, pancreatic cancer-related deaths increased among men was 5.8% and among women 7.4% annually [3]. The poor prognosis of PDAC is attributed to multiple factors, including

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late-stage diagnosis, early metastatic dissemination, and resistance to conventional therapies [4]. Despite advancements in surgical techniques, chemotherapy, and targeted treatments, survival rates have remained unchanged over the past few decades [5].

PDAC is frequently diagnosed at an advanced stage due to the absence of early symptoms and effective screening strategies [6]. The majority of patients present with locally advanced or metastatic disease, significantly limiting curative treatment options. Standard-of-care treatment includes surgical resection in eligible patients, followed by adjuvant chemotherapy with regimens such as FOLFIRINOX or gemcitabine plus nab-paclitaxel [7,8]. Only a small subset of patients (15-20%) qualify for resection; regardless of the surgical technique used, mortality and recurrence rates remain high even after surgery [9]. For those with unresectable or metastatic disease, systemic chemotherapy remains the primary treatment modality, but its efficacy is significantly compromised by intrinsic and acquired drug resistance [10].

A defining feature of PDAC is its ***tumor microenvironment (TME)***, which plays a pivotal role in therapy resistance and disease progression. The desmoplastic stroma makes up to 80% of the tumor mass and acts as both a physical and biochemical barrier, which limits drug penetration and creates an immunosuppressive environment [11,12]. In addition, PDAC cells undergo profound metabolic reprogramming, including enhanced glycolysis, altered lipid metabolism, and resistance to oxidative stress, all of which contribute to tumor persistence and chemoresistance [13]. The immune landscape of PDAC is characterized by a profoundly suppressive environment that impedes antitumor immune responses and facilitates immune evasion [9]. The presence of myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages

(TAMs), regulatory T cells (Tregs), and cancer-associated fibroblasts actively inhibit cytotoxic T-cell activity, rendering immune checkpoint inhibitors largely ineffective in clinical trials [14].

Given these challenges, recent research efforts have focused on deciphering the molecular and cellular dynamics within the TME to develop novel therapeutic strategies. Emerging approaches include targeting stromal components, reprogramming immune responses, and exploiting tumor metabolism to circumvent resistance mechanisms and improve patient outcomes [15]. While immunotherapy has revolutionized the treatment landscape for several malignancies, its success in PDAC remains limited due to the highly immunosuppressive nature of its microenvironment [16]. However, novel combination strategies, including immune checkpoint blockade, personalized cancer vaccines, and adoptive cell therapies, are under active investigation to enhance immunotherapeutic efficacy in PDAC [17].

This review provides a comprehensive analysis of the latest advancements in understanding the PDAC tumor microenvironment and its implications for chemoresistance, metastasis, and immunotherapy. We comment on the current therapeutic challenges, ongoing research efforts, and future directions in the development of innovative treatment strategies to improve clinical outcomes for PDAC patients. Furthermore, we discuss the potentials of precision medicine, biomarker-driven approaches, and novel drug delivery systems in overcoming therapeutic barriers in this highly lethal malignancy.

2. MAJOR CHALLENGE: CHEMORESISTANCE

Chemoresistance in pancreatic ductal adenocarcinoma (PDAC) remains a major challenge, significantly limiting

treatment efficacy. This resistance can be categorized as ***intrinsic***, which exists before therapy, or ***acquired***, which develops during treatment. A combination of genetic mutations, stromal interactions, metabolic adaptations, and immune evasion mechanisms contributes to the highly refractory nature of PDAC, making it difficult to treat with conventional therapies [18].

Genetic alterations play a crucial role in PDAC chemoresistance (**Table 1**). *KRAS* mutations, present in over 90% of cases, drive tumor growth and activate survival pathways such as MAPK, PI3K-AKT, and RAS signaling, reducing the effectiveness of chemotherapeutic agents like gemcitabine [19]. Similarly, *TP53* mutations, found in approximately 75% of PDAC cases, disrupt apoptotic pathways, allowing tumor cells to evade chemotherapy-induced cell death [20]. Additionally, *BRCA1* and *BRCA2* mutations in a subset of patients render tumors initially sensitive to platinum-based chemotherapy and PARP inhibitors. However, resistance can develop through secondary mutations that restore BRCA function, leading to treatment failure [21]. Given these genetic drivers, precision medicine approaches targeting mutant *KRAS*, defective DNA repair mechanisms, or apoptosis regulators are actively being explored to overcome chemoresistance [22].

Table 1. Genetic Alterations in PDAC

Gene	Type of Population	References
ATM	US Caucasian	38
BRCA1	Italian, Ashkenazi Jews	39, 40, 41
BRCA2	Italian, Ashkenazi Jews, US Caucasian, German	39, 40, 41, 42, 43, 44
CDKN2A/p16	Italian, US Caucasian, German	45,46, 47
MEN1	Italian, Korean, Japanese, German	48, 49, 50, 51
MLH1	Italian	52
MSH2	Italian, Northern European, Ireland	52, 53, 54
PALB2	European (including German, UK, Latvian, Italian, Greek, Hungarian and Spanish), Canadian,	55, 56,57

	US Caucasian	
PRSS1	US Caucasian	58, 59
KRAS	US American	60
HER2	Australian	61
NRG1	Canadian	62
BRAF	US American	60
RET	US American	63
ALK	US American	64
NTRK1	US American (White/Asian/African)	65
MET	US American (White/Hispanic/African)	66
ARID1A1	US American (White)	67
MDM2	Asian	68
TP53	US American	69
FGFR	US American (White/Black), Danish	70
CLDN18.2	Asian	71
MSLN	US American	72

Another major contributor to chemoresistance is the extensive ***desmoplastic stroma*** in PDAC, which acts as a physical and biochemical barrier to drug delivery. The extracellular matrix (ECM), composed of collagen, fibronectin, and hyaluronan, increases tissue stiffness and restricts the penetration of chemotherapeutic agents [23]. Cancer-associated fibroblasts (CAFs) further exacerbate resistance by secreting growth factors such as hepatocyte growth factor (HGF) and insulin-like growth factor (IGF), which activate tumor cell survival pathways [24]. Strategies targeting stromal components, including hyaluronan inhibitors and agents that deplete fibroblasts, have shown promise in improving chemotherapy delivery. However, clinical trials evaluating stroma-targeting approaches have yielded mixed results, highlighting the complexity of stromal interactions and the need for more refined therapeutic strategies [25].

Epithelial-mesenchymal transition (EMT) also plays a critical role in PDAC chemoresistance. During EMT, epithelial tumor cells acquire mesenchymal properties, increasing their migratory and invasive potential while becoming resistant to apoptosis [26]. This process is associated with the upregulation

of drug efflux pumps such as ABCB1 and ABCC1, which reduce intracellular drug concentrations, and anti-apoptotic proteins like BCL-2 and survivin, which enhance tumor cell survival [27]. Additionally, ***EMT-driven tumor plasticity*** allows cancer cells to transition between epithelial and mesenchymal states, further complicating treatment. Targeting key EMT regulators such as Snail, Zeb1, and Twist is being explored as a potential strategy to enhance chemosensitivity and reduce metastatic progression [28].

Metabolic reprogramming is another hallmark of PDAC that contributes to therapy resistance. PDAC cells exhibit increased reliance on aerobic glycolysis (the Warburg effect), enabling rapid ATP production and fueling biosynthetic processes necessary for tumor growth [29]. In addition to glucose metabolism, PDAC cells exploit alternative nutrient sources, including glutamine and lipids. Increased glutamine metabolism supports redox balance and nucleotide synthesis, while lipid uptake and synthesis provide essential membrane components for tumor cell proliferation [30]. These metabolic adaptations allow PDAC cells to withstand the stress conditions induced by chemotherapy. Given the critical role of metabolic alterations in chemoresistance, inhibitors targeting glycolysis, glutamine metabolism, and lipid synthesis are being investigated as potential therapeutic strategies. The expression of the fatty acid synthase (FASN) gene and the resulting FASN activity are significantly higher in cancer cells compared to adjacent normal cells. Therefore, inhibition of FASN is considered a potential selective therapeutic approach in cancer treatment. The potential application of FASN as a therapeutic target is supported by numerous studies demonstrating that pharmacological inhibition of this enzyme exerts both cytostatic (growth-inhibiting) and cytotoxic (cell-killing) effects in various tumor cells. In addition, pharmacological inhibition of other key enzymes

involved in the lipogenic pathway—including ATP citrate lyase (ACLY), acetyl-CoA carboxylase alpha (ACCA), stearoyl-CoA desaturase (SCD), and acyl-CoA synthetase—has also been suggested as an effective strategy for cancer therapy. C75, a synthetic analogue of natural cerulenin, is a potent FASN inhibitor frequently used in experimental models. Green tea polyphenols (like EGCG) and plant flavonoids (such as luteolin) also suppress FASN. Luteolin, a natural flavonoid, inhibits FASN *in vitro* and shows cytotoxic effects in breast, prostate, and liver cancer cells. Both C75 and luteolin have reduced the proliferation of prostate cancer cells.Luteolin's efficacy stems from decreasing fatty acid and nucleic acid synthesis, and lowering energy production. Other natural inhibitors, like quercetin and resveratrol, are effective at higher concentrations and primarily affect glycogen metabolism[31]. The combination of metabolic inhibitors with chemotherapy may help disrupt tumor metabolism and enhance treatment efficacy [32]. The ***immunosuppressive TME*** of PDAC further limits the effectiveness of therapies, including immunotherapy. Regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs) contribute to immune evasion by creating an immune-excluded environment that protects tumor cells from cytotoxic immune responses [33]. Additionally, PDAC is characterized by a ***low tumor mutational burden*** (TMB), which results in poor neoantigen presentation and reduced recruitment of cytotoxic T lymphocytes (CTLs) [34]. Furthermore, the **overexpression of immune checkpoint molecules** such as PD-1, PD-L1, CTLA-4, LAG-3, and TIM-3 induces T-cell exhaustion, rendering immune checkpoint inhibitors largely ineffective in PDAC [35]. To overcome this immune resistance, ***combination therapies*** incorporating immune checkpoint blockade with stromal remodeling agents, metabolic inhibitors, or cancer vaccines are being investigated [36]. Early-phase clinical trials suggest that modifying the tumor

microenvironment to promote immune activation may enhance the efficacy of immunotherapies in PDAC [37].

3. CONCLUSION

Pancreatic ductal adenocarcinoma (PDAC) exemplifies a paradigm of multidimensional chemoresistance, shaped by intricate genetic aberrations, a desmoplastic stroma, epitelial-mesenchyma transition, metabolic reprogaming, and robust immune evasion. Addressing these interdependent resistance networks via precision-targeted therapeutics, metabolic interference, and immunomodulatory strategies holds promise for improving treatment outcomes in PDAC patients. Future therapeutic succes will rely not only on isolated strategies but on integrative, biomarker-guided interventions that adapt to the evolving tumor landscape of PDAC.

Appendix 1. Relevant Genes and Mutations

Gene	Mutation
ATM	c.8266A>AT p.K2756X (only confirmed germline mutation) c.170G>GA p.W57X c.3214G>GT p.E1072X c.6095G>GA p.R2032K IVS41-1G>GT c.3801delG
BRCA1	c.514delC p. Gln172AsnfsX62 c.1687C>T p.Gln563Stop c.3756_3759delGTCT p.Ser253ArgfsX10 c.5030_5033delCTAA p.Thr1677IlefsX2 185delAG 5382insC
BRCA2	c.514delC p.Gln172AsnfsX62 c.5796_5797delTA p.His1932GlnfsX12 c.6468_6469delTC p.Glu2157IlefsX18 174delT 6672insT 6819delTG 4075delGT R2034C G3076E

	10323delCins11 IVS 16-2A>G (splice acceptor site of intron 16) IVS 15-1G>A (splice donor site of intron 15) M192T K3326X 2458insT
CDKN2A/p16	p.E27X p.L65P c.201 ACTC>CTTT (promoter) p.G67R p.R144C p.G101W p.E27X −34G>T (initiation codon) c.47T>G p.L16R c.71G>C p.R24P c.192G>C L64L c.238_251del p.R80fs c.283del p.V95fs c.318G>A p.V106V c.457G>T D183spl c.324T>A p.V95E c.482G>A p.A148T c.323_324insG p.E119X
MEN1	c.304G>T p.R102S c.723 to 724 del 320 CCC to C 68 CCC to CC 179 GAG to GTG c.249–252 del c.183G>A p.W61X c.196G>T p.V66F c.482delG c.1213C>T p.Q405X c.969C>A p.Y323X c.973G>C p.A325P 210–211insAGCCC c.712delA p.K201R c.CCT>CCGG, p.55fs64aaX c.GAG>AAG, p.E26K c.AGC>AAAC p. 66fs50aaX c. CGG>CAG p.R171Q c.CTG>CCG p.L168P c.GTG>GTTG p.236 fs12aaX c.TAT>TAG p.T268X c.GCC>CC p.437 fs15aaX

	c.GCA>G p.510fs19aaX c.CCG>GG p.493fs65aaX
MLH1	K618A
MSH2	Q402X G322D E205Q V367I c.1046C>T p.P349L c.1147C>T p.R383X
PALB2	c.1240C>T p.R414X c.508-9delAG p.R170I,183X c.3116delA, p.N1039fs heterozygous 6.7kb deletion of exon 12 & 13 c. 172-5delTTGT
PRSS1	p.N29I p.R22H
KRAS	G12C G12D G12R G12V
HER2	Amplification
NRG1	Gene Fusions
BRAF	V600E , p.N486_P490del exon11, SND1-BRAF fusions
RET	Gene Fusions
ALK	Gene Fusions (EML4-ALK, STRN-ALK)
NTRK1	Gene Fusions
MET	Overexpression
ARID1A1	c.3826C>T (p.R1276X) c.5947_5948delTG Fs IVS10+1G>A Splice site c.1945_1946insT Fs c.2296dupC Fs c.5965C>T (p.R1989X) c.6287C>G (p.S2096X) c.1585C>T (p.Q529X) c.5548dupG Fs c.2402delG Fs
MDM2	Overexpression
TP53	Y220C
FGFR	R248C
CLDN18.2	Overexpression
MSLN	Overexpression

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THE ROLE OF TUMOR MICROENVIRONMENT IN PANCREATIC CANCER

CHAPTER II

COMPONENTS OF TUMOR MICROENVIRONMENT IN PANCREATIC ADENOCARCINOMA

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1. INTRODUCTION

The tumor microenvironment (TME) in pancreatic ductal adenocarcinoma (PDAC) comprises a diverse array of ***cellular*** and ***non-cellular components*** that dynamically interact and evolve to drive tumor progression, conferring resistance to therapy [1]. Key elements of the TME include ***cancer-associated fibroblasts*** (CAFs), ***immune cells***, ***the extracellular matrix*** (ECM), and ***metabolic factors***, all of which contribute to the aggressive nature of PDAC.

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2. NON-CELLULAR COMPONENTS OF TME

2.1. Extracellular Matrix (ECM)

The ECM plays an important role in the PDAC TME by offering essential structural support while serving as a regulatory framework influencing tumor progression. Its contributions are key to understanding and addressing the complexities of tumor biology. [2]. Composed primarily of collagen, fibronectin, hyaluronan, and proteoglycans, the ECM functions as a physical barrier that limits drug penetration, significantly contributing to chemoresistance [3]. ECM remodeling, orchestrated by ***cancer-associated fibroblasts* (*CAFs)*** and ***matrix metalloproteinases*** (***MMPs***), facilitates tumor invasion and metastasis. Furthermore, excessive collagen deposition increases ECM stiffness, promoting mechanical signaling pathways that enhance tumor cell survival and resistance to apoptosis [3].

Beyond its mechanical properties, the ECM serves as a ***biochemical reservoir***, sequestering growth factors such as TGF-β and vascular endothelial growth factor (VEGF), which regulate tumor progression and angiogenesis [4,5]. The interaction between tumor cells and ECM components activates crucial signaling pathways, including integrin-mediated adhesion and ***focal adhesion kinase (FAK) signaling,*** further supporting tumor growth and survival [6].

Given the ECM's role in therapy resistance, ***targeting ECM components*** (for example collagen and hyaluronic acid) has gained traction as a therapeutic strategy. Investigational approaches include enzymatic degradation of ECM elements, inhibition of CAF activity, and disruption of ECM-integrin interactions to improve drug delivery and reduce tumor aggressiveness [7,8]. By modulating ECM remodeling, these

strategies hold promise in enhancing the efficacy of both chemotherapy and immunotherapy.

2.2. Cellular Components of TME

2.2.1. Cancer-Associated Fibroblasts (CAFs)

CAFs represent one of the most abundant stromal components in PDAC and play a central role in tumor progression [9]. These fibroblasts **secrete a range of extracellular matrix proteins, cytokines, and growth factors,** which enhance tumor cell proliferation, invasion, and metastasis. Among these, transforming growth factor-beta (TGF-β) promotes epithelial-to-mesenchymal transition (EMT) and induces an immunosuppressive tumor microenvironment, while fibroblast growth factors (FGFs) stimulate angiogenesis and further support tumor growth and dissemination [10]. Moreover, CAFs promote chemoresistance by ***increasing tissue stiffness***, restricting drug penetration, and creating an immunosuppressive microenvironment. CAFs also ***interact with immune*** cells to suppress cytotoxic responses, further ***accelerating PDAC progression*** [11].

Due to their critical role, targeting CAFs is undeniably a promising and effective therapeutic strategy. Approaches under investigation include depleting CAFs, modulating their phenotype, or disrupting CAF-tumor cell interactions, all of which have shown potential to enhance treatment efficacy and improve responses to immunotherapy [12].

2.2.2. Immune Cells and the Immunosuppressive TME

The immune landscape of PDAC is marked by a highly ***immunosuppressive environment*** that fosters tumor immune evasion. Several key immune cell populations contribute to this phenomenon:

Myeloid-derived suppressor cells (MDSCs) actively inhibit T-cell responses and support tumor progression by secreting TGF-β and interleukin-10 (IL-10), which suppress antitumor immunity. Furthermore, MDSCs modulate metabolic pathways by upregulating factors like arginase-1 (ARG1) and producing reactive oxygen species (ROS), leading to T-cell dysfunction and anergy. Elevated MDSC infiltration in PDAC is correlated with poor prognosis and resistance to immunotherapies, making them potential targets for novel strategies [13].

Tumor-associated macrophages (TAMs), predominantly of the M2 phenotype, further promote immune evasion, angiogenesis, and ECM remodeling, while also inhibiting cytotoxic T-cell activity. M2-Tams also secrete TGF-β and IL-10,reinforcing the immunosuppresive milieu by suppressing cytotoxic T-cells. Additionally, TAMs facilitate tumor metastasis via the colony stimulating factor 1 receptor (CSF1R) and C-C motif chemokine ligand 2 (CCL2) axes, enhancing tumor cell migration and invasion. Given their pro-tumorigenic role, TAMs represent a promising therapy target. Strategies aiming transition of M2 to M1 phenotype, via TLR4 activation or CSF1R inhibition, has a potential in restoring anti-tumor immunity [14].

Regulatory T cells (Tregs) suppress antitumor immune responses through direct cell-cell interactions and the secretion of immunosuppressive cytokines. Regulatory T cells (Tregs) are a subset of $CD4^+$ T-cells that suppress immune responses and promote immune evasion in PDAC. They express checkpoint molecules like CTLA-4 and PD-1, and produce high levels of TGF-β and IL-10. High Treg infiltration correlates with poor prognosis and limited response to immune checkpoint blockade, highlighting the need for strategies that deplete Tregs while preserving general immune function. [15].

Natural killer (NK) cells, which are responsible for tumor cell clearance, exhibit functional impairment in PDAC, reducing immune surveillance capacity. However, they in PDAC their function is severely impared due to multiple inhibitory signals in TME. Overespression of PD-1 and TGF-β by tumor cells and suppresive immune cells leas to NK cell exhaustion and reduced cytotoxicity. Strategies to restore NK cell function include IL-15 and IL-21 based therapies, which enhance NK cell proliferation and activaation, as well as TGF-β inhibitors that reverse NK cell suppression [16].

Moreover, PDAC tumors display high levels of immune checkpoint proteins especially programmed death-ligand 1 (PD-L1), which induce T-cell exhaustion and contribute to the limited success of immune checkpoint inhibitors in clinical trials [16].

Despite these challenges, novel therapeutic strategies are being explored to enhance antitumor immunity. Combination approaches, integrating immune checkpoint blockade, cytokine modulation, and TME reprogramming, are under investigation to counteract immune suppression and improve treatment responses. Understanding the complex interactions between immune cells and stromal components remains critical for the development of effective immunotherapies in PDAC.

2.3. Metabolic Factors

Hypoxia is a hallmark feature of the PDAC microenvironment, driven by rapid tumor cell proliferation and insufficient vascularization. The resultant hypoxia-induced stress leads to profound metabolic adaptations that support tumor progression [17]. One of the most well-characterized ***metabolic shifts*** in PDAC is ***anaerobic glycolysis***, commonly referred to as the ***Warburg effect***. This phenomenon is characterized by the substantial increase in glucose uptake and

lactate production, occurring even when oxygen is present and mitochondria are fully functional [18]. This adaptation allows PDAC cells to generate ATP rapidly while producing metabolic intermediates essential for biosynthesis and proliferation [19].

In addition to glycolysis, PDAC tumors exhibit ***altered lipid metabolism***, with upregulated lipid synthesis pathways and increased fatty acid oxidation, which help sustain high energy demands. These metabolic alterations enable PDAC cells to withstand nutrient deprivation and oxidative stress, further reinforcing their survival under hypoxic conditions [20].

Furthermore, hypoxic environment within tumors activates critical transcription factors such as hypoxia-inducible factor-1 alpha (HIF-1α), regulating expression of genes involved in angiogenesis, metastasis, and therapy resistance [21]. Besides tumor cells, PDAC-associated cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) reinforce this metabolic network by releasing metabolites (e.g., lactate, adenosine) and growth factors (e.g., VEGF, HGF, IGFs) that promote tumor progression and support immune evasion.

3. CONCLUSION

The metabolic plasticity of PDAC constitutes a centrral axis of the therapeutic resistance, enabling tumor cells to thrive in nutrient-deprived and immunosuppresive microenvironments. Strategic disruption of glycolytic flux, glutamine dependency and abberant lipid metabolism holds promise not only for sensitizing tumors to existing treatments but also for unveiling new vulnerabilities [59]. Integrating metabolic inhibitors into rationally designed combination regimens may shift may shift the therapeutic paradigm and render PDAC more amenable to long-term disease control.

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THE ROLE OF TUMOR MICROENVIRONMENT IN PANCREATIC CANCER

CHAPTER III

TARGETED APPROACHES AND CURRENT OBSTACLES IN PANCREATIC ADENOCARCINOMA THERAPY

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1. INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) has demonstrated ***limited response*** to immune checkpoint inhibitors, unlike other malignancies that have benefited significantly from these therapies. The highly immunosuppressive tumor microenvironment (TME), characterized by dense stroma, low tumor mutational burden (TMB), and poor infiltration of cytotoxic T lymphocytes, plays a crucial role in immune evasion [1]. Additionally, the presence of immunosuppressive cells, including myeloid-derived suppressor cells (MDSCs), tumor-

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associated macrophages (TAMs), and regulatory T cells (Tregs), further inhibits effector T-cell activation and function [2].

PDAC tumors typically exhibit low PD-L1 expression compared to other cancers that respond well to checkpoint inhibitors, further limiting therapeutic efficacy [3]. Moreover, metabolic reprogramming within the TME creates a nutrient-deprived environment that suppresses T-cell activity, making it even more challenging to achieve robust immune responses [4]. To overcome these barriers, researchers are investigating combination therapies that ***integrate checkpoint blockade with immune-modulating agents***, such as chemokines, cytokines, and targeted therapies against stromal components [5].

Recent studies have explored combination approaches involving anti-PD-1 or anti-CTLA-4 antibodies alongside tumor vaccines, adoptive cell therapy, or metabolic inhibitors to enhance immune activation. Although clinical trials have faced challenges, ongoing efforts in developing novel immunotherapy combinations continue to offer potential strategies for improving PDAC treatment outcomes [6].

A major obstacle in PDAC immunotherapy is T-cell exhaustion, which leads to impaired immune surveillance. Chronic antigen exposure in the TME results in T-cell dysfunction, characterized by reduced cytokine production, impaired proliferation, and decreased cytotoxic activity [7]. This exhaustion is driven by the persistent expression of inhibitory receptors including PD-1, TIM-3, and LAG-3, which suppress T-cell function and limit antitumor responses [7]. In addition to inhibitory receptor signaling, immunosuppressive cytokines like transforming growth factor-beta (TGF-β) and interleukin-10 (IL-10) contribute to T-cell exhaustion by maintaining an immune-tolerant environment [8]. Moreover, tumor-associated macrophages (TAMs) and MDSCs release factors including

arginase-1 and reactive oxygen species, which metabolically impair T cells and further suppress their activation [9].

Efforts to reverse T-cell exhaustion have focused on immune checkpoint blockade, targeting PD-1/PD-L1 and TIM-3 pathways. However, due to the highly immunosuppressive nature of the PDAC TME, these treatments have shown limited success as monotherapies. Combination approaches incorporating metabolic reprogramming, T-cell reinvigoration strategies, and immune checkpoint inhibitors are currently under investigation to enhance T-cell function and improve immune responses in PDAC [10].

2. COMBINATORIAL APPROACHES FOR OPTIMIZING THERAPEUTIC MODALITIES

Given the challenges associated with immune checkpoint blockade in PDAC, emerging therapeutic strategies focus on ***combining*** checkpoint inhibitors with stromal modulation and vaccine-based therapies to optimize the therapeutic interventions **(Table 1)**. One promising approach involves ***pairing immune checkpoint blockade*** with agents that target the tumor stroma, such as ***hyaluronidase inhibitors***. These agents degrade extracellular matrix components, improving immune cell infiltration and enhancing the efficacy of checkpoint inhibitors like anti-PD-1 and anti-CTLA-4 antibodies [11].

Another innovative strategy involves using ***personalized cancer vaccines*** alongside ***checkpoint inhibitors***. Neoantigen-based mRNA vaccines aim to stimulate the immune system to recognize and attack tumor-specific antigens. Early clinical trials combining checkpoint blockade with personalized vaccines have demonstrated increased T-cell infiltration and promising immunogenicity in PDAC [12].

Additionally, ***metabolic reprogramming strategies*** are being explored to enhance immune responses. Targeting glutamine metabolism, a key energy source for PDAC cells, has emerged as a promising approach. Glutaminase inhibitors, such as CB-839, have shown the ability to deplete glutamine levels within the TME, thereby synergizing with ***checkpoint inhibitors*** to restore T-cell function [13].

Other metabolic pathways, such as ***fatty acid oxidation*** and mitochondrial ***oxidative phosphorylation***, are also being investigated. Inhibitors of carnitine palmitoyltransferase 1 (CPT1), such as etomoxir, aim to block fatty acid oxidation, a crucial metabolic adaptation that supports PDAC cell survival under stress conditions [14]. Similarly, mitochondrial oxidative phosphorylation can be targeted using complex I inhibitors like IACS-010759, which have demonstrated efficacy in preclinical models by reducing ATP production and promoting tumor cell apoptosis [15].

Furthermore, metabolic plasticity in PDAC enables tumor cells to switch between glycolysis and oxidative phosphorylation, necessitating combination approaches that concurrently target multiple metabolic pathways. ***Dual inhibitors*** that suppress both glycolysis and mitochondrial respiration are being explored to reduce tumor adaptability and enhance therapeutic susceptibility [16]. Identifying patient subgroups with specific metabolic dependencies may help tailor metabolic interventions and improve treatment efficacy in PDAC [17].

Lactate accumulation within the TME has also been implicated in immune suppression. As a byproduct of aerobic glycolysis, lactate inhibits T-cell activation while promoting the activity of immunosuppressive cells such as Tregs and MDSCs. ***Inhibiting lactate dehydrogenase*** (LDH) has been proposed as a

potential strategy to restore immune function and enhance ***immunotherapy*** responses in PDAC [18].

Preclinical and early-phase clinical trials are currently evaluating the efficacy of ***dual inhibition*** strategies targeting metabolic pathways and immune checkpoints. These combination approaches, integrating immune modulation, metabolic intervention, and stromal remodeling, hold promise for overcoming immune resistance and expanding therapeutic options for PDAC patients [19].

The immunosuppressive nature of the TME in PDAC presents significant challenges for immunotherapy. However, novel combination strategies that incorporate immune checkpoint blockade with stromal remodeling, metabolic interventions, and personalized cancer vaccines are under active investigation. By addressing multiple resistance mechanisms simultaneously, these approaches aim to enhance immune responses and improve patient outcomes in PDAC. Continued research into the interplay between metabolic reprogramming, immune evasion, and TME modulation will be critical in developing more effective immunotherapeutic strategies for this aggressive malignancy.

2.1. Emerging Therapies In PDAC

2.1.1. Adoptive Cell Transfer (ACT) Therapies

Adoptive cell transfer (ACT) therapies, including ***chimeric antigen receptor T-cell (CAR-T)*** and ***T-cell receptor-engineered T-cell (TCR-T)*** therapies, are being actively explored as potential treatments for PDAC [20]. CAR-T cell therapy involves genetically modifying a patient's T cells to express chimeric antigen receptors (CARs) that recognize specific tumor-associated antigens, thereby enhancing tumor targeting and destruction. However, the identification of effective CAR-T targets in PDAC has been challenging due to

the lack of highly specific tumor antigens. Nevertheless, promising targets namely mesothelin (MSLN), prostate stem cell antigen (PSCA), and carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) are currently under investigation [21]. In pancreatic ductal adenocarcinoma (PDAC), the cell surface protein mesothelin (MSLN) plays an active role in peritoneal metastasis. Membrane-bound MSLN interacts with MUC-16, facilitating tumor cell clustering and peritoneal colonization. Although excessively secreted soluble MSLN (sMSLN) competitively inhibits this interaction, thereby reducing cell–cell adhesion and metastatic spread, it paradoxically promotes peritoneal metastasis through a pro-inflammatory signaling mechanism independent of MUC-16. Given MSLN's dual role in regulating cell clustering and facilitating tumor dissemination via inflammation, its therapeutic targeting could be a promising strategy to control peritoneal metastasis in PDAC [22].

TCR-engineered T-cell therapy, on the other hand, modifies T-cell receptors (TCRs) to recognize tumor-specific antigens presented by major histocompatibility complex (MHC) molecules. This approach is particularly advantageous for targeting intracellular tumor-associated proteins that are not accessible to CAR-T cells. Recent advances include the development of engineered TCRs against mutant KRAS, a key oncogenic driver in PDAC [23].

Despite their potential, both CAR-T and TCR-T therapies face significant hurdles in PDAC, including the highly immunosuppressive tumor microenvironment and poor T-cell infiltration. Strategies to overcome these challenges involve combination approaches with immune checkpoint inhibitors, cytokine modulation (e.g., IL-6 receptor blockade with tocilizumab or TGF-β signaling inhibition using galunisertib), and tumor stroma remodeling (e.g.,hyaluronidase treatment with

PEGPH20 or inhibition of CAF activation via hedgehog pathway inhibitors like vismodegib) [24]. Clinical trials are ongoing to evaluate the efficacy and safety of adoptive cell therapies, including chimeric antigen receptor T-cell (CAR-T) therapy targeting mesothelin or other tumor-associated antigens, as well as tumor-infiltrating lymphocyte (TIL) therapies in PDAC patients. These studies aim to overcome the immunosuppressive tumor microenvironment and optimize therapeutic potential by enhancing T-cell persistence, specificity, and cytotoxicity of these adoptive cell therapies in PDAC patients, aiming to optimize their therapeutic potential [25].

2.1.2. Stroma-Targeting Agents

Given the dense fibrotic stroma of PDAC, targeting the ECM and reprogramming CAFs are critical strategies for improving drug delivery and immune response [26]. One key strategy to overcome fibrotic stroma barrier involves targeting matricellular proteins [27].

Recent research has highlighted several promising strategies for enhancing cancer treatment. One approach involves targeting hyaluronic acid, ***using PEGylated recombinant hyaluronidase (PEGPH20) which*** has been shown to enhance drug penetration and improve responses to chemotherapy [28]. Another effective strategy targets ***CAFs*** that express fibroblast ***activation protein (FAP).*** This can be achieved through small-molecule inhibitors (e.g., talabostat, PT-100, and PT-630) or monoclonal antibodies (e.g., sibrotuzumab, RO6874813, and FAP5-DM1) aiming to reduce fibrosis and immune suppression [29]. Additionally, recent studies have explored the use of vitamin D analogs (e.g., calcipotriol, paricalcitol, and doxercalciferol) to induce the reprogramming of CAFs from a tumor-promoting to a tumor-restraining

phenotype, thereby making tumors more susceptible to immunotherapy and chemotherapy [30].

Combination strategies integrating stroma-targeting agents with immune checkpoint inhibitors or metabolic inhibitors are currently under investigation to achieve synergistic antitumor effects. By disrupting stromal barriers and reprogramming fibroblasts, these therapies hold significant potential in improving PDAC treatment outcomes, making previously resistant tumors more responsive to conventional and emerging therapies [31].

ADAM (A Disintegrin and Metalloproteinase) family members are zinc-dependent transmembrane proteases. They play a role in the proteolytic cleavage of cell membrane-bound substrates and in regulating processes associated with the extracellular matrix. ADAM proteases are critical in embryonic development, tissue regeneration, and immune response regulation [32].

Specifically, ADAM8, ADAM9, ADAM10, ADAM12, and ADAM17 are overexpressed in most cancer types, leading to poor prognoses [33]. These proteases (especially ADAM10 and ADAM17) have crucial functions in regulating cell proliferation, invasion, angiogenesis, immune evasion, and treatment resistance mechanisms. ADAM10 weakens CD8+ T cell responses by releasing immune evasion molecules like PD-L1, while ADAM17 triggers EGFR signaling by playing a role in activating critical growth factors like TNF-α and TGF-α. They cause cancer progression by facilitating the shedding of various growth factors, receptors, and cell adhesion molecules from the cell surface. This shedding mechanism releases ligands that bind to other cells, triggering autocrine/paracrine signaling and promoting cell proliferation [34].

Furthermore, ADAM proteases facilitate invasion by degrading the extracellular matrix (ECM) and help tumors evade immune surveillance by shedding molecules from the surface of immune cells. These proteases can also play a role in activating angiogenic factors and receptors, such as vascular endothelial growth factor (VEGF), which are involved in angiogenesis. This creates a tumor microenvironment that supports blood vessel formation, aiding tumor nourishment and growth [35].

Despite ADAM proteases being promising targets in cancer treatment, significant challenges need to be overcome. These include potential side effects due to their essential roles in normal physiological processes, a lack of specific targeting, and their expression patterns in cancer cells [36]. However, in recent years, specific small molecule inhibitors, monoclonal antibodies, and antibody-drug conjugates (ADCs) targeting ADAM proteases have been developed. BK-1361, a peptide derivative that reduces metastasis and tumor volume in pancreatic cancer, has been shown to inhibit ADAM8 multimerization. IMGC936, an ADC developed against ADAM9, provides selective elimination of tumor cells. ADAM10 and ADAM17 inhibitors (GI254023X, INCB3619, INCB7839) and antibody-based agents like MEDI3622 show promising clinical potential [37,38].

A better understanding of the biological functions of ADAM proteases and the development of more specific inhibitors could lead to new and more effective strategies in cancer treatment. New research will further elucidate the roles of ADAM proteases in complex signaling networks, enabling the development of personalized and targeted cancer therapies.

2.1.3. Metabolic Reprogramming Strategies

Targeting metabolic vulnerabilities in PDAC, such as glutamine dependency, is another area of active research. PDAC

tumors exhibit a highly adaptive metabolism, allowing them to thrive in nutrient-deprived and hypoxic environments [39]. One of the most well-studied metabolic alterations in PDAC is its reliance on glutamine metabolism. Glutamine serves as a crucial substrate for nucleotide synthesis, antioxidant defense, and energy production in PDAC cells. Inhibitors of glutaminase, such as CB-839, have shown potential in preclinical studies by disrupting glutamine metabolism and sensitizing tumors to chemotherapy and immunotherapy [40].

Lactate accumulation promoting T-cell exhaustion, additionally contributes to immune evasion. Targeting lactate dehydrogenase (LDH) with inhibitors like FX11 aims to restore immune cell function and enhance antitumor immunity. In addition to LDH inhibition, blocking monocarboxylate transporters (MCTs), which mediate lactate export, using agents including AZD3965, has also shown promise in reducing lactate-induced immunosuppression. Moreover, targeting other metabolic enzymes involved in lactate production and utilization, such as pyruvate dehydrogenase kinase (PDK) with inhibitors like dichloroacetate (DCA), can shift tumor metabolism towards oxidative phosphorylation, thereby lowering lactate levels and improving immune cell infiltration and activity [41].

Recent research has focused on combining metabolic inhibitors with immune checkpoint blockade to improve immune infiltration and restore antitumor responses. Given PDAC's metabolic plasticity, multi-target approaches that simultaneously disrupt glycolysis, glutamine metabolism, and lipid metabolism may hold the key to more effective treatment strategies.

3. FUTURE PERSPECTIVES

Overcoming PDAC resistance requires integrating multiple strategies, including targeted therapies, metabolic inhibitors, immune modulation, and stromal remodeling **(Table 2)**. Advances in single-cell sequencing and spatial transcriptomics are revolutionizing our understanding of the tumor microenvironment in PDAC [42]. Spatial transcriptomics enables high-resolution mapping of gene expression patterns within tumor tissues, providing critical insights into the spatial distribution of immune cells, stromal components, and metabolic activity [43]. This technology allows researchers to identify tumor subpopulations, uncover heterogeneity in immune infiltration, and pinpoint therapy-resistant niches within the tumor architecture .

By integrating spatial transcriptomic data with single-cell RNA sequencing, a more comprehensive picture of tumor evolution, therapy response, and potential vulnerabilities can be developed. Advances in this field are paving the way for biomarker-driven therapeutic strategies that enhance patient stratification and treatment outcomes in PDAC [44].

Combination therapies that address tumor heterogeneity and immune evasion hold promise for improving treatment responses and extending patient survival. Ongoing clinical trials are evaluating novel combinations of immunotherapy, chemotherapy, and metabolic inhibitors [44]. Future research must continue refining these strategies to optimize patient selection and treatment efficacy.

As our understanding of PDAC biology deepens, new therapeutic targets and strategies will emerge, offering hope for more effective treatments and improved patient outcomes. Continued interdisciplinary research integrating immunotherapy, metabolism, and tumor microenvironment modulation will be

crucial in advancing precision medicine approaches for PDAC [45].

4. CONCLUSION

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies, driven by its aggressive tumor biology, a fibrotic and immune-excluding stroma, and profound immunosuppresion. Despite significant advancements in elucidating its complex pathophysiology, conventional therapies continue to deliver limited success, highlighting the urgent need for transformative treatment paradigms.

Recent breakthroughs in immunotherapy, metabolic reprogramming, and stroma-targeting approaches have broadened the therapeutic landscape. Combinatorial strategies – integrating immune checkpoint inhibitors with vaccines, adoptive cell therapies, and metabollic disruptors – show promise in reversing immune evasion and enhancing sensitivity.

Simultaneously, targeting cancer-associated fibroblasts and extracellularmatrix componenets is emerging as a key tactic to improve drug penetration and reprogram the TME. Metabolic interventions disrupting glutamine dependence, fatty acid oxidation, lactate driven immunosuppression are gaining traction, particularly when coupled with immunotherapies.

Looking ahead, high-resolution tools such as single-cell sequencing, spatial transcriptomics, and multi-omics technologies are expected to redefine our approach to patient stratification and biomarker–driven treatment. These technologies will accelerate the transition from generalized protocols to personalized, mechanism–based therapies.

In the coming decade, these emerging approaches are poised to move from experimental concepts to clinically viable,

personalized treatments that not only extend survival but also enhance patients' quality of life. Achieving this will require sustained interdisciplinary collaboration, innovative trial designs, and a continued commitment to translational research.

Table 1. New Therapeutic Approaches in PDAC

Category	**Therapeutic Approach**	**Examples / Mechanisms**
Combination Therapies	Chemo-Immunotherapy	Synergistic effects of chemotherapy and immunotherapy
	Multi-Target Strategies	Dual inhibition (e.g., KRAS + TME modulation)
Targeted Therapies	KRAS Inhibitors	Sotorasib, targeting KRAS mutations
	PARP Inhibitors	Olaparib, Rucaparib, Niraparib Inhibiting DNA repair pathways
	MET and HER2 Targeted Agents	Targeting growth factor receptors
Immunotherapies	Checkpoint Inhibitors	PD-1, CTLA-4 inhibitors (immune evasion blockade)
	CAR-T Cell Therapy	Genetically engineered T cells targeting tumor antigens
	Cancer Vaccines	Stimulating immune response against tumor antigens
Stromal Modulation	TME-Targeting Drugs	Hedgehog inhibitors, disrupting tumor-stroma interactions
	Fibrosis-Reducing Agents	Modulating extracellular matrix components
	Microbiota Modulation	Influencing immune response via gut microbiome
Drug Delivery Innovations	Nanoparticle-Based Drug Delivery	Enhancing drug bioavailability and targeting
	Localized Drug Release	Direct intratumoral injections for precision therapy

Table 2. New therapy approaches

Active Ingredient	Target	Mechanism	Characteristics of the patient group or tumor	Status
Targeted Therapies				
-Sotorasib [47]	KRAS G12C	Locks KRAS G12C in its GDP-bound (inactive) state, thereby inhibiting downstream oncogenic signaling.	KRAS G12C+	Phase 1-2
-Adagrasib [47]	KRAS G12C	Binds to the KRAS-GDP complex, halting aberrant cell growth signaling.	KRAS G12C+	Phase 1-2
-Olaparib [46]	PARP1/2	Inhibits DNA repair mechanisms, triggering apoptosis in cells with defective BRCA1/2.	BRCA1/2 mutations.	FDA approved
-Rucaparib/ Niraparib [46]	PARP	Disrupt DNA repair pathways, leading to accumulation of lethal DNA damage.	DNA repair defects/ BRCA mutations.	Clinical trials
-Dabrafenib + [45] Trametinib	BRAF V600E + MEK	Dabrafenib inhibits mutant BRAF and Trametinib blocks MEK,thereby preventing MAPK pathway activation.	BRAF V600E+	FDA approved
-Larotrectinib [48]	TRK (NTRK fusions)	Selectively inhibits TRKA, TRKB, and TRKC fusion proteins, blocking downstream oncogenic signaling.	NTRK fusion+	FDA approved
-Entrectinib [48]	TRK, ROS1, ALK	Inhibits TRK fusion proteins as well as ROS1 and ALK, thereby disrupting multiple oncogenic pathways.	NTRK fusion+ & ROS1+	FDA approved
-Repotrectinib [48]	TRK, ROS1, ALK	Next-generation inhibitor targeting TRK, ROS1, and ALK, effective against resistance mutations.	Advanced solid TRK fusion+ tumors/resistant to first-line inhibitors.	Phase 1/2

-Selpercatinib [48]	RET mutations	Selectively inhibits RET kinase, thereby preventing downstream oncogenic signaling.	RET+	FDA approved
Immunotherapy				
-Pembrolizumab/ Durvalumab [50]	PD-1 / PD-L1	Blocks PD-1 (Pembrolizumab) or PD-L1 (Durvalumab) signaling, restoring T-cell–mediated anti-tumor responses.	MSI-H/dMMR; high PD-L1 expression.	Pembrolizumb: FDA approved; Durvalumab: Clinical trials in PC
-Dostarlimab-gxly [50]	PD-1	Checkpoint inhibitor that targets PD-1, restoring T-cell activity.	MSI-H/dMMR	FDA approved
-Nivolumab/ Ipilimumab [49]	PD-1 + CTLA-4	Dual blockade enhances anti-tumor T-cell response by inhibiting both PD-1 and CTLA-4 pathways.	MSI-H/dMMR or tumors with high TMB.	Under investigation in PC
-CAR-T [56]	Mesothelin	Genetically engineered T cells target and destroy tumor cells overexpressing mesothelin.	Mesothelin overexpression	Phase 1
Stromal-targeting Therapies				
-PEGPH20 [51]	Hyaluronic Acid	Degrades hyaluronic acid in the TME, thereby reducing the stromal barrier and improving drug delivery.	High stromal barrier/ hyaluronic acid levels.	Phase 3 unsuccessful; new strategies under investigation
-FAK Inhibitors [27]	FAK	Modulate the tumor stroma to enhance T-cell infiltration into the tumor.	High FAK activity.	Phase 1-2
RNA-based Therapies				
-siRNA [53]	KRAS, MYC	Silences specific mRNAs to reduce oncogenic protein production.	KRAS or MYC overexpression.	Preclinical studies
-NT122 (mRNA Vaccine) [54]	Neoantigens	Induces an immune response against neoantigens derived	High neoantigen load.	Phase 1-2

		from individual tumor mutations.		
New Combination Therapies				
-FOLFIRINOX &Immunotherapy [27]	DNA Replication & Immune Response	Combines chemotherapy-induced tumor burden reduction with immunotherapy-driven immune activation.	General PC population undergoing combined treatment strategies.	Phase 3
-Galunisertib [58]	TGF-β	Inhibits TGF-β signaling, enhances immune response	Overactive TGF-β signaling.	Phase 2

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OXIDATIVE STRESS IN AGING AND DISEASE: MECHANISMS AND BIOMARKERS

Nur KALUÇ[1]

1. INTRODUCTION

The term oxidative stress refers to the imbalance between reactive oxygen species (ROS) and cellular antioxidant molecules, which disrupt cellular homeostasis. Since antioxidants typically control ROS levels, excessive ROS formation can be driven on by radiation, toxins, inflammation, or metabolic processes. As a result of elevated ROS levels, biomolecules such as DNA, proteins, and lipids became oxidatively damaged, which eventually compromised the integrity and functionality of the cells1. By selectively targeting nitrogenous bases and the sugar-phosphate backbone, elevated ROS levels can result in chromosomal abnormalities, DNA single and double-strand breaks, and mutations. These genetic alterations disrupt the control of essential biological processes, including DNA repair mechanisms, apoptosis, and the cell cycle (Juan, Pérez de la Lastra, Plou, and Pérez-Lebeña, 2021).

Oxidative stress is a major factor in the consistent loss of tissue and organ function associated with aging (Flatt, 2012). According to the oxidative stress theory of aging, cellular senescence—a process in which cells stop dividing in response

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to damage—is caused by the accumulation of ROS-induced damage to macromolecules(Liguori et al., 2018). Oxidative stress may be the primary cause of aging and age-related diseases by triggering chronic inflammation and interfering with crucial signaling pathways, which accelerates the progression of metabolic, neurological, and cardiovascular disorders. Moreover, chronic oxidative damage hinders cellular repair systems and antioxidant defense mechanisms, aggravating tissue deterioration and raising susceptibility to age-related illnesses(Bondy and Campbell, 2018).

2. THEORIES AND MECHANISMS OF OXIDATIVE STRESS IN AGING

The most significant cause of aging is expected to be oxidative stress, as it conflicts with numerous cellular functions and results in functional impairment. Elevated ROS levels are associated with aging through dysregulated autophagy, telomere shortening, mitochondrial DNA damage, and inadequate detoxification. To highlight their involvement in the aging process, this section will focus on the theories related to aging, oxidative stress-induced telomere instability, antioxidant system depletion, and impaired cellular waste removal.

2.1. The Free Radical Theory of Aging

The free radical theory of aging is one of the first theories in the aging concept, proposed by Denham Harman in 1954(6). This theory suggests that aging results from free radical reactions, which cause cumulative damage to cellular macromolecules, leading to functional decline and age-related diseases. This idea has been strengthened and expanded over the decades by numerous studies.

2.2. The Oxidative Stress Theory of Aging

The oxidative stress theory of aging, a more contemporary and expanded version of this concept, suggests that an imbalance between oxidants and antioxidants results in aging. This theory centers on oxidative damage, which is triggered by excessive ROS levels. As a result, oxidatively damaged macromolecules, such as proteins, lipids, and DNA, reduce cellular function and promote aging. Rather than contradicting earlier theories, the oxidative stress theory of aging strengthens the idea that aging is caused by damage from free radicals by emphasizing the role of antioxidant defenses in preventing oxidative damage. In addition to free radicals, this theory provides an expanded perspective on oxidative damage and its involvement in aging by considering the harmful effects of other reactive species, such as reactive oxygen and nitrogen species. Furthermore, the theory highlights the significance of mitochondrial malfunction and oxidative stress-induced signaling pathways in age-related degeneration, along with the decline of the antioxidant system(7).

2.3. The Mitochondrial Theory of Aging

The mitochondrial theory of aging suggests that mitochondrial dysfunction is a major contributor to aging(8). Since mitochondria are the center of oxidative phosphorylation, they continuously produce ROS as byproducts of this process. Unlike nuclear DNA, mtDNA lacks protective histones and has limited repair mechanisms; therefore, mtDNA is highly susceptible to oxidative damage. Unintended ROS damage mtDNA, and mutations may occur. This situation may impair electron transfer chain (ETC) function and ATP production. Thus, the vicious cycle of mitochondrial impairment and ROS production leads to

progressive cellular dysfunction, energy depletion, and increased oxidative damage, ultimately contributing to aging and age-related diseases.

The only polymerase for mtDNA replication and repair is DNA polymerase gamma (Pol-γ), which is also responsible for its integrity(9). Pol-γ is encoded by the POLG gene and possesses an intrinsic 3'→5' exonuclease activity. Thus, it corrects misincorporated nucleotides and ensures high fidelity during replication. Mutations in POLG, which may result from oxidative DNA damage, impair Pol-γ function and lead to increased mutations in mtDNA. Indeed, mice expressing mutant Pol-γ have been shown to accelerate mutation accumulation in mtDNA, which results in premature aging phenotypes apart from excessive ROS levels(9). This finding suggests that mtDNA integrity alone is sufficient to drive aging, independent of ROS overproduction, challenging the classical free radical theory of aging. The accumulation of mtDNA mutations leads to reduced ATP production and an increase in ROS production due to the defective respiratory chain complexes and also triggers apoptosis via mitochondrial permeability transition pore (mPTP) activation(10). This dysfunction contributes to age-related diseases, including neurodegenerative disorders, cardiovascular diseases, and metabolic decline. Therefore, maintaining Pol-γ fidelity and preventing mtDNA mutations are crucial for mitochondrial health and longevity, making mtDNA stability a key target for anti-aging interventions.

2.4. Impact of Telomeres in Aging

Telomeres are specialized nucleoprotein complexes that protect linear chromosomes from degradation and improper DNA repair responses. They are composed of repetitive TTAGGG sequences, associated with the shelterin

complex, and located at the ends of linear chromosomes(11). The primary function of telomeres is to maintain chromosomal integrity by preventing end-to-end chromosome fusions. With each cell division, telomeres naturally shorten due to the end-replication problem. Eventually, when the telomere reaches a critically short length, replicative senescence occurs. To cope with this problem, cells utilize the enzyme telomerase, which extends chromosomes by adding telomere sequences to the end of chromosomes. However, the enzyme is inactive in most of the somatic cells, contributing to progressive telomere shortening over generations(12).

Due to the high guanine content, telomere sequences are particularly vulnerable to oxidative base damage. ROS generated during regular cellular metabolism may cause oxidative damage to telomeric DNA, as well as to the shelterin complex. Thus, oxidative stress plays a crucial role in accelerating telomere damage and shortening and promotes aging(13). Repair capacity of telomeric DNA is limited; therefore, telomere sequences are prone to oxidative-stress induced DNA strand breaks. This limited repair capacity leads to accelerated telomere loss and premature cellular senescence, independent of cell division. Moreover, damaged telomeres function as signaling mechanisms to arrest the cell cycle in order to prevent DNA replication in a mutagenic environment, rather than to be repaired(14).

Oxidative stress also affects the protective mechanism of telomere structure by modifying of shelterin proteins. These structures both prevent telomere fusion and regulate DNA damage response in chromosome ends. TRF2 is a subunit of the shelterin complex, and when oxidative damage disrupts the TRF2 subunit, the shelterin complex mistakenly recognizes the telomeres as DNA double-strand breaks (DSBs). Following this recognition, DNA damage response (DDR)

pathways are activated and lead to cell cycle arrest to prevent the replication of damaged DNA, triggering cellular senescence or apoptosis(11).

When DSBs occur at telomeres and cannot be fixed, permanent DNA damage sites known as telomere-associated foci (TAF) form(12). TAF is known to increase with age in various tissues, and oxidative stress is the major contributor to these persistent DNA damage sites. A typical DSB region throughout the DNA can be repaired by DSB repair mechanisms, while these regions in telomere sequences cannot be repaired due to the protective role of the TRF2 subunit of the shelterin complex. By suppressing spontaneous or induced DSBs within the telomeres, TRF2 leads to the accumulation of TAF over generations(15). Formation of TAF is directly dependent on DNA damage but independent of telomere length and telomerase activity. Additionally, their incidence increases with age. The major contribution of TAF to cellular aging and senescence is to halt the cell cycle and prevent further DNA replication by prolonged DDR signal(16).

Oxidative stress-induced shortening of telomeres contributes to numerous age-related diseases, including cardiovascular diseases(17), neurodegeneration(18), and metabolic disorders(19). While telomere shortening in vascular smooth muscle cells and endothelial cells is known to be associated with atherosclerosis and cardiovascular diseases, telomere loss in neurons is capable of inducing cognitive decline, as well as Alzheimer's disease. Telomeres serve as a molecular clock for aging in cells, but oxidative stress accelerates their shortening and forces the cells into senescence and dysfunction. Numerous age-related diseases are driven by this process, thus rendering telomere preservation and antioxidant defenses potential targets for longevity therapies.

2.5. The contribution of Oxidative Stress to the Aging Process

As previously hypothesized, oxidative stress occurs when the balance between oxidant and antioxidant molecules is disrupted in favor of oxidants. Oxidant molecules, including hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), superoxide anions ($O_2•^-$), and peroxynitrite ($ONOO^-$), can originate from both endogenous and exogenous sources. While endogenous sources primarily include mitochondrial respiration, enzymatic reactions (e.g., NADPH oxidase, xanthine oxidase), and inflammatory responses, exogenous sources include numerous chemical, physical, and environmental agents. For example, ultraviolet (UV) radiation, air pollution, cigarette smoke, heavy metals, and industrial chemicals are known to generate ROS in cells. Emerging environmental pollutants, such as nanotoxicants, have gained attention due to their significant contribution to oxidative stress. Among them, nanoplastics, derived from the degradation of larger plastic particles, are potential oxidative stress inducers. By inducing ROS generation and dysregulating antioxidant enzyme genes, they are capable of disrupting cellular redox homeostasis and further exacerbating oxidative damage(20). Both exogenous stressors and endogenous mechanisms collectively contribute to an increased oxidative burden, leading to cellular dysfunction, aging, and disease progression.

Contrary to oxidant molecules, antioxidants function to neutralize these reactive molecules. They are classified as enzymatic and non-enzymatic antioxidants. ROS are converted into less harmful molecules by enzymatic antioxidants such as glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD). Non-enzymatic antioxidants, such as vitamins C and E, glutathione,

flavonoids, and polyphenols, directly scavenge free radicals. For cellular homeostasis to be maintained, these conflicting forces must be balanced inside cells. Disruption in antioxidant balance is implicated in both pathological conditions and the aging process.

SOD, catalase, and GPx are the most important cellular antioxidant enzymes, which are crucial for detoxifying ROS and maintaining cellular redox equilibrium. To prevent oxidative damage, SOD catalyzes the conversion of superoxide anions ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2). Then, to alleviate its adverse consequences, catalase actively breaks down $H2O_2$ into water (H2O) and oxygen (O_2). By reducing $H2O_2$ and lipid peroxides by oxidizing glutathione (GSH), a vital cellular antioxidant, glutathione peroxidase (GPx) provides an extra mechanism for defense. However, as individuals age, the expression and activity of their antioxidant enzymes gradually decrease, which causes an imbalance between the formation of ROS and its detoxification.

2.6. Cellular Clearance Machinery and Aging

Cellular clearance machinery is essential for preserving cellular homeostasis, especially in aging and oxidative stress conditions. Autophagy and the ubiquitin-proteasome system (UPS) are the two major processes that cells use to get rid of oxidatively damaged macromolecules to maintain homeostasis(Li, Li, and Wu, 2022; Pohl and Dikic, 2019; Yun et al., 2020).

Autophagy is a self-degenerating process that recovers damaged macromolecules by the autophagosome-lysosome system. By removing oxidized macromolecules, aggregated proteins, and damaged organelles, autophagy maintains cellular homeostasis(24).

An accumulation of defective cellular components, which may otherwise lead to cellular stress, inflammation, and the development of diseases, is prevented by this process, which is highly essential. Mitophagy, a crucial subtype of autophagy, selectively targets and degrades damaged mitochondria, minimizing oxidative stress and hindering excessive ROS production(25). Since mitochondria dysfunction is a major driver of aging and neurodegenerative diseases, mitophagy is crucial in preventing disorders like Parkinson's disease because the formation of dysfunctional mitochondria results in the loss of dopaminergic neurons(26). Furthermore, Alzheimer's disease is linked to defective autophagy because neuronal degeneration is exacerbated by a failure to eliminate accumulated tau and amyloid-β proteins(27). In addition to neurodegeneration, autophagy mechanism also plays a protective role in both metabolic and cardiovascular diseases by eliminating damaged macromolecules that contribute to disease development(28). As a major defensive mechanism against aging-related disorders, autophagy promotes the effective removal and recycling of cellular waste. Thereby, autophagy may serve as a viable target for therapeutic interventions aimed at prolonging cellular health and longevity.

Short-lived, misfolded, and oxidized proteins are selectively removed by the ubiquitin-proteasome system (UPS), a crucial non-lysosomal protein degradation process, by ubiquitination and subsequent 26S proteasome destruction(29). By preventing the formation of hazardous protein aggregates that may affect cellular survival and function, this system is essential for maintaining the quality of proteins. By contrast to autophagy, which mainly targets large protein clusters and damaged organelles, the UPS has been programmed to degrade soluble proteins rapidly while

ensuring proteostasis. The UPS's effectiveness is especially crucial in neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's, where abnormal protein accumulation (such as tau, α-synuclein, amyloid-β, and huntingtin) impairs neuronal function(30).

Since oxidative modifications to proteasomal subunits and impaired ubiquitination mechanisms reduce the UPS's capacity to remove damaged proteins, dysfunction of the UPS has also been linked to aging. The robust loss of cellular function, tissue degradation, and inflammation seen in age-related diseases is mainly influenced by this decline(31). Increasing UPS activity may be an effective treatment strategy for diseases linked to aging because of its crucial role in maintaining protein homeostasis and cellular longevity.

Despite their protective properties in aging, both autophagy and UPS are highly susceptible to oxidative stress and age-related malfunction. This leads to a vicious cycle that worsens cellular damage. The proteins ATG3 and ATG7, which are responsible for autophagosome formation during autophagy, are directly oxidized by chronic oxidative stress which hinders autophagy(32). In the same manner, oxidative changes to proteasomal subunits and compromised ubiquitination mechanisms cause proteasomal activity to decrease with age, resulting in ineffective protein degradation(33,34).

An achievable treatment approach for aging-related disorders may be to target autophagy and UPS activation, as both processes are crucial for minimizing damage caused by oxidative stress. It has been demonstrated that increasing autophagy via mTOR inhibition, AMPK activation, or caloric restriction prolongs cell survival and delays the development of disease(35). In a similar fashion, chaperone-based

treatments and proteasome activators attempt to increase the effectiveness of protein clearance while decreasing hazardous protein aggregation(36). Therefore, regulating autophagy and UPS activity may be a therapeutic strategy to reduce oxidative stress, promote cellular homeostasis, and prevent diseases related to aging.

3. OXIDATIVE STRESS AND AGE-RELATED DISEASES

3.1. Neurodegenerative Diseases

The pathophysiology of neurodegenerative illnesses, including Parkinson's disease (PD) and Alzheimer's disease (AD), is significantly influenced by oxidative stress. Oxidative stress promotes cellular damage in both diseases, which in turn leads to neuronal malfunction and death. Oxidative stress causes tau protein hyperphosphorylation and amyloid-β (Aβ) plaque formation in Alzheimer's disease, which damages synapses and impairs cognitive function(Gouras, Almeida, and Takahashi, 2005). Furthermore, oxidative stress can be rendered severe by mitochondrial malfunction and lipid peroxidation, which interfere with brain energy metabolism and exacerbate neurodegeneration. The brains of AD patients have been found to have elevated quantities of oxidized proteins, lipids, and DNA, underscoring the role that oxidative stress plays a major role in the progression of the diseases(Yu, Shan, and Ding, 2021).

Similarly, oxidative stress has a major role in the degeneration of dopaminergic neurons in the substantia nigra, which is the hallmark of Parkinson's disease(Chan, Gertler, and Surmeier, 2010). Excessive ROS generation from mitochondrial dysfunction, particularly complex I inhibition in the electron transport chain, oxidatively damages dopamine-

producing neurons(Dias, Junn, and Mouradian, 2013). Moreover, oxidative stress promotes α-synuclein to misfold and aggregate, forming Lewy bodies that are toxic to neurons. Oxidative damage in Parkinson's disease is further exacerbated by the decreased efficiency of antioxidant defense enzymes, such as glutathione and SOD. In order to prevent neurodegeneration and enhance patient outcomes, treatment approaches focusing on ROS suppression, mitochondrial protection, and antioxidant supplements are being investigated. Because oxidative stress plays a fundamental role in both disorders(41).

The hallmarks of neurodegenerative diseases—protein misfolding, aggregation, and neuronal cell death—are all significantly influenced by oxidative stress. By oxidizing amino acid residues, ROS alter the structure and functionality of proteins, resulting in the formation of misfolded and aggregated proteins that are resistant to destruction. ROS cause amyloid-β (Aβ) plaques to aggregate and tau proteins to become hyperphosphorylated in Alzheimer's disease (AD), impairing neuronal function and leading to synapse loss. Similar to this, oxidative stress causes α-synuclein to aggregate in Parkinson's disease (PD), resulting in toxic Lewy bodies that compromise neuronal survival and mitochondrial function. Cellular toxicity and apoptosis result from the accumulation of these oxidized proteins, which overwhelms proteasomal and autophagic clearance processes. Furthermore, lipid peroxidation products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) exacerbate protein aggregation and misfolding, exacerbating neuronal stress(42). Reducing oxidative stress is a crucial therapeutic target in neurodegenerative diseases because ROS-induced damage speeds up cell death, neuroinflammation, and disease progression since neurons have a limited capacity for

regeneration. ROS-induced protein aggregation and neuronal cell death.

3.2. Cardiovascular Diseases

One of the primary contributors to cardiovascular diseases (CVDs), including atherosclerosis, hypertension, and heart failure, is oxidative stress. Vascular endothelial dysfunction, chronic inflammation, and vascular remodeling are caused by excessive production of ROS in cardiac myocytes, smooth muscle cells, and vascular endothelial cells(43). ROS promote the oxidation of low-density lipoprotein (LDL) in atherosclerosis, triggering an immune response that causes plaque accumulation, foam cell development, and arterial constriction(44). In the same manner, oxidative stress causes hypertension by decreasing the bioavailability of nitric oxide (NO), which inhibits vasodilation and raises vascular resistance(45). This results in persistently elevated blood pressure. Mitochondrial dysfunction and ROS accumulation accelerate cardiac remodeling and contribute to the development of heart failure by causing cardiomyocyte death, fibrosis, and contractile dysfunction(46). For this reason, reducing oxidative stress is an important target in treatment, such as drug treatments, lifestyle changes, and antioxidant therapy, due to its pivotal role in CVD pathophysiology.

LDL oxidation is a major mechanism that links oxidative stress to endothelial dysfunction and vascular rigidity. When LDL particles are oxidatively modified, they become atherogenic and pro-inflammatory, attracting macrophages and leading to foam cell development. Additionally, oxidized LDL (ox-LDL) inhibits the action of endothelial nitric oxide synthase (eNOS), which lowers NO levels and inhibits vascular relaxation. Furthermore, ox-LDL

promotes the synthesis of matrix metalloproteinases (MMPs), which cause stiffness in the arteries by breaking down collagen and elastin in the artery wall(47). Vascular stiffness raises systolic pressure and cardiac workload, which in turn raises the risk of cardiovascular events and hypertension. Additionally, oxidative stress increases vascular damage by activating NADPH oxidases (NOX) and angiotensin II signaling(Nguyen Dinh Cat, Montezano, Burger, and Touyz, 2013). To delay the development of atherosclerosis, enhance endothelial function, and lower cardiovascular risk is to target LDL oxidation and oxidative stress-related vascular alterations.

3.3. Cancer

Oxidative stress is a key factor in DNA damage and genomic instability, which plays a crucial role in cancer development and progression. Overproduced ROS leads to chromosomal rearrangements, base damage, and breaks in DNA strands, which may result in alterations in proto-oncogenes and tumor suppressor genes(Klaunig, Kamendulis, and Hocevar, 2010). The transformation of proto-oncogenes into oncogenes, where mutations in genes including RAS, MYC, and EGFR result in uncontrolled cell proliferation and tumor development, is an important stage in ROS-induced carcinogenesis(Rezatabar et al., 2019). Furthermore, loss-of-function mutations in tumor suppressor genes, including TP53, RB1, and BRCA1, may result from oxidative stress, inhibiting appropriate DNA repair and apoptotic reactions(Oubaddou et al., 2023).

Two-Hit Hypothesis, proposed by Alfred Knudson suggests that both alleles of a tumor suppressor gene must be inactivated or altered for the gene to lose its function and contribute to development of cancer(Knudson, 1996). The first "hit" is typically an inherited or spontaneous

mutation in one allele, while the second "hit" results from somatic mutations, oxidative stress-induced DNA damage, or epigenetic changes, which result in total loss of gene function. This process is accelerated by oxidative stress, which hinders repair processes and encourages the formation of DNA damage. Because of its loss, the tumor suppressor gene is unable to regulate apoptosis, DNA repair, or cell cycle arrest, which causes uncontrolled division of cells and the development of cancer. ROS-induced mutations eventually cause clonal expansion of mutated cells, increasing the risk of malignant transformation and metastasis.

In cancer biology, depending on their concentration and the cellular environment, ROS have two functions. They may either stimulate or limit tumor growth. Excessive ROS can cause DNA damage, mutations, and genomic instability in healthy cells. By inactivating tumor suppressor genes such as TP53 and BRCA1 and activating proto-oncogenes, including RAS, MYC, and EGFR, ROS can contribute to oncogenic transformation. However, excessive ROS generation in cancer cells may exceed their antioxidant capacity, resulting in oxidative stress-induced death via pathways including BAX activation, p38 MAPK, and JNK(Pezzella, Tavassoli, and Kerr, 2019). In order to survive in an environment with high ROS, cancer cells have enhanced antioxidant defenses, such as overexpression of NRF2, GSH, SOD, and catalase.

Numerous anticancer medications, such as doxorubicin, cisplatin, paclitaxel, and ionizing radiation, function by raising ROS levels in cancer cells, which leads to lipid peroxidation, mitochondrial malfunction, and death through the activation of caspase and cytochrome c release(Raza et al., 2017). Therefore, depending on the anticancer drug's action, the use of antioxidant therapy

requires careful evaluation. Antioxidant supplements can reduce therapeutic efficiency by preventing ROS formation if a medication depends on ROS-mediated cytotoxicity, such as elesclomol (a mitochondrial ROS inducer). Antioxidants like N-acetylcysteine (NAC) or vitamin C may help minimize treatment adverse effects without affecting effectiveness if the therapy is not dependent on ROS increase(Fuchs-Tarlovsky, 2013).

Antioxidant medicines work optimally when administered to prevent cancer rather than while the disease is actively progressing. According to preclinical research, antioxidants including sulforaphane, curcumin, and resveratrol may help halt oxidative stress-induced DNA damage by lowering the production of 8-hydroxy-2'-deoxyguanosine (8-OHdG), an indication of oxidative DNA damage(Darenskaya, Kolesnikov, Semenova, and Kolesnikova, 2023). It could be achievable to reduce the risk of cancer by preventing ROS-induced mutations in the TP53, ATM, and DNA repair genes. This emphasizes the significance of antioxidant techniques prior to oncogenic transformation(Fuchs-Tarlovsky, 2013). The time and context of antioxidant therapy must thus be carefully considered in order to optimize benefits and prevent interference with treatment efficacy, even if ROS management is still a crucial technique in cancer therapy.

3.4. Diabetes and Metabolic Syndrome

Because of affecting insulin production and β-cell function in the pancreas, oxidative stress is a major factor in the development and progression of diabetes and metabolic syndrome. The inadequate expression of antioxidant enzymes, including GPx, catalase, and SOD, renders insulin-producing pancreatic β-cells highly prone to oxidative damage(Ježek, Jabůrek, and Plecitá-Hlavatá, 2019; Newsholme, Keane,

Carlessi, and Cruzat, 2019). Chronic hyperglycemia and dysregulation of lipid metabolism produce excessive ROS, which damage DNA, the endoplasmic reticulum (ER), and β-cell mitochondria, resulting in cellular malfunction and death. By altering important transcription factors like Pdx1 and MafA, ROS also disrupt the transcription of the insulin gene, reducing insulin synthesis and release. Furthermore, oxidative stress promotes inflammation and β-cell failure by activating the JNK and NF-κB signaling pathways. Antioxidant-based treatment approaches are needed because oxidative stress causes a gradual loss of β-cell function, which leads to β-cell exhaustion and the development of type 2 diabetes(Ježek et al., 2019).

Insulin resistance, the hallmark of type 2 diabetes and metabolic syndrome, is mostly caused by mitochondrial malfunction. For glucose absorption and insulin signaling, skeletal muscle, liver, and adipose tissues need functioning mitochondria. However, inflammation, lipid deposits, and persistent oxidative stress degrade mitochondrial activity, upsetting energy balance(Talchai, Xuan, Lin, Sussel, and Accili, 2012). ROS overproduction influences key insulin signaling molecules, including IRS-1, Akt, and GLUT4, leading to oxidative damage in mtDNA, inefficiency in the ETC, and reduced ATP production(Galizzi and Di Carlo, 2022). These factors eventually decrease insulin-stimulated glucose uptake. Additionally, in insulin-sensitive tissues, mitochondrial dysfunction promotes lipotoxicity and ectopic fat deposition, which exacerbates insulin resistance and causes persistent low-grade inflammation. Also, stress kinases (JNK, IKKβ) are activated by oxidative stress and block IRS-1 phosphorylation, hence compromising insulin signaling(Tanti and Jager, 2009). Because of their significant role in metabolic diseases, mitochondrial-targeted therapeutics such as MitoQ,

CoQ10, and α-lipoic acid are being investigated to increase mitochondrial function and restore insulin sensitivity in diabetes and metabolic syndrome. These medicines include metformin, resveratrol, and mitochondrial antioxidants.

3.5. Reproductive Health

A major contributing cause to male infertility, oxidative stress severely reduces sperm motility and DNA integrity, and its effects progressively worsen with age. Because of their insufficient antioxidant defenses and high plasma membrane concentration of polyunsaturated fatty acids (PUFA), spermatozoa are especially susceptible to ROS(62). Excessive ROS, which can be caused by metabolic problems, leukocyte infiltration, mitochondrial dysfunction, or environmental pollutants, causes lipid peroxidation, which compromises acrosome response, reduces motility, and destroys membrane integrity(Tombul, Akdağ, Thomas, and Kaluç, 2025). Furthermore, ROS directly harm sperm mtDNA, impairing ATP synthesis and flagellar movement—two processes essential to successful fertilization(64). Since elderly men's seminal antioxidant capacity declines and oxidative DNA damage accumulates, they are more likely to have offspring with genetic defects and decreased fertility rates(Colasante et al., 2019). This may explain why elevated oxidative stress is frequently associated with age-related declines in sperm quality.

Beyond motility, oxidative stress is a major factor in chromatin instability and sperm DNA fragmentation, both of which become worsened with age. The genomic integrity of sperm is compromised by ROS-induced DNA strand breakage, oxidative base alterations such as 8-OHdG, and improper chromatin remodeling. These factors increase the possibility of pregnancy loss, implantation failure, and developmental

abnormalities in embryos. Reproductive capacity is further diminished by age-related increases in oxidative stress, which significantly affect sperm telomere length maintenance(66). Furthermore, aged sperm's mitochondrial dysfunction increases the generation of ROS, resulting in a vicious cycle of oxidative damage. Antioxidant-based treatments, such as vitamin C, vitamin E, coenzyme Q10, and glutathione supplements, along with lifestyle changes and lowering exposure to environmental toxins, have been suggested as ways to lessen oxidative damage and enhance sperm quality in older men.

Because it affects oocyte quality, fertilization potential, and embryonic development, oxidative stress is also a significant factor in the aging of female reproductive systems. Because oocytes are synthesized throughout fetal development and stay stalled in prophase I of meiosis until ovulation, they are more vulnerable to accumulated oxidative damage over time compared to sperm, which are continually produced. This idea, which is commonly related to the oxidative aging theory, is because aging women's decreasing oocyte quality and reproductive success are caused in part by age-related mitochondrial dysfunction, ROS accumulation, and reduced antioxidant capacity(67). As women age, chromosomal missegregation and aneuploidy are caused by oxidative damage to spindle machinery proteins, lipid peroxidation, and mutations in mtDNA, which increase the risk of diseases including numerical chromosomal abnormalities such as Down syndrome and repeated abortions. Oxidative stress additionally lowers overall fertility by affecting oocyte maturation, early embryonic development, and zona pellucida hardening(67). Therapeutic approaches, including mitochondrial-targeted antioxidants (MitoQ, CoQ10), melatonin, and resveratrol, have been studied to prevent

oxidative damage and enhance oocyte quality in older women, as antioxidant defense systems weaken with age.

4. CONCLUSION AND RECOMMENDATIONS

Aging and the development of many age-related diseases, including metabolic syndromes, cardiovascular disease, neurodegenerative diseases, and reproductive aging, are significantly influenced by oxidative stress. Age-related functional decline is caused by accumulated cellular and molecular damage resulting from an imbalance between the generation of ROS and antioxidant defenses, in favor of ROS. Given the widespread prevalence, oxidative stress indicators such as MDA and 8-OHdG need to be identified and used clinically for early disease detection, prognosis, and therapy monitoring. In order to minimize oxidative damage and delay the progression of diseases, future research should focus on the development of specific antioxidant pharmaceuticals, mitochondrial protective agents, and changes in lifestyles. Furthermore, incorporating oxidative stress indicators into standard clinical practice would improve personalized medicine methods and optimize age-related disease treatment strategies.

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