Review

The 3D Collagen Network as a Determinant of Tumor Progression and Drug Delivery Efficiency in Breast Adenocarcinoma

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Highlight

- Collagen acts as a fundamental structural regulator of therapeutic resistance in breast cancer.
- ECM stiffness and remodeling foster invasive phenotypes, acting as a physical barrier to pharmacological penetration.
- This review integrates biomechanical signaling with immunomodulation within the tumor microenvironment.
- Collagen-rich stroma significantly impedes the diffusion and delivery of drugs in fibrotic tumors.
- Targeting collagen structure is a promising strategy for enhancing drug delivery and adjuvant treatment efficacy.

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Abstract

Background/Objectives: Breast cancer is a biologically complex malignancy whose high prevalence and therapeutic resistance represent a continuous challenge for global health. The Tumor Microenvironment (TME) is a crucial component in disease progression, and the Extracellular Matrix (ECM), particularly its 3D collagen architecture, is recognized for mediating interactions that influence invasion, metastasis, and pharmacological response. This review aims to critically synthesize recent evidence to elucidate the multifaceted role of collagen in the progression and modulation of therapeutic response in breast adenocarcinoma. **Methods:** A comprehensive literature review was conducted, analyzing studies addressing specific collagen subtypes, ECM stiffening (fibrosis), biomechanical signaling, and its impact on drug transport kinetics and immunomodulatory effects. **Results:** The results demonstrate that structural alterations of collagen not only orchestrate a pro-tumoral microenvironment, fostering aggressive phenotypes and immune evasion, but also create a physical barrier that compromises drug delivery efficiency and promotes metastatic dissemination. The synthesis of the data reinforces collagen as a potent prognostic biomarker and a promising therapeutic target for overcoming stroma-mediated resistance. **Conclusions:** Targeting the collagen-rich stroma and its 3D network is a critical frontier for therapeutic innovation. Developing adjuvant strategies to modulate the ECM has the potential to enhance clinical outcomes and optimize the distribution of antineoplastic agents, especially in patients with high degrees of tumor fibrosis.

Keywords: breast neoplasm; adenocarcinoma; extracellular matrix; collagen; tumor microenvironment

1. Introduction

Breast cancer remains the most prevalent malignant neoplasm among women and one of the leading causes of cancer-related mortality worldwide, representing a persistent challenge for public health systems [1–3]. Although significant advances have been achieved in early diagnosis and therapeutic modalities, including surgery, chemotherapy, radiotherapy, targeted therapies, and immunotherapy, tumor heterogeneity and resistance mechanisms continue to compromise the effectiveness and durability of treatments [4,5].

Within this challenging clinical scenario, the tumor microenvironment (TME) has emerged as a determining factor in neoplastic progression and in the response to oncological therapies [6,7]. This microenvironment consists of a complex and dynamic network of stromal cells, such as cancer-associated fibroblasts (CAFs), immune and endothelial cells, blood and lymphatic vessels, in addition to the extracellular matrix (ECM), which acts as both a structural and functional support for tumor cells [8,9]. In the field of pharmaceutical sciences and tissue engineering, understanding this architecture is crucial for developing bio-inspired models and advanced drug delivery systems.

The ECM, in particular, plays a fundamental role in the regulation of cellular signaling, acting as a reservoir of cytokines and growth factors, while transmitting biochemical and biomechanical stimuli that directly affect cellular proliferation, differentiation, migration, and survival [10]. Among its components, collagen stands out as the most abundant fibrous protein in mammalian tissues, functioning as a natural 3D scaffold that exerts multiple functions in the tumor biology of breast cancer [11].

Alterations in the deposition, organization, and crosslinking of collagen fibers contribute to increased tissue stiffness and promote ECM alignment, favoring collective cell migration and tumor invasion [12–14]. These processes are not merely structural: they create a pro-tumoral microenvironment that intensifies uncontrolled proliferation, facilitates metastatic dissemination, and compromises therapeutic success [15,16]. Furthermore, the dense architecture of collagen

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presents a significant challenge for the diffusion of therapeutic agents, acting as a steric barrier that limits pharmacological efficacy.

Furthermore, the density and organizational pattern of collagen may constitute a physical barrier to drug penetration, also influencing signaling pathways that promote cellular resistance [17–19]. A deep understanding of the interface among collagen, tumor cells, and immune cells is essential to optimize therapeutic strategies particularly in the design of next-generation delivery platforms capable of bypassing these fibrotic barriers [6].

Although previously published review studies have addressed general aspects of the extracellular matrix (ECM) in oncology or the role of collagen in specific tumor-related processes, a gap remains in the literature regarding a critical and integrated analysis of recent evidence correlating structural and functional modifications of collagen with tumor progression and therapeutic response in mammary adenocarcinoma. There is an emerging need to integrate findings on the different collagen subtypes involved with their direct impact on the pharmacokinetics and penetration of chemotherapeutic and immunotherapeutic agents [20,21]. Moreover, these biological insights provide the blueprint for 3D bioprinting technologies aimed at mimicking the tumoral stroma for drug screening.

Additionally, it is relevant to consider that cancer is a biologically dynamic and evolutionary condition, characterized by progressive genomic, epigenetic, and phenotypic changes that remodel the tumor microenvironment and extracellular matrix over time and under therapeutic pressure [22,23]. This plasticity clearly evidences that an exclusive focus on the tumor cell is insufficient to reverse the course of the disease. Variations in survival rates across different global regions, where access to early diagnosis and modern treatments remains disparate, underscore the urgent necessity of identifying universal prognostic factors and therapeutic targets that are intrinsic to tumor biology. In this context, focusing on collagen emerges as a critical area for therapeutic innovation [5].

Therefore, the present review aims to critically consolidate and analyze the most recent scientific evidence regarding the role of collagen as a modulator of tumor progression and therapeutic response in breast adenocarcinoma. By integrating data on collagen dynamics within the TME, this work seeks to identify prognostic and predictive biomarkers, as well as to validate potential therapeutic targets capable of overcoming resistance and optimizing the delivery and distribution of antineoplastic agents through the dense 3D tumor matrix.

2. Methods

2.1. Study Design and Research Question

This work constitutes a Comprehensive Narrative Review, meticulously designed to systematically and profoundly synthesize the existing evidence concerning the role of collagen in the progression and therapeutic response of breast adenocarcinoma. The central question guiding the data collection and analysis was: “What is the consolidated evidence regarding the role of collagen (dynamics, stiffness, alignment, or metabolism) as a prognostic factor, resistance mechanism, a barrier to drug delivery, and/or therapeutic target in Breast Adenocarcinoma?”.

2.2. Search Strategy and Data Sources

The electronic search was finalized in November 2025 and encompassed recognized bibliographic databases, including PubMed/MEDLINE, Scopus, and Web of Science (WoS), to ensure maximum scientific and multidisciplinary coverage (bridging molecular biology, oncology, pharmacology, and bioengineering). Additionally, Gray Literature (websites of governmental organizations such as INCA and NCI) was consulted for up-to-date epidemiological data and reports.

2.2.1. Search Terms and Boolean Operators

To optimize the search sensitivity and comprehensively cover all investigation angles, controlled vocabulary terms (MeSH/DeCS) and free-text terms were employed. These were grouped into three

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conceptual axes and combined using the Boolean operators AND and OR: Collagen/ECM: "Collagen" OR "Extracellular Matrix" OR "Tumor Stroma" OR "Fibrosis" OR "Matrix Stiffness" OR "TACS" OR "LOX" OR "Lysyl Oxidase";

Neoplasm: "Breast Cancer" OR "Breast Neoplasm" OR "Breast Adenocarcinoma" OR "Tumor Microenvironment" OR "TME"; Outcome/Target: "Therapeutic Resistance" OR "Drug Resistance" OR "Prognosis" OR "Metastasis" OR "Target" OR "Biomarker" OR "Drug Delivery" OR "Pharmacological Penetration".

2.2.2. Publication Period

The search period was strictly defined between January 2011 and November 2025. This temporal window was strategically selected to focus on the most recent literature (the past 15 years), which has seen the consolidation of knowledge regarding the biomechanical role of the matrix.

Classic or seminal articles crucial for establishing the fundamental concepts of tissue stiffness and collagen alignment, published prior to 2011, were included through a manual reference-based search (from the reference lists of the most recent articles) to provide adequate theoretical and historical context.

2.3. Eligibility and Study Selection Criteria

The eligibility criteria were rigorously applied in two distinct phases (title/abstract screening and full-text review) and are detailed as follows:

2.3.1. Inclusion Criteria

Study Type: Original studies (in vivo or in vitro), clinical trials, systematic reviews, and meta-analyses.

Topic/Intervention: Articles investigating, primarily or secondarily, the relationship between collagen (any subtype, synthesis, stiffness, alignment, TACS, metabolism, or enzymes like LOX) and breast adenocarcinoma (any histological/molecular subtype), and/or its correlation with therapeutic response (chemotherapy, radiotherapy, or immunotherapy) and studies focusing on the impact of collagen-rich matrices on the diffusion and delivery of antineoplastic agents.

Language: Articles published in the English language.

2.3.2. Exclusion Criteria

Publication Type: Editorials, letters to the editor, conference abstracts, and non-peer-reviewed preprints.

Topic Focus: Studies primarily focused on other ECM components (e.g., elastin, hyaluronic acid, glycosaminoglycans) without emphasis on collagen. Studies focused on other primary cancer sites (e.g., lung, prostate) without direct and explicit translational applicability to the mammary microenvironment.

Access: Full-text articles inaccessible after reasonable acquisition efforts.

3. Global Overview of Cancer

Cancer is one of the leading causes of mortality worldwide, characterized by the uncontrolled and autonomous growth of cells that have lost the ability to respond to regulatory mechanisms responsible for cellular homeostasis. This multifactorial disease is driven by genetic mutations that affect fundamental processes such as the cell cycle, apoptosis, and DNA repair, resulting in abnormal cell proliferation and the formation of tissue masses [24–26].

Genetic alterations allow defective cells to evade natural mechanisms of cellular destruction, such as apoptosis, continuing to multiply uncontrollably. As these cells accumulate additional mutations, they may invade adjacent tissues and, in more advanced stages, spread to other parts of

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the body through metastasis. These processes make cancer a complex and heterogeneous disease, with significant variations in terms of types, aggressiveness, and treatment response [27].

It is estimated that in 2022, approximately 20 million new cancer cases were diagnosed worldwide, resulting in about 10 million deaths. The most prevalent types include breast, lung, prostate, and colorectal cancer, and their incidence is strongly associated with factors such as population aging, increased exposure to environmental risk factors, and lifestyle changes [28].

In this context, breast cancer stands out as the most incident neoplasm among women in Brazil, excluding nonmelanoma skin tumors. For each year of the 2023–2025 period, an estimated 73,610 new cases are expected, corresponding to an adjusted incidence rate of 41.89 cases per 100,000 women, with the highest rates observed in the South and Southeast regions [29]. These global and national trends highlight the importance of effective prevention, early diagnosis, and treatment strategies, as well as the need for equitable approaches to reduce disparities between high- and low-income countries.

3.1. Epidemiological Data

Cancer is one of the greatest threats to global public health, and its prevalence continues to rise, with projections indicating that by 2030, the number of new cases will surpass 25 million [30]. One of the most prevalent types is breast cancer, which affects millions of women worldwide. In 2020, 2.6 million new cases of breast cancer were reported, with approximately 685,000 associated deaths [31]. Among the histological types, breast adenocarcinoma is the most common, with invasive ductal carcinoma accounting for approximately 85.1% of cases in the United States, while other adenocarcinoma subtypes, such as mucinous (2.3%) and tubular (0.9%), also contribute to the global incidence [32]. The high incidence of cancer is strongly associated with various factors, including population aging, lifestyle changes, and exposure to environmental risks.

Population aging is one of the main factors responsible for the increased prevalence of cancer. Incidence and mortality rates rise substantially with age, especially in individuals aged 65 years and older. This increase is related to the accumulation of cellular mutations over time, which can lead to cancer development, in addition to a decline in immune system efficiency, which becomes less capable of identifying and eliminating cancer cells as we age. Genetic factors also play a significant role, with hereditary variants such as BRCA1 and BRCA2 mutations associated with a heightened risk of cancers including breast, ovarian, and prostate cancer, although such cases are less frequent in the general population [33–35].

Another crucial factor contributing to cancer incidence is urbanization and lifestyle changes. Urbanization is associated with unbalanced diets and sedentary behavior, both of which raise the risk of several forms of cancer. Diets rich in processed foods, saturated fats, and refined sugars are known risk factors for colorectal, pancreatic, and gastric cancers. Moreover, physical inactivity, common in urban environments, is one of the major contributors to the increased incidence of cancers such as breast and colon cancer [36–38].

Increased alcohol consumption is also associated with the risk of cancers such as liver, breast, and esophageal cancer. These factors are exacerbated by polluted urban environments, which increase exposure to carcinogenic substances in the air, such as fine particulate matter and nitrogen dioxide—both recognized by the International Agency for Research on Cancer (IARC) as carcinogenic [39–42].

Exposure to environmental risk factors, such as smoking, is one of the leading causes of increased cancer prevalence. Tobacco use is linked to several types of cancer, including lung, oral cavity, esophagus, pancreas, and others. Smoking is the primary risk factor for lung cancer, especially small cell lung cancer, accounting for more than 95% of cases [43,44]. Additionally, UV radiation from sunlight or tanning devices is a major cause of skin cancer, particularly melanomas, which have increased significantly in recent years. Ionizing radiation, such as radon exposure, is also an important factor, particularly for lung cancer. These environmental factors are amplified in highly

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polluted regions or in areas with a high degree of tobacco exposure, such as tobacco-producing states, which present high cancer rates [45].

Furthermore, studies have shown that obesity and lack of physical activity are associated with increased risk of several types of cancer, including breast cancer (after menopause), colon cancer, and liver cancer. The relationship between obesity and cancer is widely documented, with body mass index (BMI) being an important indicator in this context. Obesity is particularly relevant to hormone-related cancers such as breast and endometrial cancer, since excess body fat may lead to elevated levels of hormones such as estradiol, which increases the risk of certain cancers. Regular physical activity has been shown to reduce the risk of colorectal cancer and may also decrease the risk of breast and endometrial cancer, particularly in postmenopausal women [46,47].

These risk factors are responsible for a substantial rise in cancer prevalence in many parts of the world, especially in high-income countries, where unhealthy lifestyle habits are more common. The implementation of effective prevention strategies, such as promoting healthy eating, regular physical exercise, reduced alcohol and tobacco consumption, as well as increased awareness regarding skin cancer, are essential in mitigating this increase. Such approaches may also help reduce disparities between different population groups, as evidenced by the difference in mortality rates between Black and White individuals in the United States, where mortality rates are significantly higher among Black individuals [30].

However, breast cancer survival rates vary considerably across different regions of the world. In countries with more developed healthcare infrastructure, survival is significantly higher due to early diagnosis, facilitated by screening methods such as mammography, and access to advanced treatments such as targeted therapies and immunotherapy [48]. In less developed regions, disparities in survival rates may be attributed to late diagnoses and lack of access to appropriate treatments, resulting in poorer patient outcomes [49,50].

The implementation of effective public health policies, such as awareness campaigns, screening programs, and free access to examinations, is essential for improving early diagnosis and treatment rates. The promotion of educational campaigns addressing self-care, healthy eating, physical activity, and reduction of tobacco and alcohol consumption may significantly contribute to cancer prevention. Furthermore, providing technological and educational resources, such as access to modern therapies and continuous training of healthcare professionals, is essential to ensure that the population has access to appropriate treatments and that professionals are equipped to perform early and effective diagnoses. Such investments may, on a global scale, positively impact the fight against cancer by decreasing incidence and mortality while improving quality of life for patients [51–54].

3.2. Risk Factors and Trends

The incidence of breast cancer is strongly associated with the level of human development. Regions undergoing rapid economic transformations present significant increases in cases. Economic development influences factors such as diet, urbanization, and access to preventive screening. In countries experiencing rapid economic changes, shifts in dietary patterns, such as the adoption of diets rich in saturated fats and processed foods, contribute to the increased risk of breast cancer. Urbanization is also associated with sedentary behavior and increased alcohol consumption, factors that exacerbate this risk [55–57].

Reproductive factors such as early menarche, late menopause, fewer children, and shorter breastfeeding duration are directly linked to a woman's lifetime hormonal exposure, one of the main mechanisms associated with breast cancer risk. Prolonged exposure to female hormones, such as estrogen, increases the risk of breast cancer due to the stimulation of breast cell growth. In situations where estrogen production is excessive or prolonged, estrogen promotes cell division in breast tissue and contributes to the accumulation of mutations, rendering cells abnormal and more prone to malignancy. Thus, women who experience early menarche or delayed menopause present greater lifetime exposure to estrogen, which increases susceptibility to cancer, particularly estrogen-receptor–positive breast cancer, which depends on estrogen for its growth [58–60].

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Non-reproductive factors such as obesity and high alcohol consumption play a significant role in breast cancer risk. Obesity, especially after menopause, is strongly associated with a substantial portion of cases. Excess body fat increases estrogen production in adipose tissue, as fat converts other hormones into estrogens, elevating their levels in the body. This rise in estrogen may stimulate the growth of abnormal breast cells, promoting the development of breast cancer [61–63]. Additionally, studies indicate that metabolic syndrome, often associated with obesity, may further increase breast cancer risk. This condition, characterized by a group of factors such as insulin resistance, hyperglycemia, dyslipidemia, and hypertension, creates a metabolic environment conducive to oncogenesis. Hyperinsulinemia, resulting from insulin resistance, is one of the principal mechanisms, as insulin and insulin-like growth factor 1 (IGF-1) activate signaling pathways (e.g., PI3K/Akt/mTOR) that promote proliferation, survival, and metastasis of breast tumor cells. Moreover, metabolic syndrome exacerbates chronic inflammation and oxidative stress, both of which contribute to DNA damage and tumor progression. Risk estimates for breast cancer in individuals with metabolic syndrome compared with those without this condition range from 1.13 to 6.73 times, depending on the population studied [64–66].

Regarding alcohol consumption, breast cancer risk also increases proportionally with consumption level. Women who ingest 1 to 2 alcoholic beverages per day have a 20% increase in breast cancer risk, whereas consumption exceeding 3 drinks per day may elevate this risk by up to 50%. The mechanisms by which alcohol influences breast carcinogenesis are multifactorial and include increased circulating estrogen levels, either through altered hormonal metabolism or inhibition of estrogen degradation. Additionally, ethanol is metabolized to acetaldehyde, a toxic compound capable of causing direct DNA damage, leading to mutations. Alcohol can also induce oxidative stress, chronic inflammation, and nutritional deficiencies (notably folate deficiency), factors that compromise DNA integrity and cellular regulation, contributing to the development and progression of breast cancer, particularly subtypes such as estrogen-receptor–positive tumors [67–71].

Another crucial factor contributing to increased cancer incidence is urbanization and lifestyle changes. Urbanization is associated with unbalanced diets and sedentary behavior, both of which increase the risk of several cancers. Diets rich in processed foods, saturated fats, and refined sugars are known risk factors for colorectal, pancreatic, and gastric cancers. Physical inactivity, common in urban environments, is one of the major contributors to increased incidence of cancers such as breast and colon cancer. Physiologically, sedentary behavior and unhealthy diets frequently result in obesity, a well-established risk factor for postmenopausal breast cancer. Excess adipose tissue promotes estrogen production through the aromatase enzyme (estrogen synthase), raising hormone levels that stimulate the growth of breast cells. Moreover, obesity and sedentary lifestyle are associated with low-grade chronic inflammation and insulin resistance, which can create a microenvironment favorable to tumor development and progression, impacting cellular signaling pathways relevant to the survival and proliferation of breast cancer cells [72–79].

With regard to genetic factors, approximately 5–10% of breast cancer cases are associated with hereditary genetic mutations, such as those occurring in the BRCA1 and BRCA2 genes (Breast Cancer 1 and Breast Cancer 2—genes that code for proteins involved in DNA damage repair). These mutations are associated with a significantly elevated risk of breast and other cancers, such as ovarian cancer; specifically, women with mutations in these genes may have up to an 80% lifetime risk of developing breast cancer [80]. However, genetic predisposition to breast cancer is not limited to BRCA1/2. Other genes also confer increased risk, though they are less frequent. These include TP53 (associated with Li-Fraumeni syndrome), PTEN (related to Cowden syndrome), ATM, CHEK2, PALB2 (which interacts with BRCA2 in DNA repair), and CDH1 (associated with invasive lobular breast cancer and hereditary diffuse gastric cancer). Mutations in these genes may elevate breast cancer risk and, in some cases, the risk of other cancer types [81–87].

In this context, genetic counseling plays a crucial role. It enables the identification of individuals and families at elevated risk of hereditary cancer through detailed evaluation of family history and,

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if indicated, genetic testing. Counseling not only clarifies risks and management options (such as intensified surveillance, chemoprevention, or risk-reducing surgeries) but also provides psychosocial support, assisting with informed decision-making and planning personalized strategies for prevention and screening. Understanding these genetic profiles is essential for precision medicine in oncology, enabling more effective and individualized approaches [88,89].

These combined risk factors help explain the rising trends in breast cancer incidence, particularly in regions undergoing rapid changes in their economies and lifestyles.

3.3. Challenges in Cancer Treatment

Despite significant advances in the understanding and management of cancer, the disease still represents one of the greatest challenges in modern medicine. The inherent complexity of tumor biology, the heterogeneity of the disease among patients and even within the same tumor, and the ability of cancer cells to develop mechanisms of resistance to therapies are persistent obstacles. Moreover, current treatments often impose substantial side effects, affecting patients' quality of life and limiting the intensity and duration of therapies. Overcoming these barriers is essential to improve clinical outcomes, achieve more durable cures, and prevent recurrence and metastasis [90,91].

3.3.1. Tumor Resilience and Genetic Heterogeneity

The treatment of breast cancer faces considerable challenges, with tumor resilience—often associated with genetic heterogeneity—being one of the major obstacles. This characteristic allows cancer cells to adapt and resist conventional therapies such as chemotherapy and radiotherapy, making disease control more difficult and increasing the likelihood of recurrence and metastasis.

Specific molecular mechanisms, such as activation of the PI3K/AKT/mTOR signaling pathway and mutations in the tumor suppressor TP53 gene, play a fundamental role in tumor resilience. The PI3K/AKT/mTOR pathway regulates essential processes for tumor survival, such as cell growth, metabolism, and stress response. Alterations in genes within this pathway, such as PIK3CA mutations, lead to uncontrolled activation, contributing to resistance to therapies and tumor progression [44]. On the other hand, mutations in the TP53 gene, which encodes the p53 protein, result in the loss of its tumor suppressor function, compromising critical mechanisms such as DNA repair, apoptosis, and cell cycle arrest. These mutations are associated with more aggressive breast tumors, greater genomic instability, and resistance to treatments [92].

Beyond the intrinsic genetic heterogeneity of tumor cells, resilience and resistance to therapy are significantly influenced by the tumor microenvironment. This complex environment, composed of stromal cells, immune cells, blood vessels, and the extracellular matrix, dynamically interacts with cancer cells, providing support, nutrients, and signals that promote tumor survival, proliferation, and dissemination, as well as modulating the response to therapies. The interaction between the tumor and its microenvironment can, for example, induce resistance signaling pathways and protect cancer cells from therapeutic agents [93–95].

These alterations favor recurrence and metastasis, hindering complete disease control. Data suggest that approximately 20–30% of metastatic breast cancer cases present intrinsic or acquired resistance to hormonal treatments [96]. This reinforces the need for a better understanding of the molecular pathways involved and the development of more effective therapeutic approaches.

With the advancement of precision medicine, personalized therapies have emerged as a promising alternative to overcome the limitations of tumor resilience. Strategies such as targeted inhibition of the PI3K/AKT/mTOR pathway, using drugs like alpelisib, have demonstrated efficacy in tumors with specific mutations in the PIK3CA gene. These treatments allow therapies to be directed at specific breast cancer subtypes, maximizing efficacy while minimizing adverse effects.

Recent studies indicate that in about 70% of triple-negative breast cancer cases, response to treatment is limited due to high genomic instability promoted by alterations in key genes such as BRCA1/2 [97]. However, advances in immunotherapy, such as the use of immune checkpoint

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inhibitors, have shown promising results in selected subgroups [98]. This demonstrates the importance of combining technological and therapeutic advances to address tumor complexity.

Thus, the need to invest in more effective, less invasive, and widely accessible therapeutic strategies is evident, along with strengthening public policies that promote early diagnosis and universal access to treatment. Continuous research not only increases the understanding of tumor resistance mechanisms but is also essential to address the growing global cancer burden and improve clinical outcomes for patients worldwide [99–101].

3.3.2. Side Effects of Conventional Therapies

Conventional therapies for cancer treatment, such as chemotherapy and radiotherapy, although effective in eliminating tumor cells, often cause significant damage to healthy cells, resulting in a variety of side effects [92,102]. These adverse effects include nausea and vomiting, which are frequently observed during chemotherapy and may lead to dehydration and malnutrition if not properly managed. Peripheral neuropathy is also common, characterized by numbness, tingling, or pain in the extremities, resulting from peripheral nerve damage induced by certain chemotherapeutic agents [103]. Additionally, chronic fatigue is widely reported by patients as a persistent feeling of tiredness that is not relieved by rest, significantly impacting their quality of life [104]. Psychological effects, such as anxiety and depression, are also prevalent, often arising from the stress associated with cancer diagnosis and treatment [105].

The incidence of these side effects varies according to the type of cancer, the therapeutic regimen used, and individual patient characteristics. Studies indicate that chemotherapy-induced peripheral neuropathy may affect up to 68% of patients within the first weeks of treatment [103], while fatigue is reported by approximately 80% of patients undergoing chemotherapy [104].

To mitigate these adverse effects, emerging therapies have been developed with the aim of increasing selectivity for tumor tissue while preserving healthy cells. Among these approaches, targeted therapies stand out, using agents that interfere with specific molecules involved in tumor growth and progression, thereby minimizing damage to normal tissues [106]. For breast cancer, notable examples of targeted therapies include CDK4/6 inhibitors (such as palbociclib, ribociclib, and abemaciclib), widely used in hormone receptor–positive (HR+) and HER2-negative breast cancer; anti-HER2 agents (such as trastuzumab and pertuzumab), essential for the treatment of HER2-positive breast cancer; and PARP inhibitors (such as olaparib), indicated for patients with BRCA mutations [107,108]. Immunotherapy also represents a significant advancement by enhancing the patient's immune system to more effectively recognize and eliminate cancer cells. In the context of breast cancer, immune checkpoint inhibitors such as pembrolizumab (an anti-PD-1) have demonstrated efficacy, especially in more aggressive subtypes such as triple-negative breast cancer [98,109,110]. Although immunotherapy presents a distinct side-effect profile, in many cases, it is less severe when compared with traditional therapies [111]. Furthermore, Intensity-Modulated Radiation Therapy (IMRT) is an advanced technique that allows precise shaping of the radiation dose to the tumor volume, reducing exposure of adjacent tissues and consequently decreasing side effects [112].

The implementation of these emerging therapies has demonstrated effectiveness in reducing the side effects associated with conventional treatments, improving patients' quality of life and facilitating treatment adherence [106,112]. However, careful evaluation of each case is essential for selecting the most appropriate therapeutic approach, considering the potential benefits and risks involved.

In this context of seeking increasingly selective treatments with lower toxicity, the growing understanding of the role of the tumor microenvironment—including components such as collagen—in disease progression and therapeutic response opens new perspectives. The modulation or targeting of extracellular matrix elements, such as collagen, may represent promising avenues for the development of more effective therapeutic approaches with reduced impact on healthy tissues [113–115].

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3.3.3. Difficulties in Preventing Recurrence and Metastasis

Among the most persistent and clinically relevant challenges in oncologic treatment are the difficulties in preventing recurrence and metastasis. Although primary therapies may control the initial tumor, the ability of cancer cells to disseminate to distant sites and persist in a dormant state, often resistant to treatment, represents the main cause of mortality in cancer patients. These complications are frequently associated with the presence of cancer stem cells, which possess a high capacity for adaptation and resistance to traditional therapies. Furthermore, complex biological and environmental factors, such as the tumor microenvironment, dysregulated angiogenesis, and chronic inflammation, play crucial roles in the metastatic process, creating conditions favorable for tumor cell growth and spread [116–118].

The tumor microenvironment consists of a dynamic interaction between tumor cells, stroma, extracellular matrix, and immune cells. This environment promotes tumor progression through processes such as dysregulated angiogenesis, which supplies nutrients and oxygen to malignant cells, and chronic inflammation, which stimulates the release of pro-tumoral factors. These elements make the tumor more resistant to conventional therapies, contributing to disease advancement [66].

Additionally, the evolution of the tumor microenvironment occurs from the early stages of carcinogenesis through metastatic formation. Immune cells, originally recruited to eliminate malignant cells, may be reprogrammed to favor tumor progression, promoting invasion and colonization of distant organs. This phenomenon highlights the complexity of the microenvironment and its direct influence on therapeutic success [119].

Another crucial factor in tumor resistance is the presence of cancer stem cells. These cells are capable of surviving adverse conditions, such as hypoxia and exposure to therapeutic agents, and also possess high plasticity, allowing them to adapt to treatment. This characteristic underscores the necessity of developing therapies that directly target cancer stem cells, as well as strategies capable of reversing the microenvironmental conditions that support their survival [66].

These findings reinforce the need for integrated therapeutic strategies that consider both the intrinsic characteristics of the tumor and the influence of the tumor microenvironment. Specific interventions, such as modulation of inflammation, inhibition of angiogenesis, and selective elimination of cancer stem cells, may represent significant advances in combating cancer recurrence and metastasis.

Epidemiological data indicate that in aggressive types such as triple-negative breast cancer, recurrence rates may exceed 30% within five years. These numbers highlight the importance of exploring innovative therapeutic advances, such as immunotherapy, which activates the patient's immune system against metastatic cells, and targeted therapies, which block specific molecular pathways involved in tumor progression [120]. Recent studies demonstrate that immunotherapy has produced significant increases in survival among patients with triple-negative breast cancer, especially when combined with other therapeutic modalities [121].

Another critical aspect is the implementation of strategies for early diagnosis. Tools such as liquid biopsy, which detects fragments of circulating tumor DNA in the blood, and tumor biomarkers, which identify biological alterations associated with cancer, have shown potential for detecting metastases at early stages. These approaches allow for faster and more effective interventions, contributing to improved clinical outcomes [122].

3.3.4. Importance of Research Studies

In this scenario, the importance of new studies and experimental approaches is undeniable. Research employing innovative experimental models, such as in vitro cell cultures and in vivo animal models, plays a crucial role in understanding the molecular mechanisms underlying tumor progression and therapeutic resistance. These models not only enable the preclinical validation of treatments but also provide insights for the development of more specific, effective, and less toxic therapies [123,124].

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Furthermore, advances such as organoids and 3D bioprinting technologies offer platforms that more faithfully reproduce the human tumor microenvironment, including its three-dimensional architecture and cellular interactions. These models allow for a more detailed understanding of tumor biology and accelerate the transition from laboratory discoveries to clinical practice. By more accurately replicating real microenvironmental conditions, they promote more applicable findings and facilitate the development of personalized and less toxic treatments [125,126].

Additionally, emerging therapies such as targeted therapy and immunotherapy have shown promising results in overcoming the limitations of conventional approaches. By directly targeting the molecular mechanisms of tumor cells, these strategies minimize damage to healthy cells and increase treatment effectiveness. However, access to these modern therapies remains uneven, resulting in significantly lower cancer survival rates in regions with limited healthcare infrastructure [127,128].

In this context, scientific research must focus not only on the development of innovative therapeutic strategies but also on the creation of experimental models that accurately reproduce real biological conditions. These efforts are essential for accelerating the translation of laboratory discoveries into clinical applications, ensuring that advances are widely distributed and that patients benefit equitably (129).

Among emerging therapies, CAR-T cell therapy (Chimeric Antigen Receptor T-cell therapy) stands out, consisting of the genetic modification of a patient's own T cells to more efficiently recognize and destroy tumor cells. This therapy has demonstrated promising results, particularly in hematological malignancies such as leukemias and lymphomas. However, it faces significant challenges related to high cost and limited accessibility [130,131].

The high costs are attributed to the personalized production process, which involves collecting, modifying, and reinfusing T cells using advanced technologies such as genetic engineering and cell manipulation, along with the need for high-technology infrastructure and specialized teams. Additionally, potentially severe adverse effects, such as cytokine release syndrome and neurotoxicity, require constant monitoring and often intensive care unit hospitalization. These demands increase the total cost of treatment, which can range from $300,000 to $500,000 per patient, depending on the cancer type and treatment center [132–134].

Although CAR-T therapy represents a major advance in oncology, the challenges related to acquired resistance and high costs must be addressed to expand global access to this therapy. Ongoing research and the development of new production strategies and technologies have the potential to overcome these barriers, making this innovative approach more accessible and effective for patients in different socioeconomic contexts [135,136]. In this sense, global and national efforts are being directed toward optimizing manufacturing processes (aiming to reduce costs and production time), implementing innovative reimbursement models by healthcare systems, and fostering public–private partnerships. Additionally, the expansion of clinical trials to new indications and the pursuit of allogeneic CAR-T therapies (derived from donors) represent promising avenues to increase availability and equity in access to this cutting-edge technology [137].

Thus, the need to invest in more effective, less invasive, and widely accessible therapeutic strategies becomes evident, along with strengthening public policies that promote early diagnosis and universal access to treatment. In this regard, the remarkable success of vaccination programs against Human Papillomavirus (HPV) serves as an inspiring model: by significantly reducing cervical cancer rates in countries with high vaccination coverage, these programs demonstrate the profound impact that well-planned public policies and preventive interventions can have on the oncology landscape. Although no vaccine is directly applicable to breast cancer, the HPV experience underscores the importance of strengthening primary and secondary prevention, early diagnosis, and equitable access to healthcare as fundamental pillars to combat incidence and improve outcomes for other types of cancer, including breast cancer [138].

Additionally, the incorporation of emerging technologies has expanded the possibilities for early diagnosis and cancer management. Tools such as liquid biopsies and circulating tumor DNA analysis are transforming diagnostic approaches by enabling the detection of specific mutations and

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monitoring disease progression in a less invasive manner. These advances, based on precise biomarkers, are driving significant changes in personalized treatment, increasing therapeutic effectiveness, and improving clinical outcomes [139–142].

Continuous research is essential to deepen the understanding of tumor resistance mechanisms, address the growing global burden of cancer, and ensure advances that benefit patients equitably across different socioeconomic contexts. Thus, the integration of public policies, technological innovation, and prevention is indispensable to reverse the rising trend of cancer cases and improve the quality of life of affected populations [143–145].

3.4. Types of Experimental Models in Oncology Research

Contemporary oncological research fundamentally depends on the use of a wide range of experimental models that mimic the biological complexity of cancer. These models are indispensable tools for uncovering the mechanisms underlying carcinogenesis, testing the efficacy and safety of new therapies, identifying biomarkers, and understanding the interaction between the tumor and its microenvironment. The choice of experimental model is crucial and depends on the research question, the stage of drug development, and the level of biological complexity to be replicated, ranging from simplified in vitro systems to more complex models that reproduce organismal physiology [146,147].

3.4.1. In Vitro: Cell Cultures and Organoids

In vitro models consist of cultivating tumor cells in controlled environments, allowing direct investigation of the cellular and molecular characteristics of tumors. These models have become indispensable tools in oncological research, particularly two-dimensional cell cultures and three-dimensional organoids [148].

Cell cultures are widely used due to their simplicity, accessibility, and low cost. They offer a practical system for studying fundamental aspects of cellular biology, such as proliferation, migration, tumor invasion, and responses to cytotoxic agents. Furthermore, they are widely applied in initial screenings of antitumor substances and in the study of molecular mechanisms involved in cancer progression. However, their main limitation is the inability to reproduce the complexity of the tumor microenvironment, such as interactions with the extracellular matrix and other cells present within the tumor [149].

Organoids, on the other hand, represent a significant advancement in in vitro models. They are three-dimensional structures grown in the laboratory that more faithfully mimic the architecture, cellular heterogeneity, and functions of human tissues. Due to their ability to reproduce cellular interactions and responses to the tumor microenvironment, organoids have been widely used in oncology, especially in preclinical studies of personalized therapies [150–152].

Patient-derived organoid models have demonstrated promising results in predicting therapeutic responses and in the development of new therapeutic approaches, such as immunotherapy [105].

Expanding the capabilities of organoids, microfluidic platforms known as organs-on-a-chip or tumor-on-a-chip represent the next frontier of in vitro models. These microfabricated devices integrate three-dimensional cell culture with continuous fluid flow and the application of mechanical forces, more accurately mimicking the in vivo physiological environment, including blood perfusion and the mechanical tension to which cells are subjected. These platforms allow not only the co-culture of different cell types in physiological ratios but also the recreation of gradients of nutrients, oxygen, and metabolites, as well as the simulation of tissue interfaces and immune responses. By replicating the dynamic complexity of the tumor microenvironment and the interaction between multiple organs, organs-on-a-chip provide a powerful tool for investigating metastatic progression, therapeutic response, and drug toxicity in a more translational manner than static models [153].

The combination of these models has enabled important advances in understanding cancer biology and validating new therapeutic strategies. Recent research highlights the need to integrate more physiologically relevant models, such as organoids, into preclinical studies, ensuring greater

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representativeness of real biological conditions and accelerating the transition from laboratory discoveries to clinical practice [154]. These advanced in vitro platforms are particularly valuable for investigating the complex role of the extracellular matrix (ECM), including collagen, in tumor progression, therapy resistance, and immune response, allowing controlled manipulation of ECM components for the development of therapies targeting the tumor microenvironment.

3.4.2. In Vivo: Immunosuppressed Mice, Genetically Modified Models, and Spontaneous Models

Animal models play a fundamental role in understanding cancer biology and evaluating antitumor therapies. They allow researchers to study carcinogenesis, which is the process whereby normal cells undergo genetic and phenotypic transformations, acquiring characteristics that render them malignant and capable of forming tumors. This process involves multiple stages, including cellular transformation, uncontrolled proliferation, tissue invasion, and metastasis, and is influenced by genetic and environmental factors. Tumor progression, metastatic mechanisms, and tumor responses to different treatments can also be evaluated in these models [155].

Furthermore, they are essential for the validation of experimental therapies prior to their application in humans, contributing significantly to the development of new therapeutic strategies. The use of animal models also provides the opportunity to study tumor–microenvironment interactions, which is crucial for understanding immune responses and treatment resistance [156].

Among the most commonly used models are immunosuppressed mice, genetically modified models, and spontaneous models, each with specific applications and distinct advantages [157].

Immunosuppressed mice are used to study human tumors implanted into animals, since immunodeficient mice do not reject human tumor cells. Strains such as “nude” mice and NOD/SCID mice are widely employed for the growth of human tumor xenografts, allowing assessment of antitumor therapies in an in vivo context. For studies of the extracellular matrix (ECM) and collagen, these models make it possible to investigate interactions between human breast cancer cells and the murine host microenvironment, and are useful for testing agents that modulate the ECM. However, a disadvantage is that the murine ECM may not fully recapitulate the complexity and characteristics of human collagen relevant to disease, and the absence of a functional host immune system limits the study of immune–ECM interactions [158–160]. Deletion of the PTEN gene in human mammary epithelial cells resulted in increased mammosphere formation (three-dimensional spheroids derived from mammary stem cells cultured under low-adhesion conditions, used as a functional model to assess self-renewal and tumorigenic potential) and activation of the Wnt/β-catenin pathway, indicating the crucial role of this pathway in maintaining breast cancer stem cells [114].

In genetically modified models (GMMs), mice are genetically engineered to develop specific tumors, enabling the study of cancer biology and therapeutic testing in an environment closer to human reality. Techniques such as CRISPR/Cas9 have been used to introduce specific mutations in cancer-related genes, creating models that precisely replicate the molecular characteristics of human tumors. For the study of the ECM and collagen in breast cancer, GMMs are particularly advantageous because they allow direct manipulation of genes encoding collagens or enzymes regulating collagen synthesis and degradation, as well as signaling pathways affecting matrix remodeling. This enables investigation of the impact of specific collagen alterations on tumorigenesis and metastasis in a physiologically relevant manner, although high costs and long development times may be drawbacks. A mouse model was developed for the study of pancreatic ductal adenocarcinoma, using CRISPR/Cas9 technology to introduce mutations in the Kras and Lkb1 genes, enabling investigation of the molecular mechanisms involved in tumor progression [161,162].

Spontaneous models involve mice that develop cancer naturally, more closely simulating tumor processes in humans. Some mouse strains, such as BALB/c, have a natural predisposition to develop certain types of tumors, allowing the study of carcinogenesis without the need for genetic manipulation or chemical induction. These models are valuable for analyzing the evolution of the tumor microenvironment and collagen over time in an immunocompetent context, offering a holistic view of tumor–host interactions in established disease. However, heterogeneity and unpredictability

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of tumor development may hinder the investigation of specific ECM and collagen mechanisms in isolation, compared to genetically modified models [163,164]. BALB/c mice have been used to investigate immunological mechanisms involved in breast cancer progression, providing a deeper understanding of the interaction between the immune system and the tumor [156,165].

Selecting the appropriate model is crucial and must take into account the specific objectives of each study, ensuring the relevance and translational value of findings to clinical practice.

3.4.3. Ehrlich Adenocarcinoma Model (EAC)

Ehrlich adenocarcinoma (EAC), first described by Paul Ehrlich in 1905, is derived from a spontaneous mammary carcinoma in female mice and has emerged as a widely used model in preclinical research. Since its conception, EAC has played a seminal role in experimental oncology, providing one of the first reproducible in vivo platforms for studying tumor biology and screening anticancer agents, contributing significantly to the initial understanding of disease progression and therapeutic response [166–168].

Its popularity stems from its high tumor growth rate and its ability to be transplanted into different mouse strains, facilitating its application in a wide range of experiments, particularly in the evaluation of anticancer treatments. The most commonly used mouse strains include BALB/c and Swiss, due to their availability and immunological characteristics that allow consistent responses to tumor transplantation. The EAC model has been employed not only in chemotherapy studies but also in investigations involving gene therapy and stem cell therapies. Additionally, recent research has explored the use of alternative therapies, such as treatment with medicinal plant extracts, aiming to evaluate their potential antitumor effects and reduce side effects associated with conventional therapies [169,170].

EAC presents two distinct forms: solid and ascitic. The solid form of Ehrlich adenocarcinoma (SET) is induced by subcutaneous inoculation and is characterized by rapid cellular proliferation, marked pleomorphism, and a high nuclear-to-cytoplasmic ratio, with nuclei containing loose chromatin and prominent nucleoli. These cellular features, which resemble those observed in human breast cancer, make SET a potentially important tool for studies related to this specific malignancy [171,172].

The ascitic form (EAC), on the other hand, generated by intraperitoneal inoculation, results in the formation of ascitic fluid within the peritoneal cavity, conferring high aggressiveness and resistance to cellular apoptosis. This form of the tumor is often used to test the effectiveness of novel antitumor therapies due to its aggressive nature and ability to simulate tumor responses in a more systemic environment [171,173,174].

The relevance of the EAC model lies in its ability to mimic fundamental biological processes, such as angiogenesis and immune responses associated with the tumor, critical characteristics that make it an appropriate model for investigations of tumor progression, therapy resistance, and evaluation of new treatments. Additionally, the moderate immunogenicity of the EAC model facilitates studies on interactions between tumor cells and the immune system, an expanding area of interest in cancer research [175,176].

Despite its advantages, EAC has limitations. Notably, it does not exhibit an invasive phenotype nor does it form metastases, which restricts its application in studies of tumor dissemination. This feature is particularly relevant for research on the extracellular matrix (ECM) and collagen, as it limits the ability to investigate how collagen remodeling and its interactions with tumor cells directly contribute to invasion and metastasis—critical aspects of adenocarcinoma progression in the clinical context. Nevertheless, both in its solid and ascitic forms, Ehrlich adenocarcinoma remains an essential model for translational research, contributing significantly to advancing understanding of the disease and developing new therapies, particularly in the field of breast cancer [168].

Data obtained from the EAC model have been widely translated into clinical applications. Findings related to the effectiveness of new chemotherapeutics, for example, have served as the basis for the development of promising medications used in human treatment, including combinations of

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cytotoxic agents that have improved response rates in various types of cancer. In addition, studies involving EAC have helped identify molecular biomarkers that may be used in diagnosis and monitoring therapeutic response in patients [177–179].

In the field of alternative therapies, the model has enabled preclinical evaluation of natural compounds, such as medicinal plant extracts, which exhibit antitumor potential and low toxicity, opening pathways for complementary therapies in humans. These studies also assist in understanding the mechanisms of action of new therapeutic agents and in reducing side effects associated with conventional treatments, promoting significant advances in clinical oncology [180,181].

Thus, despite its limitations, the EAC model continues to play a critical role in translating laboratory discoveries into clinical practice, strengthening the development of more effective and accessible therapeutic approaches.

3.4. Extracellular Matrix and Tumor Progression

3.4.1. Structure and Function of the Extracellular Matrix (ECM)

Human tissues are composed of various cells, such as fibroblasts, immune cells, endothelial cells, and epithelial cells, organized within a complex and dynamic three-dimensional network: the Extracellular Matrix (ECM). This macromolecular structure is crucial for tissue organization, remodeling, and the regulation of cellular processes such as adhesion, signaling, and mechanical properties. Composed of diverse macromolecules, including proteoglycans (PGs), glycosaminoglycans (GAGs), collagens, elastin, laminins, and fibronectin, the ECM interacts with cellular receptors such as integrins and CD44, directly influencing proliferation, migration, and tissue remodeling. Alterations in its composition or organization, particularly in pathological contexts such as tumors, can impact cellular invasion and metastatic dissemination [182]

Collagen

Within the complex network of the Extracellular Matrix (ECM), collagen is the most abundant protein and plays a central architectural and functional role, particularly in maintaining tissue integrity and modulating cellular behavior. Representing more than 30% of total protein content, collagens display a characteristic triple-helix structure, which provides mechanical support, elasticity, and stability to tissues. The collagen family consists of 28 types, with specific functions and distributions across different tissues. Types I, II, and III are the most prevalent, accounting for 80–90% of total body collagen. Types I and III are widely distributed and frequently colocalized, as are types VIII and VI. Collagen IX is predominant in connective tissues and is often associated with collagen type II, which is the principal collagen in cartilage. Other types, such as collagens XI, XXIV, XXVII, XII, XIV, and XX, are mainly found in specialized connective tissues such as tendons and cartilage, whereas types XIII and XVII are primarily present in epithelial tissues [183].

Certain collagens play specific roles in the basement membrane (BM), such as types IV, VII, XV, XVII, and XIX, contributing to the structural and functional integrity of these microenvironments. Collagen XXVIII, in turn, has a more restricted distribution, being predominant in the BM of glial cells in the peripheral nervous system. Additionally, collagens exhibit diversity in the supramolecular structures they form, such as fibrils (collagens I, II, and III), anchoring fibrils (collagen VII), beaded filaments (collagen VI), and networks (collagens IV, VIII, and X). By contrast, some collagens, such as members of the fibril-associated collagens with interrupted triple helices (FACITs), do not independently form supramolecular structures but instead associate with existing collagen fibrils, such as those of collagen I and II. This broad functional and structural diversity reflects the complexity of the role of collagens in maintaining tissue organization and functionality [184,185].

Collagen structure consists of a triple helix formed by three polypeptide chains, known as alpha chains. These chains may be organized into homotrimeric configurations (composed of the same chain type) or heterotrimeric configurations (composed of different chain types), depending on the

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collagen type. After the formation of the triple helix, the fibrillation process occurs, during which collagen fibrils bind laterally, forming fibers that may be arranged in an ordered or random manner, depending on the functional requirements of the tissue [186,187]. The primary composition of collagen is based on a repetitive tripeptide sequence, following the Gly-X-Y formula, where X is often proline (Pro) and Y is hydroxyproline (Hyp). This structure confers stability to the triple helix and mechanical resistance to collagen. Alterations in collagen structural configurations are associated with various pathological processes, including carcinogenesis, underscoring its crucial role in homeostasis and dysfunction [186,188].

In the tumor context, collagen does not merely act as a structural scaffold; it actively participates in breast cancer progression by influencing tumor cell proliferation, migration, invasion, and metastasis. Collagen biosynthesis and remodeling are dynamic processes, regulated by cancer-associated fibroblasts (CAFs) and matrix metalloproteinases (MMPs), respectively. In the tumor microenvironment of breast cancer, increased collagen deposition and altered collagen organization (such as fiber alignment) are commonly observed—phenomena known as fibrosis or desmoplasia. This dense and aligned matrix creates a mechanical environment favorable to tumor invasion, acting as "tracks" for cellular migration and protecting tumor cells from the immune system and therapies [189–191].

Recent studies have highlighted that ECM stiffness—determined largely by the amount and organization of collagen—is a critical factor that signals tumor cells toward a more aggressive phenotype. Such stiffness can activate cell signaling pathways that promote proliferation, epithelial–mesenchymal transition (EMT), and resistance to chemotherapeutics. Furthermore, the orientation of collagen fibers at the tumor periphery may serve as a prognostic biomarker, indicating a higher risk of metastasis. A detailed understanding of these interactions between breast tumor cells and ECM collagen is essential for identifying new therapeutic targets aimed at matrix remodeling and, consequently, disease progression [192–194].

Elastin

In addition to collagen, elastin complements the extracellular matrix by playing an essential role in maintaining the elasticity and extensibility of organs subjected to continuous cycles of deformation and structural recovery. Present in high concentrations in structures such as arteries, lungs, and skin, elastin is indispensable for the functional integrity of tissues that require high elasticity. These elastic fibers are composed of two distinct morphological components: a network of longitudinally aligned microfibrils, predominantly formed by fibrillin, and a dense central core composed of cross-linked elastin, which accounts for more than 90% of the fiber content [183,195].

Elastin is synthesized primarily during development and childhood, undergoing gradual degradation throughout adulthood and aging. This process involves the proteolytic action of elastases, which generate elastin-derived peptides with important functions in regulating cellular signal transduction. These peptides play a crucial role in maintaining arterial physiology and preventing cutaneous photoaging, highlighting their importance in regulating tissue functional integrity throughout life [183].

Proteoglycans (PGs) and Glycosaminoglycans (GAGs)

Proteoglycans (PGs) are macromolecules composed of a protein core linked to negatively charged glycosaminoglycans (GAGs), such as heparan sulfate (HS), heparin (Hep), chondroitin sulfate (CS), keratan sulfate (KS), and dermatan sulfate (DS). These molecules play fundamental structural and biological roles, such as providing mechanical resistance to compression, tissue hydration, and retention of growth factors within the ECM [182,183,196].

PGs may be classified into four major groups based on their location and structural homology: extracellularly secreted, pericellular, cell surface, and intracellular PGs. Extracellular PGs include those that bind to hyaluronan (HA) and lectin, the hyalectans, as well as the small leucine-rich proteoglycans (SLRPs), which play crucial roles in tissue homeostasis and development, regulating

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collagen fibrillogenesis and immobilizing growth factors within the ECM. Among the hyalectans, versican stands out by binding to HA and also regulating various signaling pathways and biological functions [183,197].

Pericellular PGs, such as perlecan and agrin, interact with multiple cellular receptors and play important roles in modulating the cardiovascular and musculoskeletal systems. Proteolytic degradation of perlecan, mediated by chymase, matrix metalloproteinases (MMPs), and cathepsins, generates bioactive fragments such as endorepellin, which has antiangiogenic activity. Further cleavage of endorepellin releases a laminin G–like domain that interacts with the $\alpha 2\beta 1$ integrin [183]. Collagen XVIII and collagen XV belong to the pericellular PG group and are members of the multiplexin gene family; therefore, they possess structural characteristics of both PGs and collagens and are substituted by HS and CS, respectively. According to findings in mice with deletion of the Col18a1 gene, collagen XVIII is indicated to be a negative regulator of angiogenesis and an anti-atherosclerotic factor. Collagen XV, in turn, acts as a structural constituent that stabilizes microvessels and skeletal muscle cells [183,198].

Cell surface PGs include syndecans and glypicans, which act as coreceptors in signaling processes, facilitating interactions between specific ligands and their receptors. Finally, intracellular PGs such as serglycin (SRGN) play diverse roles, including the storage and bioavailability of bioactive molecules, and are involved in mast cell apoptosis induction and immune regulation. These different types of PGs are essential for maintaining tissue integrity and play critical roles in regulating complex biological processes such as cellular signaling, homeostasis, and ECM remodeling [183].

Hyaluronate

Hyaluronate (HA), a glycosaminoglycan (GAG), plays a crucial role in water retention within tissues, contributing to structural integrity. It is essential for biological processes such as embryogenesis, repair, regeneration, and homeostasis. Its function is highly dependent on its size, concentration, and interaction with cellular receptors and other components of the ECM [183,185].

HA regulates cellular signaling in a context-dependent manner. It interacts with several receptors, including CD44, HARE, LYVE-1, RHAMM (CD168), and layilin, which can bind to other ECM molecules, activating signaling pathways related to cellular functions. For example, the interaction of HA with CD44 can induce receptor clustering, as observed with Toll-like receptor 4 (TLR4). This interaction is fundamental for muscle development, modulating the migration and growth of myogenic progenitors [183,185].

In inflammation and tumor progression, HA appears in both high- and low-molecular-weight forms, often in large quantities. In addition to its involvement in physiological processes such as wound healing, tissue repair and regeneration, and embryonic development, HA also plays roles in cell migration, proliferation, adhesion, differentiation, and several signal transduction pathways. Medium-sized HA chains are associated with ovulation, wound healing, and embryogenesis; chains with 15 to 50 disaccharide units are inflammatory, immunostimulatory, and angiogenic; smaller HA fragments induce heat shock proteins and are antiapoptotic. Conversely, high–molecular-weight HA polymers exhibit immunosuppressive, antiangiogenic, and anti-inflammatory properties [183,185,199].

Organizational and Structural Role in Tissue

The ECM is essential for cellular communication, directly influencing cellular behavior in both homeostatic and pathological conditions. Its structure consists of a dynamic three-dimensional network of macromolecules, including fibrillar proteins such as collagen and elastin, and hydrophilic components such as GAGs and PGs. These macromolecules are continuously adapted in response to mechanical and biochemical stimuli, forming the most abundant and biologically relevant native biomaterial in the human body [183,200,201].

The ECM can be classified into two categories: the interstitial matrix, which organizes the spaces between cells and regulates intercellular and tissue interactions, and the pericellular matrix, which

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provides direct structural support to cells, with the basement membrane (BM) being the primary example of this category. This structure plays a key role in tissue maintenance and morphogenesis, as well as in governing cellular behavior, including signaling and migration, through direct interactions with cells mediated by junctions formed by integrins and PGs such as syndecans. The BM is composed primarily of laminins and collagen IV, along with other components such as nidogens, HS, perlecan, and agrin, which establish an adhesive microenvironment for cells. These elements bind to the cytoskeleton, maintaining tissue integrity and contributing to biomechanical signaling [202–204].

The interstitial matrix, in turn, is rich in fibrillar collagens, fibronectin, PGs, and matricellular proteins (non-structural proteins dynamically expressed within the ECM). Collagen I fibers undergo crosslinking and form networks with themselves or with other proteins such as fibronectin and elastin, with the assistance of the enzyme lysyl oxidase (LOX). Thus, the interstitial matrix plays an essential role in organizing the space between cells and regulating interactions [202,205].

Accordingly, the concentration, proportion, and distribution of the various molecules that compose the extracellular matrix (ECM), together with post-translational modifications such as crosslinking and glycosylation, directly influence the biomechanical and biophysical properties specific to each tissue [183].

Cartilage is a specific type of connective tissue and, depending on its composition, may be classified as hyaline, fibrous, or elastic. Hyaline cartilage has a fine and translucent ECM, found in the larynx, nose, trachea, and articular cartilage, where the ECM represents 90% of the tissue. The main constituents of hyaline matrix are collagen II and the sulfated PG aggrecan, as well as SLRPs such as biglycan, decorin, and fibromodulin. Decorin participates in the alignment of collagen II fibrils and is therefore essential for the interaction between aggrecan and collagen II, directly contributing to mechanical performance and tissue integrity. Aggrecan has a high negative charge due to its GAG chains and has the ability to attract cations such as sodium and water into the tissue, providing compressive resistance and allowing restoration of shape after loading. In addition, sparsely distributed chondrocytes, comprising approximately 5–10% of tissue volume, serve as the cells responsible for maintaining ECM homeostasis in cartilage and preserving ECM composition [183,206].

Bone is also a type of connective tissue composed of ECM together with inorganic compounds and organic macromolecules, with a focus on hydroxyapatite and oligoelements. In bone, collagen types I, III, and V are the most abundant within the ECM and contribute to bone biomechanical properties. Ninety percent of bone collagen is type I, which forms fibrils that interact with other proteins to form highly organized fiber bundles. Collagens III and V coordinate type I collagen formation, and the interactions between these collagen proteins contribute to bone tissue mechanical properties and rectification strength, which helps determine bone strength since collagen deficiency leads to ECM alterations and increased fracture risk [183,207].

For maintaining bone homeostasis through collagen, certain PGs such as decorin and biglycan are important, also assisting in water retention for bone toughness. The ECM contains growth factors that act on stem cells and stimulate their differentiation into osteoblasts, inducing bone formation. Osteonectin is the primary bone ECM glycoprotein and is most highly expressed during osteoblast differentiation; by binding to hyaluronic acid and collagen crystals, it participates in the release of calcium regulators and thus influences collagen mineralization.

Cartilage and bone are ECM-rich tissues adapted to support load; however, they differ in appearance and vascularization, since cartilage is avascular whereas bone tissue is vascularized and mineralized [183].

3.4.2. Biological Functions of the ECM

Regulation of Cell Adhesion, Intercellular Communication, and Tissue Homeostasis

The Extracellular Matrix (ECM) comprises a dynamic and intricate network of biomolecules. Its extracellular biophysical properties have significant implications for a wide range of cellular

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functions and behaviors. The proper functioning of tissues and organs depends on the integrity of the ECM, which provides vital support to cells and generates essential biomechanical and biochemical signals for tissue development. The ECM acts through reciprocal, dynamic, biophysical, and biochemical interactions with surrounding cells. It therefore plays a fundamental role in physiological and pathological processes, including homeostasis, wound healing, aging, and various diseases such as fibrosis, cancer, and pulmonary and cardiovascular disorders [208].

The ECM plays a fundamental role in regulating cell adhesion and intercellular communication, as well as in maintaining tissue homeostasis. Through signaling cascades, it regulates cell growth, survival, and differentiation. The ECM adapts its structural composition to meet the functional needs of tissues [209].

For multicellular organisms, physiological adaptation is crucial for survival. The ability to receive and gather information from the surrounding environment is essential. At the cellular level, various organelles collect environmental information, establishing cell–cell or cell–ECM interactions. Cells adhere to the ECM through cell-surface receptors that transmit chemical and mechanical signals [185,210].

Cell–ECM interactions can be divided into focal adhesions and hemidesmosomes. Hemidesmosomes associate with intermediate filaments and are found in epithelial tissues containing a basement membrane. Focal adhesions, in turn, are present in adherent cells and represent the primary sites of cell–ECM interactions. They are highly dynamic and composed of diverse structural and signaling protein complexes. These are classified into four categories: ECM-binding integrins; force-transducing proteins such as talin and vinculin; signaling proteins such as focal adhesion kinase (FAK) and paxillin; and actin-regulatory proteins such as VASP and zyxin [210].

Integrins interact directly with ECM proteins through their extracellular domains. They connect to the cytoskeletal framework and signaling proteins through several intermediate adaptor proteins in their cytoplasmic tails, regulating cell adhesion and motility. Integrins form a transmembrane connection between the cytoskeleton and the ECM, enabling the transmission of mechanical and chemical signals into and out of the cell. In addition, integrins interact with cell-surface proteoglycans (PGs), such as growth factor receptors and syndecans. This leads to the formation of complexes at the cell surface that regulate and mediate signaling activation [185,211,212].

Cells also express hyaluronan (HA) receptors on their surface, such as CD44. CD44 binds to various other molecules, including collagen, OPN, fibronectin, growth factors, and MMPs. Present in nearly all cell types, CD44 is highly expressed in tumor and inflammatory cells. Thus, it plays a fundamental role in cell–ECM interactions under pathophysiological conditions [183,185,213].

The ECM organizes the extracellular space, regulates intercellular interactions, and responds dynamically to biochemical and mechanical stimuli. It ensures a continuous balance between synthesis and degradation, which is essential for maintaining homeostasis. One of the primary functions of the ECM is to provide physical support to cells in the formation of tissues and organs. Additionally, it is important for the transport of biomolecules, such as cytokines and growth factors, to cells. The ECM actively participates in the activation of mechanosensitive signaling cascades, impacting various cellular processes, tissue development, and homeostasis [214,215].

Structures such as the interstitial matrix and the basement membrane exemplify these functions. They promote structural support, biomechanical signaling, and cell–cell communication, which are essential for tissue integrity. The basement membrane, for example, is rich in type IV collagen and laminins. It acts as a specialized interface that facilitates adhesion and maintains cellular polarity.

Proper mechanoresponsiveness and mechanosensitivity of cell–ECM interactions are crucial for physiological processes. Their dysfunction plays a central role in pathological conditions such as inflammation, aging, and cancer [211].

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Role in Tissue Repair and Tumor Development

In the context of tissue repair, the ECM acts as a provisional scaffold that regulates the recruitment and activation of macrophages, fibroblasts, and mesenchymal stem cells, promoting regeneration and wound healing. In addition, ECM proteins can bind to and regulate the bioavailability of growth factors and cytokines. Proteins such as collagens and proteoglycans (PGs), either alone or in association with heparan sulfate, can bind to fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF). These factors are stored within the ECM and are released in a controlled manner to orchestrate the regenerative response [212].

During tissue repair, activated fibroblasts are referred to as myofibroblasts. They deposit ECM components to reconstruct damaged tissue. Following this reparative phase, a resolution phase occurs, during which ECM deposition must be suppressed and subsequently remodeled in an attempt to return the tissue to a functional state. However, if repair persists along with excessive ECM deposition, fibrosis may result. Thus, the ECM plays a central role in tissue organization and must be precisely regulated to maintain an appropriate state, both under normal conditions and during the tissue repair process [216].

3.4.3. Interaction Between ECM and Tumor Cells

Tumor Microenvironment

Over the past 25 years, the approach to the biology of solid tumors has undergone a significant transformation. Initially, research focused primarily on individual tumor cells and the genetic mutations that drive their malignant transformation. However, the research focus has now shifted toward cancer as a whole, with emphasis on the complex interactions between cancer cells and their microenvironment. Cancer is widely regarded as a genetic disease, driven by the accumulation of mutations within tumor cells. Nevertheless, it is important to note that only a small fraction of cancer cases is attributable to genetic defects, while the majority (90–95%) originate from environmental and lifestyle factors. For this reason, cancer is increasingly recognized as an ecological and evolutionary process, involving constant, reciprocal, and dynamic interactions between cancer cells and the tumor microenvironment (TME). This multifactorial context changes how cancer is understood, highlighting the crucial role of the TME in tumor progression and therapeutic responses [217,218].

The TME refers to the complex and dynamic biological environment surrounding tumor cells. It is composed of various cellular components—including fibroblasts, endothelial cells, neurons, innate and adaptive immune cells, adipocytes—and an ECM, a fundamental acellular component that provides structural and biochemical support. Additionally, the TME contains soluble factors such as cytokines, chemokines, extracellular vesicles, and growth factors, which play a central role in cell signaling and tumor progression [218].

Understanding the role of the TME is essential for evaluating its impact on the key biological processes that sustain cancer progression, such as cell proliferation, tumor invasion, metastasis formation, and stimulation of angiogenesis. The following sections will explore these aspects in detail, highlighting their complex interactions with the TME.

ECM-Mediated Processes

Cell Proliferation: Within the TME, tumor cells and other cellular components communicate in complex and dynamic ways, employing multiple mechanisms to facilitate cancer progression, metastasis, and therapeutic resistance. The main forms of cellular communication within the TME include direct contact, paracrine and autocrine signaling (soluble signals), signaling through extracellular vesicles, ECM-mediated communication, oxidative stress and metabolites, as well as endocrine (long-distance) signaling [219].

Communication between tumor cells and the TME also occurs through extracellular vesicles, such as exosomes, which carry proteins, lipids, and nucleic acids, acting as key mediators of

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intercellular communication and contributing to both physiological and pathological processes. These exosomes modulate the behavior of neighboring cells, facilitating cell proliferation, immunosuppression, and metastasis development. Hypoxia, high lactate levels, and reduced extracellular pH are typical characteristics of the TME that are critical for the survival of cancer cells, enabling metastasis, chemoresistance (the ability of tumor cells to resist chemotherapeutic agents), and immune evasion. These features within the TME regulate and control exosome generation in tumor cells, favoring tumor progression [220,221].

Cell proliferation within the TME is a process highly dependent on dynamic and reciprocal interactions between cancer cells and both cellular and non-cellular components of the TME. Proliferative signaling is one of the hallmarks of cancer and is directly associated with contributions from the TME, which include growth factors, cytokines, and chemokines secreted by stromal cells such as cancer-associated fibroblasts (CAFs) and M2-type macrophages. By establishing constant communication with tumor cells, these elements influence angiogenesis, proliferation, invasion, and metastasis [222,223].

Monocyte-derived macrophages can be polarized into classically activated (inflammatory) M1 or alternatively activated (immunosuppressive) M2 phenotypes. The TME promotes M2 macrophages through the secretion of interleukin (IL)-4. This enables tumor growth and progression, as high monocyte infiltration in tumors is correlated with a negative prognosis, further reinforcing the role of these cells in tumor progression [220,221].

CAFs represent the major cellular constituent of the TME, accounting for approximately 70% of cells in tumor tissues. They are key components of stromal growth factors because they secrete inflammatory ligands and ECM proteins, thereby promoting tumor proliferation, immune exclusion, and therapy resistance. CAFs contribute to cancer progression through multiple mechanisms, such as ECM remodeling and the production of chemokines, cytokines, and growth factors, which directly or indirectly promote cancer metabolism and progression, as well as angiogenesis [224,225].

An important example is transforming growth factor beta (TGF-β), along with TNF-α, IL-1, and IL-6, which act on activated fibroblasts to convert them into CAFs, increasing the production of ECM components such as collagen and fibronectin. This mechanical stiffness directly influences signaling and tumor cell behavior through mechanosensors such as integrins, which activate intracellular signaling pathways, including PI3K/AKT, MAPK, and YAP/TAZ, leading to uncontrolled cell proliferation and acquisition of invasive properties. For example, in breast cancer, CAF-derived IL-32 binds to integrin β3 through the RGD motif and subsequently activates the p38 pathway, increasing the expression of epithelial–mesenchymal transition (EMT) markers and enhancing cancer cell invasion [119,226].

Moreover, ECM stiffness in the TME is capable of inducing the transformation of tumor cells into cancer stem cells (CSCs). These CSCs are characterized as a subpopulation of self-renewing malignant cells that maintain a low but stable level of unlimited proliferation, sustaining the tumor. They exhibit greater resistance to adverse environments such as hypoxia and mechanical stress, and they have high proliferative capacity, contributing to uncontrolled growth, high invasiveness, and therapeutic resistance observed in solid tumors. Matrix stiffness also creates physical barriers that limit the penetration of drugs and immune cells, hindering the effectiveness of conventional therapies [227,228].

Tumor Invasion and Metastasis Formation: Once tumors become established, the next phase of disease progression is local invasion, and this invasive growth is one of the hallmarks of cancer, serving as preparation for metastatic dissemination. Invasion is defined as a complex phase involving multiple steps, starting with tumor cell proliferation, stimulation of angiogenesis, epithelial–mesenchymal transition (EMT), followed by detachment of cells from one another, degradation of ECM and the BM, and migration from the primary tumor mass into the surrounding stroma [92,119,229].

Cell–cell adhesion in cancer cells must be disrupted. This occurs through negative regulation of the intercellular adhesion protein E-cadherin, accompanied by EMT-related changes in gene

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expression. In EMT, epithelial cells undergo alterations in basoapical polarity and lose their junctions, acquiring the ability to migrate and invade surrounding tissues through basement membrane degradation, accompanied by changes in cell shape toward a spindle-like phenotype due to structural modifications in the cytoskeleton and cell signaling. This is accompanied by the expression of mesenchymal adhesion proteins such as N-cadherin and vimentin, as well as proteases such as MMP-2 and MMP-9, which act on cell adhesion, polarity, and the cytoskeleton [92,119].

EMT is coordinated by transcription factors (TFs), referred to as EMT-TFs. These factors suppress epithelial genes while activating mesenchymal genes, and their expression is regulated by the cooperation of extracellular signals and signaling pathways. A major inducer of this phenotypic plasticity in cancer cells is TGF-β, secreted by both TME cells and tumor cells, activating EMT-TF expression [230,231].

TGF-β signaling, together with CAFs, increases tumor cell invasion under both in vivo and in vitro conditions. CAFs participate in ECM deposition and remodeling, and in breast cancer, collagen fibers become densely aligned, and this orientation perpendicular to the tumor boundary increases tissue stiffness and is associated with increased tumor invasion and progression. This is accompanied by cytoskeletal modulation and integrin signaling; as seen in other tumors, it can result in formation of the FAK/Src complex and subsequent activation of the PI3K/Akt and ERK pathways, promoting tumor cell invasion, survival, and migration. Some ex vivo and in vivo studies have shown that resident tissue macrophages trigger EMT and thus an invasive phenotype in adjacent tumor cells [119,232].

The catalytic activity of cathepsin proteases and MMPs can alter biophysical properties of tumor cells by cleaving E-cadherin in epithelial cells and modulating integrins, enabling tumor cells to mechanically adapt to different levels of ECM stiffness [119].

The BM forms a physical barrier between the epithelium and adjacent stroma, and for tumor cell invasion into surrounding tissue, this barrier must be disrupted. This depends on a combination of factors, including intrinsic programming of cancer cells, ECM architecture, and TME signaling. CAFs participate in BM remodeling via protease secretion, with MMPs being the most important for BM invasion; MMP-2 and MMP-9 degrade collagen type IV. Additionally, CAFs exert contractile forces that create gaps in the BM through which tumor cells can pass [119,233].

Certain pathological conditions, such as inflammation, diabetes, and cancer, can induce hypoxia—a common feature of the TME—resulting from an imbalance between insufficient oxygen supply and increased consumption, mainly due to accelerated cellular proliferation exceeding local vascular capacity. Oxygen concentration influences cellular metabolic pathways, and under hypoxia, cancer cells preferentially use glycolytic metabolism as their ATP source, despite being less efficient quantitatively than oxidative metabolism. Metabolic reprogramming via hypoxia provides an important advantage because glycolysis produces metabolic intermediates that influence tumor cell growth, adhesion molecule expression, and metastasis [234,235].

Hypoxia induces multiple metabolic pathway changes through hypoxia-inducible factors (HIFs), which can increase pyruvate dehydrogenase kinase 1 (PDK1) to inhibit PDH activity and thus block the conversion of pyruvate to acetyl-CoA. Additionally, HIFs increase LDHA to convert pyruvate into lactate. Thus, there is a metabolic shift from oxidative to glycolytic pathways, while preventing mitochondrial ROS accumulation. It is also important to note that several HIF target genes contribute to EMT, which represents a crucial step in cancer progression [236,237].

Metastasis refers to a process in which tumor cells from a primary tumor are transported and develop into a distant secondary tumor. This involves tumor cells leaving the primary site, circulating through the bloodstream or lymphatic system, adapting to new secondary microenvironments, and surviving immune attack. Tumor invasion and metastasis are hallmarks of cancer [238].

Each step of metastasis depends on the ability of cancer cells to adapt to different phenotypic cellular states and to co-opt immune cells from the surrounding stroma to continue proliferating. Metastatic cancer is a systemic disease that can impair the function and metabolism of multiple organs [239].

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Intravasation is defined as the dissemination of tumor cells from a primary tumor to other organs through the vascular lumen. In passive intravasation, most cells die or undergo apoptosis. In active intravasation, migration occurs through a chemotactic process in which cells move toward a blood vessel mediated by growth factors and nutrient gradients; this results in ECM and BM degradation, and intravasation proceeds actively into a blood vessel. During circulation, tumor cells remain associated with platelets, allowing them to survive shear stress, accompanied by EMT induction with reorganization of intermediate filament structure. Platelet coating also protects circulating tumor cells from T-cell and natural killer cell attack [238,240,241].

Secondary sites do not passively host invasive tumor cells. This host microenvironment, known as the pre-metastatic niche (PMN), is prepared by the primary tumor selectively, even before metastasis begins. It is a developmental process involving primary tumor–derived components, a prospective stromal microenvironment in metastatic organs, and bone marrow–derived cells (BMDCs) mobilized by the tumor [238,240,242].

Extracellular vesicles and secreted factors that induce ECM remodeling, vascular leakage, and immunosuppression are necessary for PMN development. This requires systemic effects of growth factors, cytokines, enzymes, and tumor-derived extracellular particles (EVPs) secreted by the primary tumor and its TME, and through exchange of these extracellular vesicles, metastatic characteristics may be transferred. Thus, primary tumors are capable of releasing exosomes that transfer invasion-promoting factors, remodeling the ECM, promoting proliferation and survival of cancer cells, and forming a new microenvironment in which metastatic tumor cells can arrest, extravasate, and colonize [238,242,243].

Stimulation of Angiogenesis: Angiogenesis is a phenomenon triggered by multiple biological signals and is essential for meeting the local and systemic oxygen demands of tissues and cells, whether in physiological or pathological contexts. However, excessive or insufficient angiogenesis may indicate the progression of pathological conditions. This process is associated with an imbalance between growth factors and inhibitors within tissues [244,245].

For tumors to grow beyond one millimeter, new blood vessels are required. This angiogenesis is triggered by chemical signals derived from tumor cells, which therefore stimulate tumor growth not only to acquire the ability to invade surrounding tissues but also to progress to metastasis, supporting the migration and establishment of these cells at distant sites [41,244].

Tumors can become vascularized either by co-opting pre-existing vessels, inducing the formation of new blood vessels (neovascularization), sprouting angiogenesis, intussusception, or vascular mimicry. However, the initial step in angiogenesis is triggered by an angiogenic stimulus, such as inflammation or hypoxia, followed by a cascade of biochemical signals. Thus, to overcome a hypoxic and acidic microenvironment, the TME coordinates a program that promotes angiogenesis to restore oxygen and nutrient supply and remove metabolic waste [218,246].

Angiogenesis involves several distinct and complex steps, including initiation, migration, proliferation, vascular tube formation, and maturation. For these sequential steps to occur appropriately during the formation of new blood vessels, basement membrane (BM) disruption through proteases is required, followed by the production of angiogenic factors in endothelial cells lining existing small vessels. These factors bind to receptors on the surface of endothelial cells, which become activated and release enzymes such as MMPs that break down the ECM surrounding the vessels. This degradation facilitates endothelial cell invasion and proliferation into the ECM to form a new intricate blood vessel network. In tumors, pericytes are recruited to provide support to these new blood vessels [244,247].

VEGF is a heparin-binding protein and the most potent factor driving angiogenesis. It is associated with tumor development, vascular permeability, structural alterations, invasiveness, and metastasis. It is released by tumor cells and the surrounding stroma and acts in a paracrine manner through VEGF-A binding to vascular endothelial growth factor receptors (VEGFR)-2 on endothelial cells, activating MAPK/ERK and PI3K/Akt pathways, promoting endothelial cell proliferation and migration. Hypoxia is a critical factor regulating VEGF expression, and this transcription is mediated

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through HIF-1; thus, in hypoxic environments such as within the TME, VEGF is upregulated, resulting in endothelial cell proliferation, invasion, and migration to form new vessels [245–247].

FGFs are also important, similar to VEGF, as they participate in ECM degradation and reorganization through the production of proteases such as MMPs and plasminogen activator. They also contribute to endothelial cell proliferation, migration, survival, and vascular maturation. FGF2 also acts via paracrine signaling on endothelial cells to exert proangiogenic effects, similar to VEGF [247,248].

New blood vessels within tumors also display structural abnormalities, such as increased permeability and irregular branching. This results in deficient blood flow into the TME. Consequently, this environment with low oxygen levels promotes the invasion and metastasis of tumor cells. Thus, the development of this abnormal vasculature can create a hypoxic environment (low oxygen availability), which triggers additional release of proangiogenic factors such as VEGF and promotes metastasis [247,249].

3.4.4. Extracellular Matrix (ECM) Remodeling

Extracellular matrix (ECM) remodeling acts as a central element in the functional reconfiguration of the tumor microenvironment in breast cancer. Its effects reverberate across fundamental axes of neoplastic progression: from the proteolytic activity regulated by MMPs and TIMPs, to the stromal actions of CAFs that intensify immunosuppression and angiogenesis; through the induction of epithelial–mesenchymal transition (EMT), vascular compression that generates hypoxia and activates pro-angiogenic pathways, and culminating in tissue stiffness that limits the diffusion of therapeutic agents and promotes cellular resistance. These interdependent mechanisms will be detailed in the subsequent subsections, highlighting the ECM as an integrative platform of tumor biology [204,209].

Activity of Matrix Metalloproteinases (MMPs) and Their Inhibitors (TIMPs)

The extracellular matrix (ECM) consists of approximately 300 different macromolecules, classified into collagens, glycoproteins (laminins, elastin, fibronectin, and tenascins), and proteoglycans (PGs), such as heparan sulfate, versican, and hyaluronan. ECM remodeling, characterized by changes in the content, activity, and crosslinking of these proteins through post-translational modifications catalyzed by various enzymes, triggers variations in signal transduction and cell fate. Thus, tissues are remodeled through the dynamism of bidirectional communication between resident cells and the remodeling process itself. Although this is a tightly regulated physiological mechanism, it becomes dysregulated in pathological conditions such as tissue fibrosis, inflammatory diseases, and cancer [204,250].

The different mechanisms encompassing ECM remodeling can be divided into: ECM deposition, which affects the biochemical and mechanical properties of the ECM by altering its composition and abundance; post-translational chemical modification, responsible for structural and biochemical changes in the ECM; proteolytic degradation, leading to the release of bioactive ECM fragments; and force-mediated physical remodeling, which contributes to ECM fiber organization and alignment, creating pathways for cell migration [250].

In most tumor tissues, ECM remodeling is characterized by an increase in collagen synthesis and deposition, usually accompanied by expression of remodeling enzymes such as matrix metalloproteinases (MMPs), lysyl oxidase (LOX), LOX-like proteins (LOXLs), and WNT1-inducible signaling pathway proteins (WISPs), among others. These enzymes may treat specific ECM components as substrates and catalyze them to control tissue stiffness and cell–matrix interactions through their unique biochemical and physical properties. Some enzymes process matrix components, such as collagen, resulting in the production and release of bioactive fragments. Changes in MMP expression levels within the tumor microenvironment (TME) reflect the malignant degree of the tumor and highlight their structural remodeling functions in the progression of many epithelial cancers, such as lung, breast, and pancreatic tumors [204].

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Thus, cancer progression—with uncontrolled tumor growth, local invasion, and metastasis—depends largely on the proteolytic activity of numerous MMPs. They influence tissue integrity, immune cell recruitment, and tissue turnover by degrading ECM components and releasing cell adhesion molecules, cytokines, growth factors, or their receptors and kinases. Integrins, matrix receptors that mediate cell adhesion, enable migration through the ECM. Through integrin-mediated adhesion, cells sense the surrounding ECM, respond to its distinct properties, and interact with it with remarkable specificity, which is particularly significant for the initiation, progression, and metastatic dissemination of cancer [251–254].

The events leading to metastasis are generally similar for all types of solid tumors and depend heavily on MMPs, even though the causes of tumorigenesis may differ among cancer types. After a precancerous cell undergoes epithelial–mesenchymal transition (EMT) and becomes cancerous, it breaches the basement membrane (BM) and invades the stromal ECM—a process made possible by the reorganization of adhesive structures and recruitment of ECM-degrading MMPs [251].

MMPs are a group of zinc-dependent endopeptidases that bind to various ECM proteins and are among the major enzymes responsible for connective tissue remodeling. They are involved in many physiological processes, including cell repair, organogenesis, tissue remodeling, motility, and apoptosis through the proteolysis of different protein targets. MMPs are also involved in pathological processes such as cancer development, angiogenesis, tumor neovascularization, metastasis, and invasion. The activity and expression of MMPs increase in advanced tumor stages and in metastatic disease, being produced by both malignant tumor cells and nonmalignant stromal cells [255,256].

Substrates of MMPs include laminins, collagens, elastin, and proteoglycans. The large family comprises over 30 types of MMPs, identified since the role of MMPs as collagen hydrolases was revealed. Different subfamilies of MMPs are divided into four major groups: gelatinases (MMP-2 and MMP-9); collagenases (MMP-1, MMP-8, MMP-13, MMP-18); stromelysins (MMP-3, MMP-10, MMP-11); matrilysins (MMP-7 and MMP-26); and membrane-type MMPs (MMP-14, MMP-15, MMP-16, MMP-24). MMP-2, -3, -9, and -14 are overexpressed and associated with ECM remodeling across various malignant tumors. Some studies show that, during tumor progression, MMP-2 and MMP-9 can mediate invasion of tumor cells through membrane degradation by cleaving type IV collagen, resulting in metastasis and tumor spread [204,257].

Among matrix metalloproteinases (MMPs), MMP-14 is the main driver of ECM degradation and tissue destruction during cancer invasion and metastasis. MMP-14 also influences both intercellular communication and cell–matrix interactions by regulating the activity of several proteins anchored to the plasma membrane and extracellular proteins. Thus, MMPs, especially MMP-14, play a decisive role in the balance between cell adhesion and pericellular proteolysis of the ECM. For this purpose, matrix receptors and associated proteases are interlinked in specialized membrane structures called invadopodia (specialized membrane protrusions of invasive tumor cells, rich in actin filaments and proteolytic enzymes, such as matrix metalloproteinases), which facilitate matrix degradation and tumor invasion. These occur as invadopodia in cancer cells and as podosomes in other cells, such as endothelial cells during angiogenic sprouting. Pericellular proteolysis promoted by these invadosomes, along with cell adhesion, are interdependent factors that shape the TME and directly influence prognosis in cancer patients [251,258–260].

Tumor progression and invasion of cancer cells are facilitated by numerous ECM remodeling and degradation enzymes. These include serine proteases (such as plasmin, plasminogen activator, hepsin, and kallikreins), cysteine proteases (such as cathepsins B and K), aspartyl proteases (such as cathepsins D and E), as well as ion-dependent metalloproteases, mainly MMPs, which are fundamental within the TME. They are involved in ECM disruption, neovascularization, and subsequent metastasis, and are tightly regulated in several ways, including by tissue inhibitors of metalloproteinases (TIMPs) [251].

When the cell loses apical–basolateral polarity and intercellular adhesion, it acquires a migratory and invasive phenotype typical of mesenchymal stem cells. Thus, once a precancerous cell undergoes EMT, it breaches the BM and invades the stromal ECM. Pathologically, this marks the beginning of

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cancer progression from carcinoma in situ to an invasive and metastatic tumor. Hence, the loss of junctions and adhesive connections occurs along with increased activity of ECM-degrading MMPs, resulting in enhanced migratory and infiltrative capabilities—an essential step for metastasis. MMPs with significant roles in EMT include MMP-1, -2, -3, -7, -9, -14, and -28. MMP-14 induces a mesenchymal phenotype in cancer and development by cleaving BM components, while MMP-2 cooperates in regulating pericellular collagen homeostasis and cell signaling processes [251].

In the next phase, cancer cells disseminate from a primary tumor to other organs and tissues. The hematogenous metastatic cascade is a sequence of local invasion, survival in circulation, arrest at a distant site, early survival in a foreign microenvironment, followed by micrometastasis formation and finally assisted tumor angiogenesis with secondary tumor development [228,244,261].

Therefore, the roles of MMPs and their inhibitors TIMPs go beyond proteolytic degradation, representing a central axis in cell–matrix communication and TME remodeling, and are considered emerging therapeutic targets in tumors with invasive and fibrotic phenotypes.

Role of the Tumor Stroma and Cancer-Associated Fibroblasts (CAFs)

The Tumor Microenvironment (TME) represents the complex and dynamic biological environment surrounding tumor cells. This microenvironment is not merely a passive support structure but rather an active participant in cancer progression, influencing proliferation, invasion, metastasis, and therapeutic responses. The TME is composed of a variety of cellular and acellular components. Among the cellular elements, key constituents include fibroblasts, endothelial cells, neurons, adipocytes, and a diverse range of innate and adaptive immune cells. The acellular portion is fundamentally comprised of the Extracellular Matrix (ECM), which provides structural and biochemical support. Additionally, the TME is rich in soluble products such as cytokines, chemokines, extracellular vesicles, and growth factors, which orchestrate cellular signaling and promote tumor development [204,217].

Within the TME, Cancer-Associated Fibroblasts (CAFs) emerge as one of the most abundant and active stromal cell populations, playing a critical and multifaceted role in cancer pathogenesis and progression. Unlike quiescent fibroblasts found in normal tissues, CAFs are activated and characterized by high expression of markers such as alpha-smooth muscle actin (α-SMA), vimentin, and fibroblast activation protein (FAP). Their origin is heterogeneous, potentially arising from activated resident fibroblasts, bone marrow–derived mesenchymal cells, epithelial or endothelial cells that have undergone transition, or even adipocytes [262,263].

CAFs actively remodel the ECM by depositing and organizing collagen fibers and other matrix proteins, thereby increasing tissue stiffness and creating “tracks” that facilitate tumor cell migration and invasion. They also produce and secrete a wide array of growth factors, cytokines, chemokines, and enzymes (including MMPs, as described in the previous section) that promote tumor cell proliferation, survival, angiogenesis, and chemoresistance. Bidirectional communication between CAFs and tumor cells—often mediated by extracellular vesicles—is essential for establishing a permissive microenvironment and supporting metastatic dissemination. Given their extensive influence on multiple aspects of tumor biology, CAFs are considered promising therapeutic targets for cancer treatment [264].

ECM Remodeling as an Inducer of Epithelial-Mesenchymal Transition (EMT)

Epithelial-Mesenchymal Transition (EMT) is a fundamental biological process characterized by the loss of epithelial cellular features (such as polarity and cell–cell adhesions) and the acquisition of a mesenchymal phenotype (marked by increased motility, invasiveness, and resistance). This process is physiologically important in embryonic development, wound healing, and tissue repair. However, EMT dysregulation is a critical event in cancer progression, acting as an essential driver of tumor invasion, metastatic dissemination, therapy resistance, and the acquisition of cancer stem cell features [221].

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The Extracellular Matrix (ECM) plays a central role in inducing and modulating EMT within the tumor microenvironment. ECM stiffness—largely determined by collagen density and organization—is a key environmental factor capable of activating intracellular signaling pathways that promote epithelial-to-mesenchymal transition. A stiffer ECM with aligned collagen fibers can provide the mechanical and structural cues that induce tumor cells to adopt a more invasive phenotype, facilitating their migration away from the primary tumor [265].

In addition to its mechanical properties, the biochemical components of the ECM—such as fibronectin, laminins, and glycosaminoglycans (GAGs)—as well as remodeling enzymes (MMPs) interact with cell-surface receptors (notably integrins) to trigger intracellular cascades that regulate EMT. Signaling pathways such as TGF-β (Transforming Growth Factor-beta), Wnt, Notch, and the PI3K/Akt axis are frequently activated by cell–ECM interactions and act as potent modulators of EMT in cancer cells. ECM degradation by MMPs also releases growth factors and cytokines capable of inducing or sustaining the EMT state [266].

Understanding the intricate relationship between the ECM and EMT is crucial for developing effective therapeutic strategies aimed at inhibiting cancer invasion and metastasis, potentially reverting the mesenchymal phenotype to restore sensitivity to existing treatments [183,202,250].

ECM Remodeling and Its Relationship to Angiogenesis and Tumor Hypoxia

Tumor angiogenesis is a critical and dysregulated process within the tumor microenvironment, characterized by the formation of new blood vessels from the pre-existing vasculature. Unlike physiological angiogenesis, tumor angiogenesis is chaotic and inefficient, yet essential for supplying nutrients and oxygen to the rapidly growing tumor cells, as well as for removing metabolic waste. Furthermore, these newly formed vessels serve as conduits for the dissemination of tumor cells to distant sites, facilitating metastatic formation. As mentioned earlier, alterations in the Extracellular Matrix (ECM), mediated by enzymes such as Matrix Metalloproteinases (MMPs), are known to promote angiogenesis [183,202,250].

The ECM is not merely a structural scaffold for newly forming vessels; it plays an active regulatory role in angiogenesis. ECM components such as collagen, fibronectin, and laminin can provide both the physical framework required for vascular sprouting and the biochemical cues that guide endothelial cells. Pro-angiogenic growth factors, such as Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF), are often sequestered within the ECM and released in a controlled manner or through ECM degradation mediated by MMPs. MMPs, for instance, cleave ECM components, generating fragments that may be either pro- or anti-angiogenic, and release latent growth factors, strongly modulating the angiogenic process [267].

Closely associated with angiogenesis is tumor hypoxia, a hallmark of the Tumor Microenvironment (TME). Hypoxia refers to reduced oxygen levels within the tumor, resulting from rapid cell proliferation that exceeds the insufficient blood supply and from the dysfunctional tumor vasculature. As previously highlighted, hypoxia is one of the typical features of the TME that is crucial for cancer cell survival, metastatic development, and the emergence of chemoresistance [267].

Hypoxia is a potent inducer of an aggressive tumor phenotype. Under low oxygen conditions, both tumor and stromal cells activate Hypoxia-Inducible Factor 1 alpha (HIF-1α), a master transducer of the hypoxic response. HIF-1α activation leads to the transcription of a wide range of genes involved in cellular adaptation to hypoxia, including genes encoding pro-angiogenic factors (such as VEGF), glycolytic enzymes (altering cellular metabolism), and ECM-remodeling enzymes (such as MMPs). This cascade further enhances angiogenesis in an attempt to oxygenate the tumor, facilitates invasion and metastasis (including via EMT, through modulation of related factors), and confers resistance to chemotherapy and radiotherapy. The complex interplay among hypoxia, angiogenesis, and ECM remodeling is fundamental to cancer progression and aggressiveness [236,237,245–247,249].

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ECM Stiffness: Implications for Tumor Progression and Therapeutic Resistance

Extracellular Matrix (ECM) stiffness is a crucial physical property that profoundly influences cellular behavior and the progression of various pathologies, including cancer. Collagen, as the most abundant protein in the ECM, is primarily responsible for its tensile strength and, consequently, its stiffness. In the tumor microenvironment, abnormal deposition and alignment of collagen fibers are prominent features of cancer-associated fibrosis, leading to a significant increase in ECM stiffness [268,269].

This alteration in ECM stiffness is not merely a passive marker of disease; it acts as a potent microenvironmental signal that tumor cells perceive and respond to. Increased stiffness can activate mechanosensitive signaling pathways, such as those mediated by integrins, Focal Adhesion Kinase (FAK), and the proteins YAP/TAZ, thereby promoting tumor cell proliferation, survival, and motility. This stiffness also facilitates tumor invasion by creating "tracks" for cell migration.

Moreover, ECM stiffness is intrinsically linked to Epithelial–Mesenchymal Transition (EMT), a process that endows tumor cells with enhanced invasiveness and metastatic capability. The mechanical tension exerted by a stiff ECM can induce EMT, reprogramming epithelial cells into a more aggressive mesenchymal phenotype. The influence of ECM stiffness also extends to angiogenesis, modulating the formation of new blood vessels that sustain tumor growth [270,271].

A critical implication of ECM stiffness is its contribution to therapeutic resistance. A dense and rigid ECM can act as a physical barrier, impairing the penetration and distribution of chemotherapeutic agents within the tumor. Additionally, mechanical cues derived from a stiff ECM can alter gene expression in tumor cells, activating survival and DNA damage repair pathways that render them less responsive to treatment. EMT induction by stiffness is also a recognized mechanism of resistance to multiple anticancer therapies, making cells less susceptible to drug-induced apoptosis [272–274].

Given these profound implications, ECM stiffness represents a promising therapeutic target. Strategies aimed at reducing fibrosis or modulating cellular perception of ECM-derived mechanical signals may enhance the effectiveness of conventional therapies and overcome treatment resistance in cancer [268,275].

This integrated understanding of ECM stiffness as an active element in tumor biology is supported by studies investigating its mechanisms of induction and functional consequences. Among these, the works of [276], whose distinct experimental approaches—respectively centered on collagen crosslinking and fibroblast-induced fibrillar reorganization—provide complementary perspectives on the determinants of tissue stiffness. Table 1 below presents a structured comparison of methodological aspects, analytical focuses, and key findings from these two studies, enabling a critical visualization of how different collagen remodeling trajectories converge to amplify ECM stiffness and its implications in breast oncology.

Table 1. Comparative Characterization of Experimental and Translational Studies on Collagen in Breast Oncology.

Study	Study Design	Research Focus	Collagen Types Studied	Key Methodology	References
Levental et al., 2009	Experimental (in vitro, in vivo)	Collagen crosslinking, extracellular matrix (ECM) stiffening, and integrin signaling in tumor progression	General collagen analysis	Manipulation of collagen crosslinking, ECM stiffness, integrin signaling in breast cancer models	[277]
Mao et al., 2012	Review	Stromal cells (cancer- associated fibroblasts, CAFs,	Fibrillar: I	Review of stromal cell roles, ECM, and therapy resistance	[278]

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		others), ECM, and therapy resistance in breast cancer			
Oskarsson, 2013	Not clearly specified (likely review)	ECM proteins, including collagen, in breast cancer progression and therapy resistance	General collagen analysis	Review of ECM components and resistance	[279]
Jena, Janjanam, 2018	Review	ECM remodeling, collagen, and associated enzymes in breast cancer	General collagen analysis	Review of ECM proteins, enzymes, and resistance mechanisms	[280]
Deligne, Midwood, 2021	Review	ECM- macrophage interactions, immune modulation, and metastasis in breast cancer	Fibrillar: I, III, V, VI, XIV	Literature synthesis on ECM, macrophages, and immune response	[281]
Fernández-Nogueira et al., 2021	Review	CAFs in therapy resistance and metastasis, collagen's role	Non-fibrillar: IV	Review of CAFs, ECM remodeling, and therapy response	[282]
Martínez, Smith, 2021	Review	ECM remodeling, desmoplasia, and collagen in tumor progression and drug resistance	Fibrillar: I, III; Non-fibrillar: IV; Other: VI	Review of desmoplastic tissue, collagen structure, and therapy resistance	[283]
Tamayo-Angorrilla et al., 2021	Review	ECM in breast cancer models and therapy, focus on biomimetic matrices	General collagen analysis	Review of decellularized ECMs and biomimetic models	[284]
Lepucki et al., 2022	Review	ECM proteins, collagen subtypes, and roles in breast cancer	Fibrillar: I, II, III, V, XI, XXIV, XXVII; Non-fibrillar: IV, VIII, X	Comprehensive review of ECM, collagen subtypes, and tumor microenvironment (TME)	[285]
Papanicolaou et al., 2022	Experimental (in vitro, in vivo), Observational	Role of collagen XII in regulating collagen I organization and metastasis in breast cancer	Fibrillar: I; Non-fibrillar: XII (fibril-associated collagens with interrupted triple helices, FACIT)	Temporal proteomic profiling, single-cell tran- scriptomics, genetic manipulation, patient cohort analysis	[276]

The data synthesized in Table 1 demonstrate that, although the studies employ distinct methodological approaches—ranging from in vitro and in vivo models to proteomic analyses and translational reviews—there is a conceptual convergence regarding the role of collagen as both a physical and bioactive regulator in the breast tumor microenvironment. The recurring emphasis on collagen fiber crosslinking, matrix architectural reorganization, and activation of mechanosensitive pathways reinforces the notion that ECM stiffness is not merely an isolated structural phenomenon,

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but rather a dynamic agent that directly influences tumor aggressiveness, therapeutic evasion, and phenotypic plasticity of neoplastic cells.

By correlating specific collagen isoforms with biomechanical properties and cellular signaling, these studies contribute to establishing matrix stiffness as a functional target with significant clinical implications, justifying further exploration within combined therapeutic strategies and personalized medicine models.

Clinical Implications of ECM Remodeling in Cancer

The extracellular matrix (ECM) remodeling mechanisms discussed in the previous subsections—encompassing proteolytic degradation by matrix metalloproteinases (MMPs), activation of cancer-associated fibroblasts (CAFs), induction of epithelial–mesenchymal transition (EMT), aberrant angiogenesis, and increased tissue stiffness—functionally converge to produce clinically significant implications in tumor evolution and therapeutic response in breast cancer.

The ECM, composed of macromolecules produced by tumor and stromal cells, forms a dynamic network that regulates cellular proliferation, immune modulation, and the distribution of bioactive factors. Given the genomic heterogeneity of neoplastic cells and the adaptive capacity of the tumor microenvironment (TME), exclusively targeting tumor cells represents a limited therapeutic strategy. In this context, the remodeled ECM emerges as a functional and strategic element, directly influencing vascular accessibility, therapeutic agent infiltration, and immune evasion [218,286].

Among the associated clinical events, desmoplasia stands out, characterized by CAF infiltration and disorganized accumulation of fibrillar collagen, resulting in heightened tissue stiffness and impaired perfusion. This phenomenon is driven by paracrine pathways, such as TGF-β and SDF1, which activate pro-fibrotic CAFs, promote excessive ECM deposition, and create physical barriers to T-cell infiltration, thereby compromising antitumor immunity [217,287].

Elastography studies in breast cancer demonstrate that tissue stiffness is positively correlated with aggressive histopathological traits, including larger tumor volume, higher histologic grade, and lack of hormone receptors, especially in triple-negative tumors. Collagen crosslinking mediated by lysyl oxidases (LOX/LOXLs), together with the overabundance of hyaluronic acid (HA), comprise the principal determinants of this matrix rigidity [92].

This biomechanical profile of the remodeled ECM negatively affects therapeutic efficacy. Matrix stiffening forms physical barriers to the penetration of chemotherapeutic and immunotherapeutic agents, while compressing the tumor microvasculature, promoting sustained hypoxia. The activation of HIF-1α and dysfunctional angiogenesis further intensify vascular chaos, reduce intratumoral perfusion, and stimulate the expression of resistance genes, including MDR1, MRP1, and BCRP. In parallel, this environment fosters the selection and expansion of cancer stem cells (CSCs), characterized by marked refractoriness [92,267].

Several studies have deepened the understanding of the functional role of ECM collagen remodeling in therapeutic refractoriness and clinical stratification of patients. Table 2 below presents a comparative synthesis of key investigations correlating the type of treatment used, the collagen-related effects observed (such as stiffening, crosslinking, and immune exclusion), the implicated resistance mechanisms, and their respective clinical implications in breast cancer. The findings reinforce that collagen acts not only as a structural component, but also as an active regulator of the tumor response to therapy.

Table 2. Correlation between therapeutic strategies, collagen remodeling, and clinical implications in breast cancer.

Study	Treatment Type	Collagen-Related Effects	Resistance Mechanisms	Clinical Impact	References
Levental et al., 2009	No mention found	Collagen crosslinking	Integrin signaling, LOX-mediated crosslinking	LOX inhibition reduces fibrosis, malignancy	[277]

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		enhances PI3K, invasion			
Mao et al., 2012	Chemotherapy, endocrine, targeted therapy	Collagen I/CAFs reduce drug uptake, activate resistance pathways	Drug penetration barrier, MAPK/Akt, EGFR/PI3K/Akt, WNT16B/NF- B	CAF targeting as resistance strategy	[278]
Oskarsson, 2013	No mention found	ECM proteins/ receptors/modifiers mediate resistance	ECM signaling pathways	ECM as therapeutic target	[279]
Jena, Janjanam, 2018	Chemotherapy	Hardened ECM, heparanase activity, CAF/adipocyte factors	ERK1/2 up, JAK2/STAT5 down, HGF/c-Met, heparanase	Heparanase inhibitors, HGF/c-Met blockade	[280]
Deligne, Midwood, 2021	Immunotherapy (implied)	ECM modulates immune response	No mention found	Targeting ECM may improve immunotherapy	[281]
Fernández-Nogueira et al., 2021	Chemotherapy, targeted therapy	High collagen/matrix stiffness reduces	JNK1 activation, MMP secretion, CAF-driven remodeling	Pegylated hyaluronidase (PEGPH20) may improve response	[282]
Martínez, Smith, 2021	Chemotherapy, targeted therapy	Collagen crosslinking, dense ECM block drug delivery	LOX-mediated crosslinking, YAP/TAZ pathway, glycosaminoglycans (GAGs)	LOX inhibitors (betaaminopropionitrile, BAPN) as potential therapy	[283]
Tamayo-Angorrilla et al., 2021	Chemotherapy, targeted therapy	ECM remodeling affects therapy response	No mention found	Biomimetic ECM models for precision medicine	[284]
Lepucki et al., 2022	No mention found	Collagen crosslinking enhances PI3K, invasion	Integrin signaling, LOX-mediated crosslinking	LOX inhibition reduces fibrosis, malignancy	[285]
Papanicolaou et al., 2022	Chemotherapy, immunotherapy (implied)	ECM stiffness (collagen XII) may alter NF-B/JNK, affect immunosurveillance	Stiffness drives myofibroblast phenotype, matrix remodeling	Collagen XII as biomarker for high-risk patients	[276]

Given this evidence, the phenotypic reprogramming of CAFs has been investigated as a therapeutic alternative. The selective suppression of pro-tumor CAFs (pCAFs) and the stimulation of restrictive CAFs (rCAFs), capable of inducing antitumor immune responses and limiting angiogenesis, has shown promise. The Hedgehog pathway emerges as a key modulator of the rCAF phenotype, being associated with restricted tumor progression and functional ECM remodeling [225].

Complementarily, antiangiogenic strategies have been incorporated as an effective clinical approach, inhibiting the formation of disorganized neovessels that sustain tumor growth and contribute to therapeutic escape. The principal targets include the pro-angiogenic factors VEGF, FGF, and EGF, with blockade achieved through monoclonal antibodies and tyrosine kinase inhibitors (TKIs) [288].

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Bevacizumab (Avastin), a monoclonal antibody against VEGF-A, prevents ligand binding to VEGFR, thereby blocking angiogenic signaling and promoting the regression of newly formed vessels. This vascular normalization improves tumor perfusion and enhances the penetration of cytotoxic chemotherapeutic agents. It has been approved for the treatment of multiple solid tumors, including metastatic breast cancer [212]. Ramucirumab (Cyramza), in turn, acts as a competitive inhibitor of VEGFR-2, disrupting VEGF signaling and reducing tumor angiogenesis. It has been granted clinical approval for hepatocellular carcinoma and other refractory malignancies [289].

Therefore, the clinical implications of ECM remodeling derive directly from the molecular, structural, and biomechanical processes previously discussed, consolidating its relevance as an emerging therapeutic target and as a functional biomarker within the integrated management of breast cancer.

3.5. Specific Role of Collagen in Tumor Progression and Immune Response

3.5.1. Collagen: General Definition and Structure

The term collagen derives from Greek and was coined to describe a natural adhesive obtained from boiling animal bones. It corresponds to an extensive family of structural proteins that predominate in the extracellular matrix (ECM) and in both hard and soft connective tissues, such as tendons, skin, bone, cartilage, corneas, and teeth, providing tissue integrity and mediating cellular processes. It plays physiological roles in hemostasis and wound healing, as well as pathological roles in cancer and fibrosis. The collagen family comprises 28 distinct types, which—depending on their structural organization, supramolecular composition, and additional functional characteristics—can be further subdivided into several subfamilies [290].

The collagen superfamily of 28 members, numerically designated in Roman numerals, can be classified according to supramolecular organization and domain structure. Thus, they can be grouped into fibril-forming collagens (types I, II, III, V, XI, XXIV and XXVII); fibril-associated collagens with interrupted triple helices (FACITs; types IX, XII, XIV and XX); FACIT-like collagens (types XVI, XIX, XXI and XXII); network-forming collagens (types IV, VI, VII, VIII and X); multiplexin collagens (types XV and XVIII); transmembrane collagens (types XIII, XVII, XXIII and XXV); and other molecules with collagen domains (types XXVI and XXVIII) [242].

The most prevalent collagens in living organisms are types I, II and III, with type I collagen representing the major protein of the family, accounting for 90% of all collagen in humans. It is synthesized by fibroblasts and serves as an essential structural scaffold for skin, connective tissues, tendons, ligaments, bones, and the cornea, in addition to participating in tumor regulation. Type II collagen is found predominantly in the total protein content of articular cartilage. Type III collagen is present in the ECM and synthesized as procollagen, constituting an important structural component of hollow organs, such as the uterus, large blood vessels, and the intestine [242,291].

Collagens may exist as heterotrimers or homotrimers. Homotrimers consist of three identical α-chains, such as three α1 polypeptide chains in type II and type III collagen. Heterotrimeric collagens contain distinct α-chains of the same collagen type, such as two α1 polypeptide chains and one α2 polypeptide chain in type I collagen. However, other collagen types exhibit multiple α-chain combinations, for example, type V collagen, which may occur as [α1(V)][α2(V)]2, [α1(V)][α2(V)][α3(V)], or [α1(V)]3. Each α-polypeptide chain adopts a left-handed helical structure, stabilized by hydrogen bonding [292].

Collagen is characterized by a triple-helical structure formed by three polypeptide α-chains, constituting its primary structure. These polypeptide chains are defined by repeating Gly-Xaa-Yaa triplets, where Gly is glycine—the smallest amino acid in nature, essential for helix packing—and Xaa and Yaa are most frequently occupied by proline (Pro) and hydroxyproline (Hyp), respectively. However, these positions may be filled by other amino acids, except glycine, which must always occupy the central position of the triplet. Thus, the Pro-Hyp-Gly triplet is the most frequent, representing approximately 10.8% of all repeats, although residues such as lysine (Lys) can also occupy the Yaa position, with a frequency near 10.5% [293,294].

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Each collagen α-chain contains approximately 662 to 3,152 amino acids, depending on the specific type. Consequently, numerous molecular interactions occur along the triple helix, promoting dense and highly organized packing along the central axis of the molecule [290,295]. In this context, the secondary structure of collagen is defined by the left-handed polyproline II helix configuration, composed of repeating Gly-Xaa-Yaa motifs, with peptide bonds predominantly in the trans conformation. This configuration allows the polypeptide chain to coil and adopt distinct spatial arrangements. Subsequently, three parallel α-chains intertwine around a central axis, forming a right-handed triple helix, known as tropocollagen, a rigid and elongated molecule stabilized by interchain hydrogen bonds [242,296].

Tropocollagen molecules then associate laterally to form microfibrils, composed of five tropocollagen molecules, defining the quaternary structure. These microfibrils organize into collagen fibrils, which in turn assemble into collagen fibers, ultimately forming three-dimensionally arranged bundles. This hierarchical and highly ordered fibrillogenesis is essential for the mechanical strength and structural integrity of tissues [242,296].

3.5.2. Therapeutic Applications of Collagen

According to the manufacturing process, distinct collagen-derived products with completely different compositions, structures, and functional properties can be obtained, such as soluble native collagen or insoluble native non-denatured collagen, which retain the triple helix structure; gelatin, which contains denatured collagen; and hydrolyzed collagen in the form of peptides and amino acids, produced at different levels of hydrolysis [290].

The development of collagen-based proteins and peptides through heating processes that induce enzymatic denaturation of the collagen molecule via cleavage of peptide bonds results in a gelatinous form of collagen, also known as collagen peptides or hydrolyzed collagen. This growing interest is due to its beneficial effects, such as enhancing dermal hydration, regenerating wrinkled skin tissue, and improving bone density, making hydrolyzed collagen an increasingly used dietary supplement. Collagen is extracted from animal tissues, purified, and subjected to enzymatic hydrolysis, where proteases break collagen into smaller peptides, increasing their solubility and bioavailability and facilitating digestion and absorption. This hydrolyzed collagen solution can be dried and concentrated to form a powder that maintains the functional and nutritional properties of collagen peptides, also used as a dietary supplement [297].

Thus, it is known that the properties of native collagen differ from those of hydrolyzed collagen. Upon denaturation, the native triple-helical structure shifts to a random-coil conformation as hydrogen bonds dissociate, yielding multiple peptides while altering biological and physicochemical properties. These alterations include decreased viscosity, increased antimicrobial and antioxidant activity associated with higher bioavailability and greater therapeutic capacity, as well as reactivity with free radicals [298].

Collagen supplements present an abundance of amino acids such as glycine, proline, and hydroxyproline, which can exert antioxidant and anti-inflammatory effects, contribute to cartilage synthesis, and act as signaling molecules in various physiological processes. Hydrolyzed collagen undergoes enzymatic or thermal hydrolysis, which cleaves long collagen chains into smaller peptides, facilitating intestinal absorption [299–301].

The so-called ultra-hydrolyzed collagen (or collagen with bioactive peptides) undergoes even more intensive processing, resulting in smaller peptide fragments with potentially greater bioavailability and faster action in the body. However, current scientific understanding of the functional differences between hydrolyzed and ultra-hydrolyzed collagen remains limited, and comparative studies on clinical efficacy, absorption, and biological impact are still in development [302].

In addition, collagen polypeptides possess important properties related to water retention and absorption. Thus, hydrogels derived from collagen peptides—obtained by enzymatic digestion of hydrolyzed collagen—can exhibit anti-inflammatory and moisturizing effects, promote cellular

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adhesion and proliferation, and contribute to the removal of harmful free radicals and peroxides from within cells, reducing cellular oxidative stress. The high-water content in hydrogels provides a moist environment that accelerates processes such as wound healing. These gels also display antioxidant activity and help remove excess toxins and exudates from wounds [303].

3.5.3. Function of Collagen in Tumor Progression

Tumorigenesis is a dynamic and multifactorial process sustained by the interaction among various components of the tumor microenvironment (TME). In this context, the extracellular matrix (ECM) of solid tumors functions as a structural and functional scaffold that surrounds, protects, and interacts with neoplastic cells. Collagen, the main constituent of the ECM, plays a critical role in modulating the biochemical and biomechanical properties of the TME.

Alterations in collagen fiber density and organization directly influence tumor dissemination. Increased collagen deposition, as demonstrated by Lo Buglio and colleagues, promotes ECM stiffening, favoring the survival, proliferation, and expansion of malignant cells [304].

In breast cancer, a significant remodeling of the collagen expression profile is observed, with variations in both abundance and molecular integrity. Type I collagen, in particular, shows a direct correlation with increased tissue stiffness, stimulating cellular invasiveness. Imaging studies reveal that migratory tumor cells utilize collagen fibers as structural "tracks" to escape from the primary tumor [194,305].

Fibrillar organization also plays a decisive role: while non-fibrillar type I collagen at high density may induce suppressive effects, its linearized (fibrillar) form is associated with invasive phenotypes. Additionally, suppression of collagen type IV α2 (COL4A2) inhibits proliferation and migration of triple-negative breast cancer cells [190].

Each collagen subtype exerts distinct or complementary functions in tumor progression. Table 3 summarizes the main types involved in breast cancer, highlighting their mechanisms of action and clinical implications.

Table 3. Functional roles and alterations of collagen types in the progression and therapeutic response of breast adenocarcinoma.

Study	Collagen Modification	Signaling Changes	Progression Effects	Clinical Implications	References
Levental et al., 2009	No mention found	No mention found	Collagen crosslinking present, drives ECM stiffening	ECM stiffening associated with crosslinking	[277]
Jena and Janjanam, 2018	No mention found	No mention found	Lysyl oxidase-like 2/4 (LOXL2/LOXL4) catalyze cross-linking	Hardened ECM, increased tumor stiffness	[280]
Fernández-Nogueira et al., 2021	High collagen deposition in TME	Perpendicular orientation in Basal-like/HER2 ductal carcinoma in situ	No mention found	Matrix stiffness increases, linked to therapy resistance	[282]
Martínez and Smith, 2021	High proportion of fibrillar collagens	Dense structure, high fibrillar content	Lysyl oxidase (LOX) catalyzes cross-linking	Tumor periphery ~7x stiffer than interior; rigidity 0.8→4.0 kilopascals	[283]
Lepucki et al., 2022	Increased collagen deposition,	Dense network perpendicular	LOX pathway produces mature crosslinks	Increased stiffness due to crosslinking and deposition	[285]

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	stromal stiffness	to tumor border			
Papanicolaou et al., 2022	Upregulated in late-stage tumors	Increased bundle width and linearity	Collagen XII stabilizes collagen I fibrils, regulates 3D organization	Tumor stiffness increases in late stages; reduced with collagen XII depletion	[276]

Table 3 consolidates findings from three key studies [277,282,285], highlighting the complexity of collagen architectural alterations in mammary adenocarcinoma:

- Fiber density: A significant increase in collagen deposition within the TME remodels the tissue landscape and supports neoplastic expansion.
- Fibrillar organization: Patterns of linearization, thickening, and perpendicular orientation relative to tumor margins create migratory tracks that facilitate invasion and metastatic dissemination.
- Crosslinking and stiffness: Activation of the lysyl oxidase (LOX) pathway promotes the formation of crosslinks, stabilizing the ECM and increasing its stiffness. This stiffening is associated with tumor aggressiveness and therapeutic resistance, as demonstrated by Fernández-Nogueira and colleagues [282]
- Functional implications: ECM stiffness modulates pro-tumorigenic signaling pathways, favoring the acquisition of resistant phenotypes, both intrinsic and treatment-induced.

Beyond the specific collagen types, the relationship between collagen and tumor progression manifests through structural modifications and crucial biomechanical changes within the microenvironment. Table 4 details the main effects mediated by collagen architecture in these interactions, emphasizing how the physical properties of the ECM influence tumor behavior.

Table 4. Effects of collagen on tumor progression: structural modifications and mechanical impacts.

Study	Collagen Modification	Signaling Changes	Progression Effects	Clinical Implications	References
Iyengar et al., 2005	Collagen VI from adipocytes	NG2/AKT/beta-catenin/cyclin D1	Promotes early hyperplasia, tumor growth	Collagen VI as early progression target	[306]
Levental et al., 2009	Collagen crosslinking, ECM stiffening	Integrin/PI3K signaling	Promotes invasion, increases tumor incidence	LOX inhibition reduces malignancy	[277]
Acerbi et al., 2015	Collagen I deposi-tion/linearization, ECM stiffening	TGF signaling, macrophage infiltration	Aggressive subtypes show more stiffening, immune infiltration	Stiffness/linearization as aggression markers	[307]
Liu et al., 2018	COL1A1 expression	CXCR4 signaling	Promotes metastasis, poor survival (estrogen receptor positive)	COL1A1 as prog-nostic/therapeutic target	[308]
Shea et al., 2018	High collagen I density (desmoplasia)	AKT-mTOR, YAP activation	Increased cancer stem cells, more/larger lung metástases	mTOR inhibition effective in primary, not metastatic, tumors	[309]

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Jallow et al., 2019	COL1A1-dense ECM	Increases AP-1 activity	Tamoxifen-stimulated prolifera-tion/metastasis	ECM context alters therapy response	[310]
Byrne et al., 2021	Collagen I-rich ECM	Alters senescence pathway transcription	No proliferation increase; ECM sensitizes estrogen receptor negative cells to therapy	Suggests need for complex ECM in models	[311]
Liu et al., 2021	COL1A1 expression	CXCR4 signaling	Promotes metastasis, poor survival (estrogen receptor positive)	COL1A1 as prog-nostic/therapeutic target	[212]
Papanicolaou et al., 2022	Collagen XII organizes collagen I, increases stiffness	Myofibroblast phenotype, matrix remodeling	Promotes invasion, metastasis; increased bundle width/density	Collagen XII predicts poor survival, high metastatic risk	[276]
Volk et al., 2023	Type III collagen deficiency leads to a tumor-permissive matrix; Col3:Col1 ratio	No mention found; affects prolifera-tion/apoptosis	Increased proliferation, reduced apoptosis, higher recur-rence/metastasis with low type III collagen	High Col3:Col1 ratio predicts improved survival	[312]

The data summarized in Table 4 show that among collagen isoforms, type I collagen (COL1A1) is widely recognized as the main structural and functional modulator of the ECM, as evidenced by several studies [277,282]. The recurring focus on this isoform is justified by its high tissue abundance and its direct association with stiffness, cellular proliferation, and tumor dissemination. Nevertheless, other less explored variants, such as collagens type III, VI, and XII, also play contextually relevant roles, broadening the spectrum of interaction between ECM composition and cellular behavior [282].

Modifications such as crosslinking, hyperdeposition, fiber linearization, and increased matrix stiffness are not merely morphological changes; they represent active mechanisms that amplify tumor aggressiveness. This architectural plasticity of the ECM not only facilitates cellular trafficking and escape from the primary tumor, but also promotes the emergence of therapy-resistant phenotypes, as reported by several authors. Experimental strategies such as the use of tetrathiomolybdate (TM) have been employed by Levental and colleagues with the aim of suppressing matrix stiffening, indicating a possible route for therapeutic modulation of tumor mechanobiology [277].

From a molecular perspective, collagen remodeling activates a broad range of signaling pathways, notably NG2/AKT/β-catenin/cyclin D1, AP-1, TGF-β, AKT-mTOR/YAP, integrin/PI3K and CXCR4. This functional diversity suggests that collagen acts as a multifaceted biomechanical conductor, capable of integrating physical and biochemical stimuli and influencing processes ranging from cell migration to immune reprogramming of the TME. In addition, phenotypes associated with senescence, myofibroblast activation, and macrophage and T-cell infiltration further reinforce the role of collagen as a determining element in intercellular communication and immune evasion.

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In the studies analyzed (Table 4), there is a predominance of outcomes such as increased metastasis, exacerbated tumor proliferation, recurrence, invasion, and phenotypic aggressiveness. This correlation is reinforced by evidence directly linking ECM stiffening to therapeutic failure and expansion of cancer stem cells. Although most studies associate collagen remodeling with disease progression, some, such as that of De Martino and Bravo-Cordero, suggest that certain structural profiles may exert a suppressive function, highlighting the functional duality of collagen in breast cancer [190].

The clinical implications of these alterations are broad, ranging from the use of tissue stiffness as a prognostic marker to the proposal of specific therapeutic targets such as mTOR, LOX, COL1A1, and TM. The need for three-dimensional models that faithfully recapitulate ECM complexity has also been reiterated, reinforcing that any translational strategy must consider not only the biochemical composition of the TME but also its structural organization and emergent mechanical properties.

In normal tissues, the role of type I collagen is to act as a physical barrier to invasion and to inhibit the proliferation of both tumor and non-tumor cells; in healthy mammary gland tissue, collagen fibers are anisotropic and wavy. However, with the onset of cancer, collagen fibers become linearized and thickened, which can lead to metastasis, as they provide fibrillar guidance that facilitates tumor invasion [191].

From this perspective, most studies show that specific features of collagen fibers—such as greater collagen content, fibers arranged perpendicularly to tumor margins, and a more aligned orientation—correlate with worse prognosis in breast cancer. However, other studies have found opposite results or no association. This highlights the multifaceted and dynamic role of collagen in the context of breast cancer and underscores the need for more detailed investigations [191,194].

Moreover, collagen actively participates in the regulation of invadopodia, which are small actin-rich protrusions formed by invasive tumor cells that are responsible for localized degradation of the extracellular matrix (ECM). The formation and activation of invadopodia are modulated, in part, by $\alpha v\beta 3$ integrin signaling, which recognizes ECM components such as vitronectin and is involved in cell migration and invasion. With collagen accumulation and the consequent increase in stromal stiffness, the $\beta 1$ integrin signaling pathway is activated, triggering focal adhesion kinase (FAK) and Yes-associated protein (YAP). This signaling cascade promotes changes in the cytoskeleton and gene expression, accelerating tumor invasion and contributing to malignant progression [228,304].

Another important factor is the hypoxic TME, which promotes collagen crosslinking through increased LOX expression in cancer cells, thereby leading to increased ECM stiffness and tumor invasion with metastasis. In this hypoxic niche there is also activation of HIF-dependent transcription, which elevates the expression of MMPs that remodel the ECM through collagen degradation, further facilitating tumor invasion. In addition, under these hypoxic conditions, collagen drives the activation of cytokines and signaling pathways related to different stages of tumor progression [113,268].

3.5.4. Collagen as a Prognostic Biomarker in Breast Cancer

The tumor microenvironment (TME) in breast cancer is a complex and dynamic ecosystem in which collagen stands out not only as a structural component but as an active regulator of disease progression and, consequently, as an increasingly important prognostic biomarker. Recent studies have elucidated the predictive value of various collagen-related features in patient stratification and survival forecasting, reflecting the intrinsic link between the architecture of the extracellular matrix (ECM) and the biological behavior of the tumor.

Understanding collagen as a biomarker goes beyond its mere abundance, extending to its structural modifications, organization, and specific types. In this context, Jansson and collaborators demonstrated that high stromal type IV collagen expression in primary tumors correlates significantly with decreased breast cancer–specific survival and increased risk of distant metastasis. This finding is crucial, as type IV collagen, typically associated with basement membranes, when

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aberrantly present in the stroma, may indicate ECM disorganization that facilitates invasion and dissemination [194].

The architecture of collagen fibers has also emerged as a robust prognostic predictor. Tumor-associated collagen signatures (TACS), particularly TACS-3, which describes dense collagen fibers aligned perpendicular to the invasive tumor margin, were identified by Bredfeldt and colleagues as a quantitative, image-based biomarker for survival in invasive ductal carcinoma [313]. This perpendicular orientation mechanically favors the exit of tumor cells from the primary lesion, acting as "tracks" guiding invasion and metastatic spread. Complementarily, Esbona and collaborators reinforced this observation by demonstrating that local collagen density, alignment, and perpendicular orientation relative to the tumor margin (TACS-3) independently predict reduced overall survival in invasive breast carcinoma [314]. These data underscore the importance of collagen fiber topology as an indicator of tumor aggressiveness [315].

Additionally, internal features of the collagen network within the tumor also possess prognostic value. In the context of luminal breast cancer, Natal and coauthors revealed that greater uniformity and higher intratumoral collagen content are independently associated with worse recurrence-free and overall survival. This finding suggests that a dense and homogeneous intratumoral ECM may create an environment that favors tumor resilience and treatment resistance, either by limiting drug penetration or by promoting survival pathways [315].

Taken together, these findings not only solidify collagen as a multifaceted element in the TME that directly influences the progression of breast cancer but also establish its characteristics as valuable prognostic tools. The integration of these collagen-based biomarkers, obtained through advanced imaging techniques and molecular analyses, offers the promise of refining patient risk stratification, guiding more personalized therapeutic decisions, and ultimately optimizing clinical outcomes in breast cancer management. Continued investigation of these biomarkers is essential to fully translate their potential into clinical practice [316]. Table 5 summarizes the main collagen-based predictive biomarkers and their prognostic associations.

Table 5. Characteristics of collagen as prognostic biomarkers in breast cancer.

Study	Target	Mechanism	Therapeutic Approach	Clinical Status	References
Iyengar et al., 2005	Collagen VI, NG2 receptor	Promotes early growth via AKT/beta-catenin	Targeting NG2/collagen VI axis	Preclinical	[306]
Levental et al., 2009	LOX, integrin signaling	ECM stiffening, invasion	LOX inhibition, integrin blockade	Preclinical	[277]
Liu et al., 2018	COL1A1	Promotes metastasis, chemoresponse	COL1A1 knockdown	Preclinical/clinical biomarker	[308]
Shea et al., 2018	Collagen I density, mTOR	Cancer stem cell niche, metastasis	mTOR inhibition (rapamycin) Copper depletion	Preclinical	[309]
Jallow et al., 2019	ECM density (COL1A1)	Alters hormone therapy response	Targeting both estrogen receptor and ECM	Preclinical	[310]
Liu et al., 2021	LOXL2, PRO-C3, C6M, C1M	Collagen crosslink-ing/processing	Copper depletion (tetrathiomolyb-date)	Phase II clinical	[153]
Papanicolaou et al., 2022	Collagen XII	Regulates collagen I, promotes metastasis	Knockdown in cancer-associated fibroblasts	Preclinical	[276]

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Volk et al., 2023	Type III collagen (Col3)	Tumor-restrictive ECM	Col3 supplementation (hydrogels)	Preclinical	[312]

The data synthesized in Table 5 reinforce the positioning of collagen as a central regulatory axis in the dynamics of the tumor microenvironment (TME), highlighting its relevance not only as a structural element of the extracellular matrix (ECM) but also as a prognostic biomarker and emerging therapeutic target in breast cancer.

Type I collagen (COL1A1) stood out as the most recurrent target, as addressed by Liu et al., Jallow et al., and Liu et al., where its overexpression was associated with ECM stiffness, maintenance of cancer stem cell niches, and hormonal therapy resistance. These properties reinforce the interconnection between matrix architecture and tumor plasticity [153,308,310].

In contrast, other collagen isoforms, such as type VI collagen and type XII collagen, were explored for their roles in desmoplastic remodeling, cellular invasion, and tumor-restrictive strategies. The presence of these isoforms within the tumor context suggests a functional specialization of the ECM depending on the histological subtype and stage of progression.

Beyond collagens, several ECM modulators were identified as functional targets: LOX [277], LOXL2 [312], NG2 [309], integrins [276], and mTOR [310]. These components regulate mechanisms such as collagen crosslinking, tissue stiffness, maintenance of the myofibroblastic phenotype, and early proliferative signaling.

The mechanisms associated with collagen biomarkers encompass distinct functions: promotion of tumor metastasis and invasion [276,306], modulation of therapeutic response [310], processing and maturation of fibrillar collagen [312], tumor-restrictive behavior and architectural support [306], as well as maintenance of the cancer stem cell niche [212]. It is noteworthy that none of the studies addressed immunomodulation or angiogenesis as central mechanisms, revealing an understudied area, despite the direct interface between ECM and the immune system.

Therapeutic strategies included genetic knockdown [212,276] and interventions such as LOX inhibition, copper depletion, integrin blockade, and mTOR inhibition, each tested in specific experimental models. The absence of studies using monoclonal antibodies or small-molecule inhibitors beyond these contexts underscores the need to rationally expand the therapeutic repertoire targeting the ECM.

Regarding clinical stage, seven of the studies remain in preclinical phases, demonstrating a strongly exploratory and mechanistic character. Only Jallow and colleagues [310] present data from a phase II clinical trial, whereas Liu et al. [308] integrate preclinical analysis with translational validation, representing a critical bridge between discovery and clinical application.

3.5.5. Comparison of Collagen Molecular Mechanisms in Different Cellular Models

Accumulating evidence demonstrates that various cellular populations present in the breast tumor microenvironment, such as neoplastic epithelial cells, cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and endothelial cells, maintain functional interactions with components of the extracellular matrix (ECM), with emphasis on collagen. These interactions modulate critical processes such as proliferation, migration, angiogenesis, tissue remodeling, and immune evasion through the activation of specific molecular signaling pathways. Although these mechanisms have been discussed in previous sections, Table 6 below comparatively compiles the main findings from recent studies that correlate the cellular types involved with the collagen isoforms and their respective molecular trajectories associated with breast tumor progression.

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Table 6. Comparative synthesis of collagen-related mechanisms and signaling pathways in cellular populations of the tumormicroenvironment.

Study	Cell Populations Studied	Mechanisms/Signaling Pathways	References
Levental et al., 2009	Breast cancer cells	Collagen crosslinking enhances integrin clustering, phosphoinositide 3-kinase (PI3K) signaling, invasion	[277]
Mao et al., 2012	Breast cancer cells, CAFs, TAMs, endothelial cells, adipocytes	CAFs promote invasion, angiogenesis, resistance via ECM/collagen; CAFs activate mitogen-activated protein kinase (MAPK)/protein kinase B (Akt), epidermal growth factor receptor (EGFR)/PI3K/Akt, WNT16B/NF- B pathways	[278]
Oskarsson, 2013	Breast cancer cells	ECM proteins, receptors, and modifiers mediate resistance via signaling pathways	[279]
Jena, Janjanam, 2018	Breast cancer cells, CAFs, TAMs, adipocytes, endothelial cells	Hardened ECM upregulates extracellular signal-regulated kinase 1/2 (ERK1/2), downregulates Janus kinase 2/signal transducer and activator of transcription 5 (JAK2/STAT5); heparanase activity, hepatocyte growth factor (HGF)/c-Met pathway	[280]
Deligne, Midwood, 2021	Macrophages, CAFs, endotelial cells	ECM modulates immune cell infiltration, polarization; matrix molecules influence tumor-associated macrophage (TAM) phenotype	[281]
Fernández-Nogueira et al., 2021	Breast cancer cells, CAFs, fibroblasts	High collagen activates JNK1, stress/inflammatory pathways; CAFs secrete matrix metalloproteinases (MMPs), modify collagen orientation	[282]
Martínez, Smith, 2021	Breast cancer cells, CAFs, macrophages, T cells, myofibroblasts	Collagen crosslinking via LOX, Yes-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ) pathway, immune exclusion, metabolic reprogramming	[283]
Tamayo-Angorrilla et al., 2021	CAFs (implied), other TME cells	ECM remodeling affects cell behavior and therapy response (mechanisms not specified)	[284]
Lepucki et al., 2022	Breast cancer cells, CAFs, T cells, TAMs, endritic cells, endothelial cells, adipocytes	Collagen crosslinking enhances integrin clustering, phosphoinositide 3-kinase (PI3K) signaling, invasion	[285]
Papanicolaou et al., 2022	Breast cancer cells, cancer-associated fibroblasts (CAFs)	Collagen XII alters collagen I organization, creates pro-invasive microenvironment; ECM stiffness may affect nuclear factor kappa B (NF- B)/c-Jun N-terminal kinase (JNK) pathways	[276]

Based on the studies analyzed and the synthesis presented in Table 6, a convergent thematic scope is observed among the publications, with emphasis on the structural and functional influence of collagen on the different cellular populations of the breast tumor microenvironment (TME) and their respective signaling pathways.

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Levental et al. inaugurate this body of evidence by directly correlating collagen crosslinking with increased ECM stiffness, integrin activation, and PI3K signaling, impacting the invasiveness of epithelial tumor cells [277]. Mao et al. and Oskarsson expand this understanding by exploring how ECM proteins and matrix modifiers modulate tumor resistance through receptors and autocrine/paracrine signaling [278,279]. Jena and Janjanam, in turn, associate collagen remodeling with the MAPK axis in breast cancer cells, highlighting aspects of cellular proliferation [280].

Deligne and Midwood deepen the immunological implications of collagen, revealing that remodeled matrix can favor macrophage (TAM) polarization and impact endothelial and CAF infiltration, creating a permissive environment for tumor progression [281]. The studies by Fernández-Nogueira et al. and Martínez and Smith establish links between collagen density, immune exclusion, and metabolic alterations, citing pathways such as JAK/STAT, NF-κB, and cellular reprogramming [282,283]. Tamayo-Angorrilla et al. reinforce the role of collagen in communication between tumor cells and CAFs, highlighting heparanase as a matrix modulator [284].

Lepucki et al. offer one of the broadest approaches, including a variety of cell types, such as dendritic cells and adipocytes, and correlating collagen with integrins and invasiveness [285]. Finally, Papanicolaou et al. present a multi-scalar and translational perspective, demonstrating how collagen XII secreted by CAFs influences collagen I conformation and promotes cell migration through modulation of fibrillar organization [276].

Taken together, these studies demonstrate that collagen, beyond being a structural element of the ECM, acts as a regulatory agent of cellular communication and molecular signaling in breast cancer, influencing matrix remodeling, immune modulation, phenotypic reprogramming, and therapeutic response. The summarized table consolidates these findings, illustrating how different authors and methodologies converge toward characterizing collagen as a functional modulator of the TME.

3.5.6. Modulation of the Immune Response by Collagen

Tumor cells interact with collagen through several classes of specialized receptors, including the mannose receptor family, glycoprotein VI, leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1), the osteoclast-associated receptor, the discoidin domain receptor family (DDR1 and DDR2), in addition to integrins. These interactions trigger a broad range of cellular responses, such as adhesion, migration, cytokine production, extracellular matrix (ECM) remodeling, and intracellular signal transduction. Such signals modulate the behavior of cancer cells, promoting tissue invasion, proliferation, metabolic reprogramming, chemotherapy resistance, and immune evasion [113].

Both cancer cells and immune cells are capable of binding to collagen through integrins. In tumors, integrin–collagen interactions enhance tumor cell motility, whereas in immune cells, these interactions are antitumorigenic and facilitate the migration of natural killer (NK) cells and T cells into the tumor. Moreover, the increased stiffness of collagen-rich ECM also provides support for antitumor cytokines. However, mechanically, this increased stiffness—resulting from the deposition of aligned, elongated, and organized collagen fibers—can impede immune cell infiltration into the tumor nest, while simultaneously creating favorable pathways for tumor cell dissemination [304].

Thus, beyond its actions on tumor cells, collagen also exerts effects on immune cells infiltrating tumors, as DDR1 activation can promote immune evasion by favoring collagen fiber alignment. In a highly immunosuppressive TME, T-cell cytotoxicity against cancer cells is suppressed due to the presence of immunosuppressive phenotypes such as polarized M2 macrophages, myeloid-derived suppressor cells, and regulatory T cells. In addition, a densely compacted collagen environment affects T-cell activity and regulates the speed of their migration into tumors [184].

In hepatocellular carcinoma, an immunosuppressive TME is established, and collagen can modulate this immune context. During tumor progression, the ECM is degraded and replaced by collagen, which can induce macrophages toward an immunosuppressive phenotype. This results in reduced T-cell proliferation and diminished recruitment of cytotoxic T cells. Furthermore, the highly

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compact collagen structure of the ECM can act as a physical barrier, thereby impairing the infiltration of CD8+ T cells and NK cells, resulting in tumor progression within an "immune desert" [291].

3.5.7. Impact of Collagen Type Differences on Cancer Development

Within the complex and dynamic interplay between tumor cells and the ECM, collagens account for approximately 30% of its composition, with 28 different types identified, several of which participate in the interstitial ECM and basement membrane. Collagens are relevant to cancer development and precancerous lesions, as recent studies have reported abnormal collagen appearance during tumor progression—such as degradation, fragmentation, remodeling, linearization, deposition, and fasciculation—thus conferring recognized diagnostic and prognostic value [114].

Depending on the context, collagen type IV, one of the ECM components, may exert either pro-tumor or anti-tumor effects; however, in the vast majority of studies, it has been correlated with reduced survival and increased metastasis risk. In vitro studies in endothelial cells have shown that canstatin and tumstatin, both derived from the α3 chain of collagen type IV, may exert pro-apoptotic actions and inhibit angiogenesis. Conversely, in breast, gastric, pancreatic, and colorectal cancers, collagen type IV fragments have been associated with lower survival and greater invasiveness. Additionally, collagen type XII secreted by CAFs can alter the structural organization of collagen type I, creating a TME that supports metastatic dissemination. Collagen type I may also initiate EMT via ILK-dependent phosphorylation of IκB, leading to Snail and LEF-1 transcription factor expression, as well as decreased E-cadherin expression [276].

In breast cancer, suppression of collagen type IV α2 chain significantly inhibits the proliferation and migration of triple-negative breast cancer cells. Moreover, variations in scarcer collagen types, such as types XV and XVIII, may be involved in angiogenic and lymphangiogenic processes within the TME. Additionally, some other low-expression collagen types may correlate with a better prognosis in breast cancer, including collagen type XXV α1, collagen type XVII α1, collagen type VI α6, collagen type IV α6, and collagen type IV α3 (317]. The complex and often distinct influences of the various collagen types and expression patterns on tumor progression are multifaceted and context-dependent. Table 7 summarizes the principal effects of collagen expression patterns and specific collagen types on cancer progression.

Table 7. Expression patterns and collagen types and their effects on tumor progression.

Study	Collagen Type(s)	Expression Pattern	Impact on Progression	Clinical Correlation	References
Conklin et al., 2011	No mention found (TACS-3)	Bundles of straight, aligned collagen perpendicular to tumor boundary	TACS-3 reported as an independent predictor of poor disease-specific and disease-free survival	TACS-3 reported to correlate with stromal syndecan-1 expression	[318]
Brodsky et al., 2016	Type X collagen alpha 1 chain (COL10A1)	Increased stromal COL10A1 in non-responders	Reported association with poor pathologic response	High COL10A1 and low tumor-infiltrating lymphocytes reported to predict poor neoadjuvant therapy response	[319]
Thangavelu et al., 2016	Collagen type XVII alpha 1 chain (COL17A1)	Underexpression in breast cancer; overexpression	Underexpression in breast câncer reported to be	COL17A1 underexpression reported to	[320]

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		in cervical cancer	linked to increased invasion and poor	predict poor distant metastasis-free, metastasis-free, recurrence-free, and overall survival	
Esbona et al., 2018	No mention found	High local density, alignment, and perpendicular orientation (tumor-associated collagen signature-3, TACS-3)	Reported to predict poor overall survival	High COX-2, CD68, CD163 and aligned collagen, reported to predict worse survival	[314]
Liu et al., 2018	Collagen type I alpha 1 chain (COL1A1)	High expression in breast cancer cells and patients	Reported to promote metastasis; knockdown reported to inhibit metastasis	High COL1A1 reported to be associated with poor survival in estrogen receptor-positive patients	[308]
Ren et al., 2018	Collagen type V alpha 1 chain (COL5A1)	Overexpressed in invasive ductal carcinoma, co-polymerizes with type I collagen	Reported association with increased cell viability, migration, invasion; knockdown reported to reduce these	High COL5A1 mRNA reported to be associated with distant metastasis-free survival; overexpression linked to estrogen/progesterone receptor status	[321]
Tomko et al., 2018	Collagen type XII alpha 1 chain (COL12A1), tenascin C (TNC), thrombospondin-2 (THBS-2	Aligned collagen fibers (TACS-3) co-localized with TNC, THBS-2	TACS-3 and associated proteins reported to correlate with poor distant metastasis-free survival	COL12A1, TNC, THBS-2 signature reported to predict poor outcome	[322]
Wang et al., 2018	Type X collagen alpha 1 chain (COL10A1), elastin	COL10A1/elastin complex forms amorphous clumps in tumor stroma	Reported correlation with poor pathologic response to neoadjuvant chemotherapy	High COL10A1/elastin expression reported to correlate with poor outcome	[323]
Wei et al., 2019	Type I collagen, PLOD2	Adipocyte-driven remodeling reported to increase alignment and	Reported to promote metastasis; PAI-1/PLOD2 axis reported to drive collagen reorganization	High PAI-1 reported to correlate with poor prognosis, especially in HER2-	[324]

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		density at invasive front		negative/estrogen receptor-negative and triple negative cancers	
Volk et al., 2023	Type III collagen (COL3A1), Type I collagen (COL1A1)	High type III to type I collagen ratio in noninvasive regions; more aligned fibers in invasive areas	Type III collagen reported as tumor-restrictive; high type III to type I collagen ratio reported to improve survival and reduce metastasis	High type III to type I collagen ratio associated with improved overall, disease-free, and progression-free survival	[312]

The structural diversity of collagens and their expression patterns within the tumor microenvironment (TME) have proven to be key determinants in modulating neoplastic progression and clinical outcomes in breast cancer. Table 7 synthesizes findings from ten studies exploring different collagen isoforms, their fibrillar architectures, and prognostic implications, revealing a complex and functionally relevant molecular landscape.

Collagen type I (COL1A1) was the most frequently investigated, appearing in three studies [308,314,318], in which its overexpression and perpendicular organization at tumor margins (TACS-3) were associated with increased invasiveness, reduced overall survival, and unsatisfactory therapeutic response. By contrast, collagen type III (COL3A1), investigated by Brodsky et al. [319], was correlated with a tumor-restraining effect, particularly when present in high proportion relative to type I, suggesting that the fibrillar composition ratio modulates tumor aggressiveness.

Other isoforms, such as COL10A1 [323,325], COL12A1 [312], COL17A1 [324], and COL5A1 [322], were examined in specific contexts, revealing associations with co-polymerization, amorphous aggregates, interactions with matrix proteins, and immunological markers. These structural variations were implicated in processes such as angiogenesis, cell migration, therapeutic resistance, and tumor recurrence.

Regarding expression patterns, five studies—including those by Conklin et al., Esbona et al., and Volk et al.—highlighted perpendicular fiber orientation as a marker of tumor aggressiveness. Two studies reported high collagen density, while others identified amorphous aggregates, co-localization with ECM proteins, and overexpression of specific isoforms, such as COL10A1 and COL17A1 [312,314,318].

In terms of clinical impact, eight of the ten studies correlated collagen-related features with unfavorable outcomes, including reduced overall survival [56,308,314,318], decreased distant metastasis-free survival [308,314,323], and poor therapeutic response [308,314]. Only Brodsky et al. [2016] reported an association with improved prognosis, reinforcing the functional complexity of collagen isoforms.

Additionally, studies by Wei et al. and Volk et al. identified correlations with immunological markers (COX-2, CD68, CD163, TILs), hormone receptors (ER/PR, HER2), and stromal syndecan-1, demonstrating that the collagen profile may reflect not only tumor mechanics but also the immunological and phenotypic composition of the TME [312,324].

Further contribution from the study by Zhang et al. indicates that COL11A1 overexpression is inversely associated with infiltration of multiple immune subpopulations, including NK cells, Tregs, Th1/Th2 lymphocytes, neutrophils, and macrophages, suggesting an immunomodulatory role of collagen in TME architecture [273]. This physical barrier appears to be related to collagen fiber alignment mediated by the extracellular domain of the DDR1 receptor, whose presence may limit infiltration of CD8+ and CD4+ T lymphocytes, as also proposed by Lo Buglio et al. [304].

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3.6. Therapeutic Approaches and Translational Innovation

3.6.1. Conventional Therapies Versus Alternative Therapies

Conventional therapies, such as chemotherapy and radiotherapy, are widely recognized for their effectiveness in cancer treatment. Chemotherapy employs potent chemical agents aimed at attacking and destroying rapidly dividing cells, affecting both tumor cells and healthy tissues [326,327].

Radiotherapy, on the other hand, uses ionizing radiation to damage the DNA of cancer cells, preventing their replication and promoting tumor destruction. In the context of breast cancer, neoadjuvant chemotherapy plays a fundamental role. Administered prior to surgery, this approach seeks to reduce tumor size, facilitating surgical resection and, in many cases, allowing breast preservation. It also helps combat subclinical metastases and enables a more precise assessment of tumor response to treatment, contributing to improved therapeutic planning [167]. Despite their effectiveness, conventional therapies have limitations that frequently impact patients' quality of life [328,329].

Chemotherapy can affect healthy cells that also have a high division rate, such as those of the bone marrow, hair follicles, and gastrointestinal tract, resulting in side effects such as immunosuppression, anemia, hair loss, nausea, and fatigue. Radiotherapy, in turn, may cause damage to tissues adjacent to the tumor, leading to inflammation, fibrosis, and other long-term adverse effects. The toxicity associated with these therapies may compromise not only physical well-being but also the psychological and social health of patients [244].

These limitations underscore the need for new therapeutic strategies that are more selective and present lower toxicity, in order to minimize negative impacts on patients' well-being. The evolution of cancer therapies reflects a continuous search for approaches that are more effective and less invasive. Historically, practices such as acupuncture and phytotherapy have been used across cultures to alleviate symptoms and improve the quality of life of oncology patients. However, the integration of these complementary therapies into conventional medicine faced challenges, including the need for standardized studies demonstrating their benefits and risks. For example, apitherapy and other complementary therapies faced resistance from the biomedical community due to the lack of robust scientific evidence [330].

In recent decades, significant advances have brought new perspectives to oncologic treatment. Immunotherapy, for example, aims to strengthen the patient's immune system so that it recognizes and combats cancer cells more effectively. Since the first reports in the late 19th century, when William Bradley Coley used bacteria to stimulate an immune response against tumors, immunotherapy has advanced considerably. Currently, immune checkpoint inhibitors, such as anti-PD-1 and anti-PD-L1 antibodies, have demonstrated efficacy in various cancers, providing durable responses and improved survival [331]. Targeted therapies represent another major innovation, focusing on specific molecules involved in tumor cell growth and survival. By targeting these molecules, damage to healthy cells is minimized, increasing treatment accuracy and effectiveness. The introduction of these approaches has revolutionized oncology by enabling more personalized treatments with fewer side effects than conventional therapies [244].

The trajectory of cancer therapies demonstrates a progression from traditional practices, such as acupuncture and phytotherapy, to modern innovations such as immunotherapy and targeted therapies. This evolution reflects a continuous commitment to improving therapeutic strategies, aiming for treatments that are more effective and have reduced impact on patients' quality of life [167].

Alternative and complementary therapies in cancer treatment, although promising, face significant challenges related to high cost and limited accessibility. Access to therapies such as immunotherapy and targeted treatments is not always feasible in low-income settings, and elevated costs may be an obstacle to large-scale implementation. According to recent studies, advances in

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high-technology therapies, although effective, require significant investments, limiting their applicability in public health systems and in countries with restricted financial resources [332].

Beyond the physical challenges, emotional and family support is essential for the recovery and quality of life of oncology patients. Emotional distress caused by physical changes, such as hair loss and altered appearance, can significantly affect self-esteem. Social isolation and emotional difficulties stemming from treatment directly impact quality of life, making psychological support a crucial component of cancer care [333].

It is important to discuss the safety and risks associated with alternative therapies. Although some have shown benefits, they may interact with conventional treatments and, in certain cases, cause adverse effects. Medical supervision is essential to ensure there are no contraindications or negative interactions. The literature emphasizes the need to carefully evaluate the biological risks associated with the use of biotechnologies and alternative therapies, implementing technical, administrative, and legal measures aimed at damage prevention and health promotion. Despite the adversities associated with conventional treatments, such as side effects and access challenges, they remain essential for eliminating cancer cells and reducing metastasis risk [62,332,333].

In this context, it is fundamental that healthcare professionals adopt an integrative approach, considering not only the clinical aspects of treatment but also the emotional and psychological health of patients. The integrative care model, which combines conventional treatments with complementary therapies and psychological support, is crucial to ensuring well-being and quality of life throughout the healing process. Additionally, treatment personalization, taking into account the individual characteristics of each patient and their cancer, is essential to optimize outcomes and minimize side effects [334,335].

The use of targeted therapies and immunotherapy, for example, has shown potential to reduce toxicity and improve treatment efficacy, although challenges such as cost and accessibility still must be overcome. With ongoing research advances, the future of cancer treatment is expected to be not only more effective and less aggressive but also more humane, respecting the physical and emotional needs of patients [336].

3.6.2. Therapeutic Potential of Collagen

Fibrillar type I collagen constitutes the main component of the extracellular matrix (ECM), representing up to 90% of the protein composition of connective tissues. In the tumor context, cancer cells actively modulate the formation of peritumoral collagen, while the mechanical properties of collagen and the tumor microenvironment (TME) play a critical role in regulating the behavior of neoplastic cells [200]. During cancer progression, the compressive mechanical forces generated by tumor growth promote an invasive phenotype and facilitate cellular migration. Simultaneously, these forces contribute to tumor hypoxia due to the collapse of lymphatic vessels and small blood vessels, as well as increased interstitial fluid pressure. TME stiffness, largely associated with increased deposition and rearrangement of ECM proteins compared with adjacent healthy tissues, is a determining factor for the heightened risk of metastasis [200].

This process is related to the deposition of proteins such as fibronectin, proteoglycans, and type I, III, and IV collagen, as well as the increase in cross-linking between ECM components. Thus, ECM stiffening not only contributes to tumor progression but also represents a relevant biological marker for understanding the interplay between ECM mechanical properties and cancer cell behavior [200].

The architecture and orientation of collagen fibers in the peritumoral stroma play an essential role in modulating cancer cell migration, directly influencing tumor progression. In tumors with high invasive potential, the ECM is characteristically stiff. However, the organization of collagen fibers is a determining factor in regulating neoplastic cell behavior. In early stages of breast cancer, the parallel arrangement of collagen fibers around the tumor forms a dense functional barrier that hinders cellular invasion. However, as the tumor expands, the mechanical pressure exerted on these fibers intensifies. This increased compressive force promotes structural alterations in collagen, facilitated by interactions with recruited stromal and tumor-associated cells, such as cancer-associated

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fibroblasts (CAFs) and tumor-associated macrophages (TAMs). These cells interact with collagen fibers through integrin-mediated binding, contributing to matrix remodeling and collagen deformation [200].

Additionally, collagen exerts significant influence on tumor development by modulating exosome secretion. These extracellular vesicles, surrounded by lipid membranes, are secreted by cancer cells to facilitate intercellular communication and interactions with the ECM. Exosomes play a critical role in regulating cellular survival, tumor growth, tissue invasion, and metastasis. In the peritumoral stroma, exosomes promote the differentiation of fibroblasts into CAFs, induce epithelial-mesenchymal transition (EMT) in cancer cells, and stimulate secretion of MMP-14. This enzyme is responsible for digesting and remodeling the collagen network, thereby facilitating tumor invasion [337,338].

The degradation and rupture of peritumoral fibers become more pronounced when matrix metalloproteinases (MMPs) are released by cancer cells and infiltrate collagen fibers. This proteolytic process, associated with structural remodeling of the stroma, favors cellular invasion, particularly when collagen fibers align perpendicularly to the tumor boundary. This ECM reorganization, combined with the effect of MMPs on collagen–proteoglycan cross-links, creates spaces between fibers and fibrils, allowing cancer cells direct access to migrate toward adjacent tissues [113,251].

Breast cancer, marked by its biological heterogeneity, has become the leading cause of cancer-related mortality among women worldwide. This scenario reflects the significant clinical challenges involved, considering the high morbidity and mortality associated with the disease. Despite substantial scientific advances, identifying specific and effective therapeutic targets remains a critical obstacle. High mammographic density, frequently associated with increased collagen content in breast tissue, is recognized as an important risk factor for breast cancer development. Recent studies suggest that modulation of collagen composition and organization in the ECM may have diagnostic, prognostic, and therapeutic implications. Furthermore, specific members of the collagen family have been identified as potential relevant biological targets, highlighting the importance of translational research in advancing breast cancer treatment [317].

Alteration of collagen expression profiles is a hallmark of breast cancer, playing a central role in tumor progression and metastasis. In this malignancy, collagen undergoes significant changes not only in abundance but also in molecular integrity, contributing to TME reorganization and modulating the phenotypic attributes of neoplastic cells. A critical aspect of breast cancer pathophysiology is the increase in collagen density, particularly type I collagen, the most prevalent stromal variant. Additionally, collagen fiber orientation and organization play distinct roles in tumor invasion: while linearized (fibrillar) type I collagen favors invasive phenotypes, high-density non-fibrillar collagen may exert tumor-suppressive effects [191,317].

In this context, the role of collagen in regulating both tumor progression and therapeutic resistance stands out. Recent studies combining in vitro, in vivo, and bioinformatic approaches have investigated the functions of type III collagen (Col3) in human breast cancer. Findings revealed that Col3 may act as a tumor suppressor modulator. Human fibroblasts deficient in Col3 produce a collagen matrix that is permissive to tumor growth, promoting cellular proliferation and inhibiting apoptosis in invasive and non-invasive breast cancer cell lines. In biopsy samples from human triple-negative breast cancer, elevated Col3 deposition was observed in non-invasive regions compared with invasive regions, suggesting a distinct role of this collagen in tumor behavior [339].

Moreover, in vivo experiments demonstrated that co-injection of murine breast cancer cells with hydrogels enriched with rhCol3 resulted in reduced tumor growth and decreased pulmonary metastatic burden compared with control groups. These results support a tumor-suppressive role for Col3 in human breast cancer, suggesting that therapeutic strategies aimed at increasing Col3 expression or supplementation may represent a safe and effective approach to limiting tumor progression and recurrence in breast cancer patients [339].

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3.6.3. Exosomes and Tumor Progression

Exosomes are extracellular vesicles (EVs) that originate from the cell membrane and are involved in several biological functions in both physiological and pathological conditions. They are produced through a process of cytoplasmic membrane invagination, resulting in the formation of multivesicular bodies (MVBs) that contain intraluminal vesicles (ILVs), which are released as exosomes measuring 40 to 160 nm in diameter. MVBs then fuse with the plasma membrane and release exosomes into the extracellular space via exocytosis. These exosomes carry cargo specific to their host cell, and when internalized via endocytosis, they are capable of releasing their content and facilitating intercellular communication [340].

In cancer, exosomes originate from tumor cells and participate in bidirectional cell-to-cell communication. They play roles in cellular migration and invasion, angiogenesis, and immune evasion, and are fundamental for interactions among cells within the tumor microenvironment (TME), transporting proteins, RNAs, and lipids [269,317].

From this perspective, exosomes have a crucial role at the interface between the TME and tumor cells, constituting a fundamental mechanism for tumor initiation and progression. Thus, exosomes can modulate the ECM and the TME through extracellular receptors, signaling pathways, and disruption of cell adhesion. Furthermore, integrins and integrin ligands can be transported by exosomes, contributing to tumor cell colonization and formation of the pre-metastatic niche. Additionally, cancer-derived exosomes may induce the differentiation of various TME cell types into cancer-associated fibroblasts (CAFs), the dominant stromal population, making exosomes critical for ECM remodeling and TME reprogramming. Exosomes derived from CAFs contain microRNAs and growth factors that act on different target cells within the TME [242].

Macrophage polarization toward an M2 phenotype can drive tumor progression, and exosomes are able to alter polarization toward tumor-associated macrophages. Exosomes carry gp130, which induces STAT3 signaling through IL-6 upregulation. STAT3 signaling is a pro-oncogenic pathway that promotes tumor growth and metastasis while inhibiting cell-cycle arrest and apoptosis. Consequently, STAT3 induces EMT, resulting in tumor invasion, poor prognosis, and chemoresistance. Additionally, exosomes containing STAT3 also carry MMP-2, MMP-9, and cyclin D1, thereby promoting cellular proliferation and tumor invasion in breast cancer [249].

Exosomes also transport essential elements that induce EMT, including TGF-β, IL-6, β-catenin, HIF-1α, caveolin-1, and nucleic acids such as microRNAs. During EMT, the ECM undergoes degradation, and tumor cells lose epithelial characteristics and transition to a mesenchymal phenotype, becoming aggressive and invasive. Moreover, tumor-derived exosomes also induce vascular formation and angiogenesis through activation of the VEGF signaling pathway, as they transport several angiogenic factors, including fibroblast growth factor (FGF), TGF-β, TNF-α, IL-8, and platelet-derived growth factor (PDGF) [220,338].

4. Conclusions and Translational Implications and Future Research

This comprehensive review successfully achieved its objective by systematically consolidating the most recent evidence positioning collagen not merely as a physical scaffold, but as a central, bioactive regulator of tumor progression and therapeutic response modulation in breast adenocarcinoma. The synthesis of findings reinforces the emerging oncological paradigm: the extracellular matrix (ECM)-rich tumor microenvironment (TME) is a crucial determinant, and stromal manipulation is essential for overcoming intrinsic therapeutic failure. The in-depth analysis revealed that alterations in collagen architecture, namely increased stiffness (fibrosis), COL1A1 hyper-deposition, and the perpendicular alignment of fibers (TACS-3) at tumor margins, function as powerful accelerators of malignancy. These structural modifications orchestrate a pro-tumoral milieu through biomechanical signaling (e.g., integrin/PI3K/Akt and TGF-β/YAP pathways) that drives Epithelial–Mesenchymal Transition (EMT), invasion, and metastatic dissemination. Crucially, from a biopharmaceutical perspective, the dense and reticulated stroma imposes a significant physical

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barrier to the efficient penetration and diffusion of chemotherapeutics and immune agents, fostering intrinsic drug resistance.

From a translational perspective, this review validates collagen as a potent prognostic biomarker and a highly promising therapeutic target. The future treatment of breast cancer in fibrotic tumors necessitates adjuvant strategies aimed at disarming the collagenous matrix, either by inhibiting cross-linking enzymes (such as LOX and LOXL2) or by modulating tumor mechanoreactivity. Therefore, these biological insights provide the essential parameters for the pharmaceutical industry to develop next-generation delivery platforms. We advocate that future investigations prioritize: 1) the clinical validation of collagen biosynthesis and cross-linking inhibitors in combination with standard chemotherapy and immunotherapy regimens; and 2) the development of advanced 3D-bioprinted tumor models that faithfully recapitulate the mechanical properties of the ECM, providing a more accurate platform for pharmacological screening and the study of drug transport kinetics. In summary, by focusing on stromal biology, this study charts an unequivocal course toward the enhancement of prognosis and therapeutic efficacy in breast cancer, shifting the exclusive focus from the tumor cell to a more integral approach that encompasses the 3D microenvironment architecture that sustains it.

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