








Review

Collagen and Its Roles in the Tumor Immune Microenvironment: Structure, Function, and Therapeutic Potential

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Abstract

Collagen, the primary structural protein of the extracellular matrix, shows marked structure–function duality in the tumor immune microenvironment (TIME). Biomechanical and biophysical alterations—matrix stiffening, viscoelastic energy dissipation, fiber alignment and tumor-associated collagen signatures, reduced pore size, and solid stress—create migration tracks, sequester signaling cues that regulate proliferation and metabolism, and form dense barriers that impede immune infiltration. These changes are sensed and transduced via mechanosensing and mechanotransduction pathways, thereby reinforcing malignant behavior and immune exclusion. Given its dynamic, spatiotemporally regulated roles, collagen is a promising therapeutic target to overcome immunotherapy resistance. This review examines the structural features, biological functions, and regulatory pathways of collagen within the TIME. Based on these insights, several clinical strategies were highlighted: targeting cancer-associated fibroblasts and profibrotic signaling to reduce fibrosis; remodeling the matrix enzymatically or physically; and inhibiting collagen receptor signaling. These approaches are often combined with immune checkpoint inhibition. Future directions will emphasize biomarker-guided stratification of collagen status; combining therapies informed by mechanobiology; and using scalable, noninvasive monitoring to optimize immunotherapy outcomes.

Keywords: tumor immune microenvironment; collagen; extracellular matrix remodeling; mechanosensing; therapeutic target; immunotherapy

1. Introduction

Collagen, comprising approximately 30% of the total protein mass in mammals, represents the most abundant structural component of the extracellular matrix (ECM). The collagen superfamily encompasses 28 distinct types (I–XXVIII) that collectively orchestrate a sophisticated three-dimensional scaffold essential for tissue architecture, biomechanical properties, and cellular homeostasis [1]. Under physiological conditions, the synthesis, deposition, and degradation of collagen are tightly regulated by a complex interplay of matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and lysyl oxidases (LOX), maintaining a precise dynamic equilibrium. However, within the tumor immune microenvironment (TIME), this exquisite balance undergoes profound dysregulation, transforming collagen from a passive structural element into an active driver of malignant progression and therapeutic resistance [2,3].

The pathological hallmark of many solid tumors is a pronounced desmoplastic reaction, characterized by excessive deposition of fibrillar collagens (predominantly types I, III, and V), aberrant cross-linking mediated by the LOX family of enzymes, and architectural remodel-

ing of the ECM [4]. Tumors characterized by dense, collagen-rich tissue include pancreatic ductal adenocarcinoma, cholangiocarcinoma, and triple-negative breast cancer [5]. By contrast, tumors with relatively low collagen content include clear cell renal cell carcinoma and glioma. This fibrotic response is primarily orchestrated by cancer-associated fibroblasts (CAFs), which are activated through multiple mechanisms, including profibrotic signaling pathways such as transforming growth factor beta (TGF- β), platelet-derived growth factor/fibroblast growth factor (PDGF/FGF), and the renin–angiotensin axis (RAS), as well as mechanical stress and metabolic reprogramming [6]. CAFs upregulate collagen type I A1/2 (COL1A1/2) and LOX enzymes to create a protumorigenic niche, including increased matrix stiffness (often exceeding 10-fold that of normal tissue), linearization of collagen fibers, and formation of tumor-associated collagen signatures (TACS) [7]. These physical changes facilitate tumor cell invasion through contact guidance and durotaxis, while simultaneously activating mechanotransduction pathways such as Yes-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ) and integrin-focal adhesion kinase (FAK) signaling that promote proliferation and stemness [8].



Critically, remodeled collagen matrix acts as a powerful immunosuppressive barrier through multiple interconnected mechanisms. Dense, linearized collagen fibers physically exclude cytotoxic T lymphocytes (CTLs) and other immune effectors from the tumor parenchyma, a phenomenon quantified by reduced CD8⁺ T cell/regulatory T cell (Treg) ratios in collagen-rich regions [9]. Beyond physical exclusion, collagen fragments and specific collagen receptors (e.g., integrins) deliver inhibitory signals that directly suppress T-cell activation and promote the polarization of tumor-associated macrophages (TAMs) toward an M2-like phenotype [10]. This creates a self-reinforcing cycle, as inflammatory cytokines and reactive oxygen species generated during frustrated immune responses paradoxically enhance CAF activation and collagen production through signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappa B (NF- κ B) pathways [11]. Distinct CAF subsets, including myofibroblastic, inflammatory, and antigen-presenting, work together to remodel the tumor's collagen-based ECM and shape antitumor immunity.

In addition to protumor roles, collagenous stroma can exert context- and stage-dependent antitumor effects. Intact desmoplastic encapsulation may mechanically contain the lesion, stabilize perfusion, and transiently limit dissemination or antigen spillover [12]; however, chronic inflammation, fibroblast reprogramming, LOX-mediated crosslinking, hypoxia signaling, and the immune system all work to break down the natural barriers, restructuring the tissue matrix into a dense, aligned network that promotes tumor invasion and excludes immune cells [13].

The clinical significance of this collagen-mediated immunosuppression cannot be overstated. Recent analyses of immunotherapy trials have identified high collagen density and specific collagen gene signatures as robust predictors of a poor response to checkpoint inhibitors across multiple cancer types [9]. Therefore, targeting the collagenous ECM has emerged as a crucial strategy for overcoming resistance to immunotherapy.

2. Structure and Characteristics of Collagen in the TIME

In physiological settings, tropocollagen, the basic building block of collagen, consists of three polypeptide chains coiled into a canonical right-handed triple helix. These monomers spontaneously self-assemble into staggered arrays, forming collagen fibrils. Subsequently, these fibrils are stabilized and endowed with tensile strength through covalent cross-linking. This porous and biomechanically compliant matrix provides critical tensional support and adhesion cues for maintaining cellular quiescence, while ensuring low-resistance pathways for nutrient perfusion and immune surveillance [1]. However, the collagen architecture within the TIME undergoes profound pathological remodeling (Fig. 1).

2.1 Reshaping of Physical Properties

The changes in collagen in terms of stiffness, spatial structure, and fiber morphology jointly promote tumor progression.

A primary alteration is the dramatic stiffening of the tumor stroma, a process driven by both the aberrant deposition of fibrillar collagens and their extensive cross-linking. Tumors frequently show higher levels and enzymatic activity of LOX enzymes, catalyzing the formation of covalent bonds that dramatically increases tissue tension [14]. Clinically, this is evidenced by the elastic modulus of breast cancer tissue, which can reach 4–12 kPa, starkly contrasting with the 0.4–2 kPa of normal mammary tissue [15]. This heightened matrix stiffness is not a passive feature but is a potent protumorigenic signal.

Concurrently, the matrix undergoes profound architectural reorganization. The physiological, porous, and randomly oriented collagen meshwork transforms into a dense, linearized architecture, resulting in a significant reduction in interstitial pore size from the normal range of 10–50 μ m down to 1–10 μ m in tumors [16]. This densified structure forms a formidable physical barrier that severely impedes the intratumoral trafficking of immune effector cells, such as CTLs, as well as the perfusion of therapeutic agents. This phenomenon, widely termed “immune exclusion”, is an important mechanism contributing to resistance against both chemotherapy and immunotherapy [5].

On a microscopic scale, the morphology of individual collagen fibers is also remodeled. Tumor-associated collagen fibers become significantly thicker and longer, with diameters increasing from 50–500 nm in healthy tissue to 200–2000 nm [17]. Critically, at the tumor's invasive front, these fibers are often realigned and bundled into taut, linear tracks oriented perpendicularly to the tumor boundary. These unique topological features are known as TACS [18].

2.2 Remodeling of Biochemical Properties

Beyond its role as a physical scaffold, the collagen matrix operates as a dynamic and potent biochemical signaling hub. On the one hand, the TME's collagen network functions as a crucial reservoir, concentrating and modulating the bioavailability of growth factors, cytokines, and chemokines. The high density of positively charged residues on collagen fibers facilitates the electrostatic sequestration of numerous anionic signaling molecules, including TGF- β , vascular endothelial growth factor (VEGF), and FGF [8]. This interaction is further stabilized and specified by intermediaries such as heparan sulfate proteoglycans, which act as co-receptors [17]. This creates a localized, high-concentration gradient of protumorigenic signals.

On the other hand, the collagen fibers themselves serve as solid-phase ligands, directly initiating intracellular signaling cascades upon binding to cell surface receptors on both cancer and immune cells. On cancer cells,

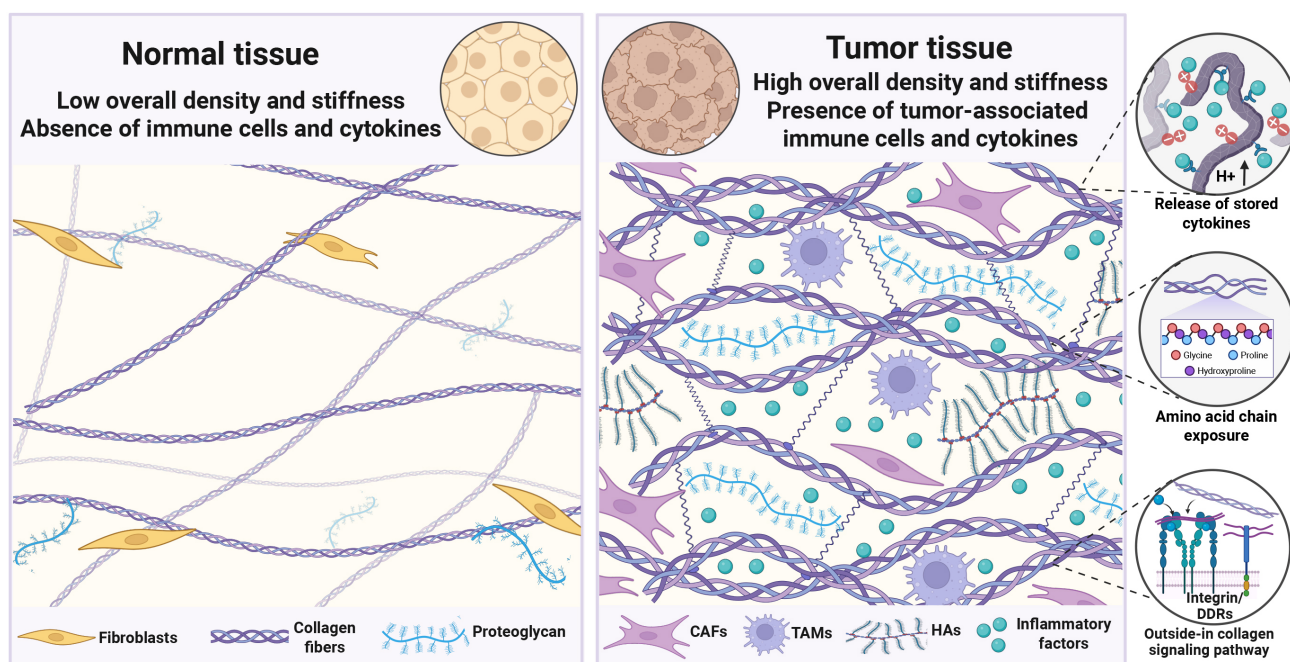


Fig. 1. Remodeling of collagen in the tumor immune microenvironment (TIME). Unlike normal tissues, collagen fibers in the TIME are characterized by increased diameter, greater density, and extensive cross-linking, leading to formation of the unique tumor ECM that is both highly dense and rigid. Collagen interacts with tumor and immune cells by releasing inflammatory factors, exposing binding sites, and recognizing collagen receptors. CAFs, Cancer-associated fibroblasts; TAMs, Tumor-associated macrophages; HAs, Hyaluronic acids.

specific cryptic binding motifs within the collagen triple helix, such as the GFOGER (for Gly-Phe-Hyp-Gly-Glu-Arg) and RGD (Arg-Gly-Asp) sequences, become exposed during matrix remodeling [19]. These motifs are recognized by integrin heterodimers and discoidin domain receptors 1 and 2 (DDR1/2), which are unique receptor tyrosine kinases [20]. This engagement establishes a pernicious feed-forward loop: activated cancer cells increase their secretion of MMPs, which further remodel the collagen matrix, exposing more binding sites and thus amplifying these protumorigenic signals. Additionally, collagen can bind to inhibitory receptors on immune cells (e.g., leukocyte-associated immunoglobulin-like receptor-1 [LAIR-1]) [21].

3. Functions of Collagen in the TIME

Structural remodeling of the collagen matrix is not just a sign of disease but is the mechanistic basis for changes to its function. Thus, within the TIME, collagen is not a passive architectural bystander but is a dynamic orchestrator of tumor malignancy and immunity (Fig. 2).

3.1 Physical Functions: Contact-Guided Migration and Permeability Barrier

The primary physical function of collagen is to provide a critical three-dimensional structural support framework for tumor tissues. Tumor cells tend to proliferate preferentially in regions with higher collagen density. These areas not only offer stable anchor points necessary for cells

to resist mechanical stress and maintain their shape but also function as migration tracks [22]. Cells can migrate along these collagen fibers through contact guidance, at speeds up to 5 to 10 times faster than free migration in the matrix. Live imaging techniques have confirmed that tumor cells preferentially invade outward along collagen fibers perpendicular to the tumor boundary (TACS-3) [18]. Notably, different types of immune cells have varying abilities to utilize these collagen tracks, which may be related to their deformability: dendritic cells and macrophages can efficiently use these tracks for migration, while T-cell migration is more dependent on the interstitial network's gaps [23].

High-density collagen deposition can form a fibrous capsule around the tumor, serving a protective function. On the one hand, this dense barrier severely hinders the effective penetration of chemotherapy drugs and antibody drugs, which is a significant cause of treatment resistance. For instance, in pancreatic cancer characterized by dense stroma, the penetration distance of drugs is often limited to 50–100 micrometers around blood vessels, far below the range required to achieve an effective therapeutic concentration [24]. On the other hand, it significantly restricts the infiltration of immune effector cells. Studies have shown that for every doubling of collagen density, the infiltration depth of CD8⁺ T cells decreases by approximately 30% [11]. Notably, some immune cells also have regulatory roles in tumor fibrosis. Research has found that TAMs, through man-

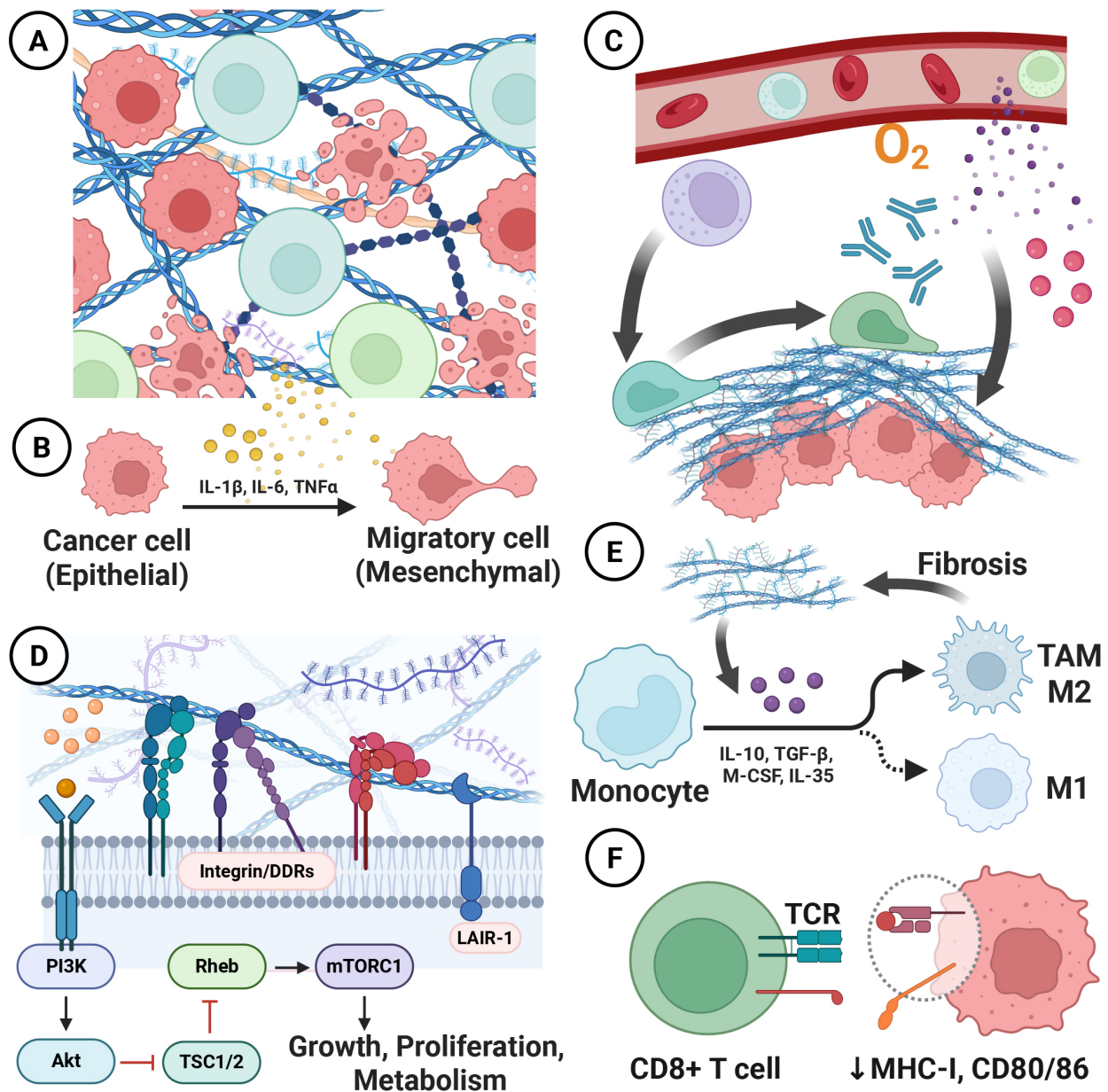


Fig. 2. Functions of collagen in the TIME. Collagen is not merely a passive bystander but is an active participant in malignancy, playing a dynamic role through its intricate “structure–function” continuum: (A) Within the tumor, the collagen fiber network provides cells with spatial guidance and movement tracks. (B) Collagen-derived cytokines promote the tumor epithelial–mesenchymal transition (EMT) and subsequently facilitate tumor metastasis. (C) At the tumor margin, the collagen barrier restricts the infiltration of immune cells, antitumor drugs, and nutrients. (D) Collagen mediates extracellular-to-intracellular signal transduction by releasing stored cytokines or binding to collagen receptors. (E,F) Collagen inhibits tumor immune responses by inducing the polarization of anti-inflammatory cell phenotypes and downregulating major histocompatibility complex class II (MHC-II) and co-stimulatory molecules.

nose receptor-mediated mechanisms, upregulate inducible nitric oxide synthase, thereby generating reactive nitrogen species to promote tumor fibrosis [25].

3.2 Biochemical Functions: Signal-Mediated Phenotypic and Immune Regulation

In the TIME, the physical functions of collagen and its biochemical functions are not independent of each other but

form a continuous spectrum through “mechano-chemical transduction”: extracellular mechanical stress is first integrated into intracellular signals, which then cascade to affect cell proliferation, metabolism, and immune regulation [26].

Mechanical cues of collagen are sensed by cell-surface receptors (integrins $\alpha2\beta1$, $\alpha11\beta1$ and DDR1/2), which activate mechanosensitive pathways such as FAK–

extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase–Akt, and the Hippo–YAP/TAZ axis [27]. Studies have shown that the stiff collagen matrix can accelerate the transition of the cell cycle from G1 to S phase by 2 to 3 times through activation of the FAK–ERK signaling pathway, thereby promoting tumor cell proliferation [13]. At the same time, collagen signals can upregulate the expression of cyclins D1 and E and downregulate the levels of cyclin-dependent kinase inhibitors p21 and p27 [28]. However, for T cells, excessive collagen signals can lead to functional exhaustion through continuous activation, thereby inhibiting their proliferation [21].

These mechanosignals further orchestrate a profound metabolic reprogramming. In response to matrix stiffness, the Hippo–YAP/TAZ signaling axis is activated, which, in concert with the hypoxia common in poorly vascularized fibrotic regions, stabilizes hypoxia-inducible factor 1 alpha (HIF-1 α) [29]. This axis collaboratively upregulates key glycolytic enzymes (e.g., facilitative glucose transporter 1, hexokinase 2, and lactate dehydrogenase A), forcing cancer cells into a state of enhanced aerobic glycolysis (the Warburg effect). This metabolic shift not only fuels rapid proliferation but also acidifies the microenvironment [30]. The stiff environment imposes a metabolic penalty on immune cells, leading to dysfunctional mitochondria in T cells that produce less ATP and forcing macrophages to develop a protumor M2-like phenotype characterized by altered oxidative phosphorylation [8].

In parallel, collagen provides precise directional guidance to immune cells by forming stable concentration gradients through its degradation products and chemokines bound to it. For instance, the degradation product PGP (Pro-Gly-Pro) of type I collagen can specifically recruit neutrophils through the C-X-C motif chemokine receptor 2 receptor, while the NC1 domain fragments of type IV collagen (e.g., tumstatin and canstatin) have anti-angiogenic activity [31]. Particularly in the TIME, the chemotactic effect of collagen tends to recruit immunosuppressive cells, such as Tregs and myeloid-derived suppressor cells. For the few effector cells that do infiltrate, the dense, stiff matrix impairs the maturation and antigen-presentation capacity of dendritic cells, evidenced by a marked reduction in MHC-II and co-stimulatory molecule (cluster of differentiation 80 [CD80]/CD86) expression [32]. Furthermore, matrix stiffness induces cytoskeletal tension in T cells, physically disrupting the formation of a stable immunological synapse. Crucially, studies have found that collagen can directly transmit strong inhibitory signals to various immune cells (including T cells and natural killer [NK] cells) by binding to LAIR-1 [8].

4. Regulation and Spatiotemporal Heterogeneity of Collagen

The collagenous matrix of the TIME is the dynamically, on-demand, and precisely regulated output of a com-

plex interplay among synthesis, enzymatic remodeling, and degradation. These processes collectively manifest as profound spatiotemporal heterogeneity that evolves with and contributes to malignancy (Fig. 3).

4.1 Regulatory Pathways of the Collagen Matrix

First, the acidic pH of the TIME (typically 6.5–6.8) can affect the collagen network by modulating charge changes. Under acidic conditions, the protonation of key amino acid residues on collagen (e.g., lysine and arginine) increases, raising the surface positive charge density by approximately 30%. This not only alters the electrostatic binding ability of growth factors but also influences the interaction between collagen fibers, potentially leading to relaxation of the fiber network. Additionally, acidic pH can directly activate pH-sensitive proteases such as cathepsins B and L, accelerating collagen degradation [33].

Second, the MMP family is the main enzymatic driver of collagen turnover. The TIME is characterized by the aberrant expression and activity of specific MMPs, including interstitial collagenases (MMP-1, MMP-8, MMP-13) that execute the initial cleavage of the native collagen triple helix, and gelatinases (MMP-2, MMP-9) that degrade the denatured products and associated basement membrane structures [34]. Secreted predominantly by CAFs and tumor cells, these MMPs form highly active “hotspot regions” at the tumor invasion front. This localized degradation not only carves out pathways for cellular invasion but also liberates bioactive collagen fragments and releases matrix-sequestered signaling molecules, which in turn regulate angiogenesis and inflammation [35].

Furthermore, oxidative stress profoundly affects the integrity of collagen. Elevated reactive oxygen species (ROS) levels in the TIME can directly oxidize proline and lysine residues in collagen, destabilizing the triple-helical structure and even causing peptide chain breaks. Additionally, ROS can activate latent MMPs, indirectly accelerating collagen degradation. This oxidative damage is highly spatially specific, mainly occurring in the hypoxia-reoxygenation junction areas within the TIME [34].

Finally, the tumor-associated inflammatory cytokine network regulates collagen synthesis and degradation. For instance, tumor necrosis factor alpha and interleukin 1 beta (IL-1 β) can upregulate MMP expression and downregulate their endogenous inhibitors, TIMPs, promoting degradation [34]. TGF- β is the most potent promoter of collagen synthesis and inhibitor of its degradation [36]. Additionally, IL-6 contributes to fibrosis through the STAT3 signaling pathway [37]. The complex spatiotemporal distribution patterns of these factors ultimately determine the overall pattern of collagen remodeling in tumors.

4.2 Temporal Evolution of the Collagen Matrix

The dynamic remodeling of collagen architecture has been systematically characterized into distinct TACS,

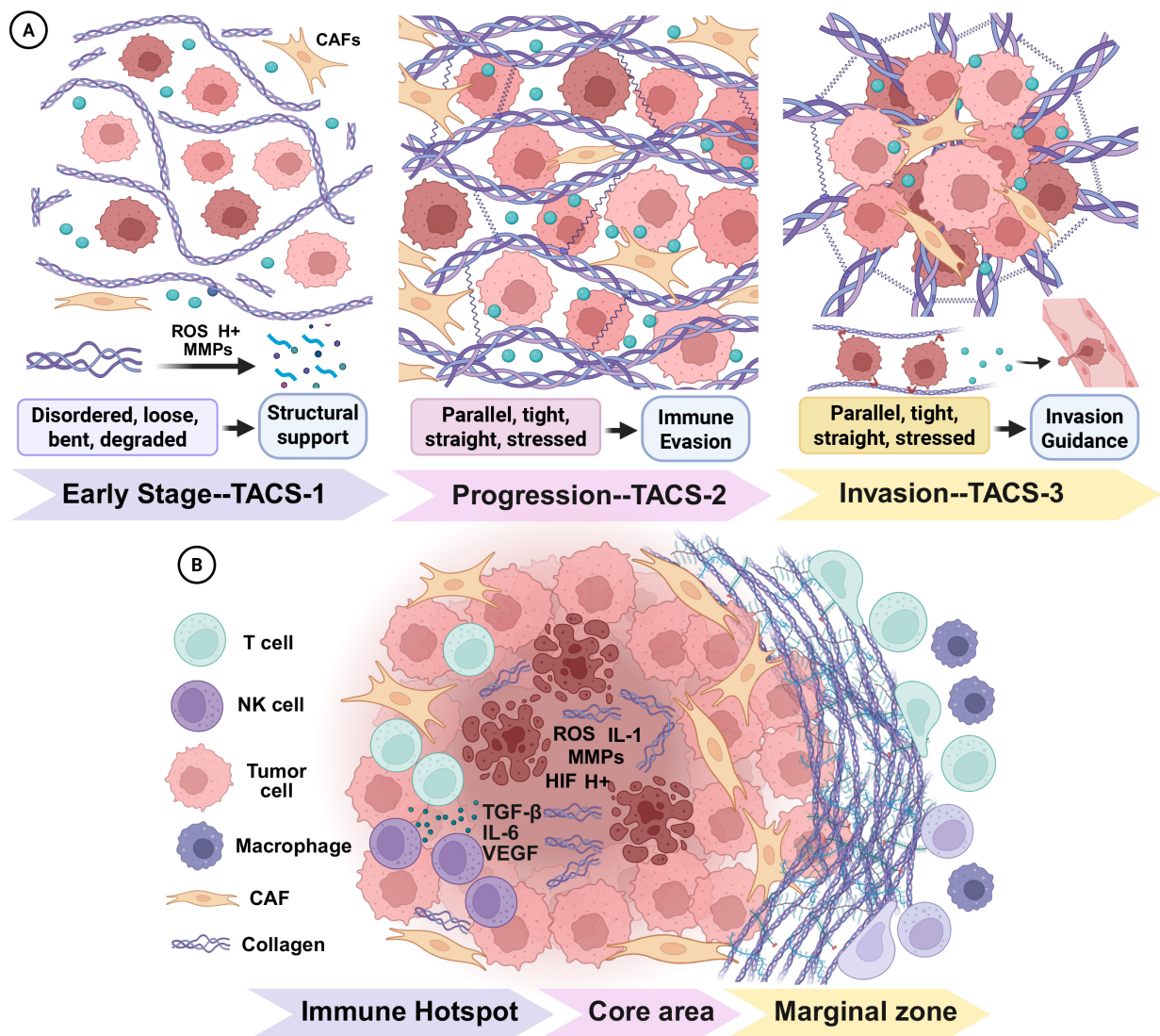


Fig. 3. Spatiotemporal heterogeneity of collagen. The physical and biochemical properties of collagen exhibit dynamic and stage-specific changes during tumor development, both temporally and spatially. These alterations are closely associated with the biological behavior of tumors: (A) In the early stages, loose collagen structures provide sufficient space for tumor cell expansion. As the tumor progresses, increased collagen density facilitates immune evasion. (B) Within the tumor core and immune hotspot regions, collagen undergoes localized degradation under the influence of inflammatory factors. By contrast, at the tumor periphery, collagen forms a dense barrier that restricts immune cell infiltration.

which serve as structural indicators of tumor invasiveness and potential biomarkers for evaluating treatment responses [7].

In the early stage of tumor occurrence (precancerous lesions and early *in situ* carcinoma), TACS-1 is defined by a locally disorganized and wavy collagen configuration. These fibers, showing only a modest increase in density, loosely encircle nascent tumor foci. This signature reflects a period of localized matrix degradation, primarily mediated by tumor cell-secreted MMPs, which creates the necessary space for initial tumor expansion and has relatively immune accessibility [38]. Early, less-aligned matrices can sequester antigens and chemokines within peritumoral niches and support local immune surveillance,

acting as a transient barrier to systemic spread; this immunoprotective compartmentalization diminishes as fibers straighten, pores narrow, and inhibitory collagen-immune receptor pathways intensify [39].

As the tumor progresses and its volume increases, it exerts significant mechanical pressure on the surrounding stroma, leading to the TACS-2 signature. This stage is characterized by collagen fibers that become linearized, bundled, and aligned tangentially (parallel) to the tumor boundary [38]. This architectural shift is dominated by the robust activation of CAFs, which become the primary source of *de novo* collagen synthesis, dramatically increasing overall matrix density and stiffness [40].

When tumor enters the invasion stage, it is marked by the emergence of TACS-3. These fibers are reorganized into thick, straightened bundles oriented perpendicularly to the tumor margin. This radial alignment creates veritable ‘highways’ that facilitate directed cell migration into the surrounding tissue. At this stage, the matrix reaches its peak density and degree of cross-linking, providing stable, pre-established conduits for invasion [13]. Critically, intravital imaging has confirmed that the vast majority of motile, invasive tumor cells migrate along these TACS-3 fibers [18]. In addition, TACS-3 also features dual physical–ligand barriers and T-cell rejection characteristics, including collagen–LAIR-1 signaling and DDR1/DDR2-directed invasion [41].

Notably, the tumor immune response itself can also have a reverse effect on the evolution of collagen. In the early stage of immune activation, local degradation of collagen occurs by effector T cells and NK cells, forming “hot zones” where immune cells aggregate [42]. However, as chronic inflammation is established, the elevated levels of T helper 2-type cytokines and TGF- β conversely promote fibrotic reactions, leading to a dense, restrictive matrix [6]. This dynamic relationship is clinically important, as the study showed a clear link between effective immune checkpoint inhibitor (ICI) therapy correlates and a measurable reduction in collagen density [9].

4.3 Regional Distribution of Collagen Matrix

The collagenous stroma within the TIME is organized into distinct spatial regions, from the peritumoral capsule to the invasive front and perivascular niches. This structural heterogeneity has functional consequences.

The central regions of many tumors are often characterized by a sparse and fragmented collagen landscape. This is typically a consequence of localized hypoxia and necrosis, which limit the metabolic activity of stromal cells and favor a degradative enzymatic environment [14]. However, in some dense tumors (e.g., pancreatic cancer), even the central region can maintain a high collagen content, which is mainly related to the tumor type and distribution pattern of CAFs [24].

Encapsulating the tumor mass is the peritumoral region or invasive front, which typically represents the zone of maximal collagen deposition. Here, a dense, highly cross-linked fibrous capsule (often 50–200 μ m thick) forms a strong physical barrier. This structure can achieve a mechanical stiffness 10- to 20-fold greater than that of adjacent normal tissue [14]. Spatially resolved transcriptomic studies have confirmed that CAFs in this area express the highest levels of collagen synthesis genes (e.g., COL1A1) and cross-linking enzyme genes (e.g., LOX). This marginal barrier is a critical determinant of both immune cell exclusion and drug penetration [9].

At the junction between tumors and normal tissues, there is a dynamic transition zone with a width of approx-

imately 100–500 micrometers. Real-time imaging shows that the collagen in this area is in a highly dynamic remodeling state, with both synthesis and degradation actively occurring simultaneously [17]. This interface represents a critical arena for tumor–immune interactions. Thus, the collagen density and structure in this area directly affect the efficiency of immune surveillance [14].

Finally, a special pattern of collagen tissue is present around the tumor neovasculature. This collagen is frequently arranged in concentric circles, forming a vascular sheath [43]. This structure, on the one hand, provides structural support for the fragile tumor blood vessels, and on the other hand, forms another barrier for the extravasation of immune cells from the blood vessels. A study has shown that a higher density of collagen surrounding blood vessels is statistically linked to lower levels of immune cell infiltration [44].

5. Therapeutic Strategies Involving the Collagen Matrix

The pivotal role of collagen in orchestrating tumor progression and immune evasion has established it as a critical therapeutic target. Preclinical and clinical efforts are largely focused on two complementary axes. The first involves anti-fibrotic approaches aimed at inhibiting excessive collagen synthesis, primarily by targeting CAFs or their activation pathways [45]. The second involves matrix remodeling, which seeks to degrade the established collagen barrier or neutralize the downstream protumorigenic signaling it elicits via cell–matrix receptors [46] (Table 1, Ref. [47–63]).

5.1 Inhibition of Collagen Biosynthesis

Direct ablation or functional modulation of CAFs represents a foundational strategy in antifibrotic tumor therapy. Talabostat (PT-100) [47], a small molecule inhibitor of fibroblast activation protein (FAP), exemplifies this approach. By targeting FAP—a serine protease highly expressed on activated CAFs that facilitates ECM remodeling—Talabostat disrupts the proteolytic activity essential for collagen turnover and CAF maintenance [64]. However, clinical translation has revealed modest single-agent efficacy, likely due to the remarkable plasticity of the stromal compartment and compensatory activation of alternative fibrogenic pathways. This limitation has catalyzed the development of combination therapies, such as combining ICIs with FAP-directed chimeric antigen receptor (CAR) T cells or antibody-drug conjugates (ADCs) [5,48]. Early-phase trials have shown encouraging tumor regression and stromal depletion, suggesting that cellular immunotherapy may overcome the barriers that hampered small molecule approaches [65].

A complementary strategy is to inhibit the signaling pathways that drive the profibrotic phenotype in CAFs. The TGF- β signaling axis orchestrates CAF activation, collagen synthesis, and matrix cross-linking through both canonical

Table 1. Clinical trials targeting collagen in the TIME.

Mechanism	Target	Drug	Medication regimen	Cancer type(s)	Phase	Outcome	Trial no.	Reference
Inhibit collagen biosynthesis	CAF	Talabostat mesylate	Single-agent vs. carboplatin or temozolomide	Brain and central nervous system, childhood germ cell, and kidney cancer	I	At the dose level of 600 µg/m ² , serum DPP-4 inhibition reached 85% at 24 h.	NCT00303940	[47]
		Simlukafusp- α (FAP-IL2v)	FAP-IL2v q3w/qw-q2w + atezolizumab	Cervical squamous cell carcinoma	II	n = 48. Of the 45 evaluable patients, 12 (27%; 95% CI: 16.0–41.0) had OR, including 3 CR and 9 PR.	NCT03386721	[48]
	TGF- β	Galunisertib (LY2157299)	80 or 150 mg bid LY2157299	Pancreatic and lung cancer	I	n = 12. 7 patients (80 mg: n = 2; 150 mg: n = 5) had TEAEs: increased brain natriuretic peptide (n = 2), leukopenia (n = 2), and rash (n = 2)	NCT01722825	[49]
			160 or 300 mg bid LY2157299 + sorafenib or ramucirumab	Carcinoma, hepatocellular	II	Median OS was 7.3 months (95% CI: 4.9–10.5) in A and 16.8 months (95% CI: 10.5–24.4) in B. (A: increased AFP, 160/300 mg; B: normal AFP, 300 mg)	NCT01246986	[50]
		Fresolimumab	1 or 10 mg/kg q3w fresolimumab + radiotherapy	Metastatic breast cancer	II	Patients with high dose of fresolimumab had a better OS compared to patients with low dose (HR = 2.73, p = 0.039)	NCT01401062	[51]
		Pirfenidone	500 mg q8h Pirfenidone vs placebo from Tipifamib trial	Plexiform neurofibroma	II	Median TTP for pirfenidone was 13.2 months compared to 10.6 months for the placebo (two-tailed p = 0.92; one-tailed p = 0.46)	NCT00076102	[52]
	PDGF/FGF	Nintedanib	200 mg bid po Nintedanib	Advanced non-small cell lung cancer	II	n = 20. ORR = 15% with 3 PR, 12 SD. 13 (65%) \geq 16 weeks. Median PFS = 4.3 months (95% CI: 1.8–7.9). Median OS = 11.3 months (95% CI: 3.5–44.2). 3 patients over 1 year	NCT02299141	[53]
		Imatinib	400 mg bid po Imatinib	Advanced melanoma	II	median TTP was 54 days and median OS was 200 days. No patient was free of progression at 6 months	NCT00027586	[54]
Enhance collagen degradation	MMP	S-3304	800, 1600, 2400, 3200 mg bid po	Solid tumors	I	n = 32. Toxicities were mostly gastrointestinal. Maximum tolerated dose was not reached	NCT00033215	[55]
	HA	PEGPH20	PEGPH20 + nab-paclitaxel/gemcitabine (PAG) vs. nab-paclitaxel/gemcitabine (AG)	Metastatic pancreatic ductal adenocarcinoma	II	PFS in PAG (HR = 0.73; 95% CI = 0.53–1.00; p = 0.049). HA-high (0.51; 0.26–1.00; p = 0.048). HA-high PAG vs. AG: ORR = 45% vs. 31% and median OS = 11.5 vs. 8.5 months (0.96; 95% CI = 0.57–1.61)	NCT01839487	[56]

Table 1. Continued.

Mechanism	Target	Drug	Medication regimen	Cancer type(s)	Phase	Outcome	Trial no.	Reference
Interfere with sig- nal transduction	lysyl-oxidase	Simtuzumab	iv gemcitabine, 1000 mg/m ² + 200 or 700 mg simtuzumab or placebo	Metastatic pancreatic adenocarcinoma	II	Gemcitabine + simtuzumab 700 mg/200 mg/placebo: median PFS = 3.7 months (HR = 1.09; 95% CI = 0.74–1.61; <i>p</i> = 0.73), 3.5 months (1.13; 0.76–1.66; 0.61), and 3.7 months. Median OS = 7.6 months (0.83; 0.57–1.22; 0.28), 5.9 months (1.07; 0.73–1.55; 0.69), and 5.7 months. ORR = 13.9%, 14.5%, and 23.5%	NCT01472198	[57]
	DDR1/2	Nilotinib (DDR1 antagonist)	400 mg bid nilotinib	Melanoma with KIT mutation (A) or brain metastasis (B)	II	3/11 in A (27%) and 1/8 in B (12.5%) achieved 4-month disease control. 2 PR in A (18.2%). Median TTP and OS was 3.3 (90% CI = 2.1–3.9 months) and 9.1 months (90% CI = 4.3–14.2 months)	NCT00788775	[58]
	Integrin	Cilengitide	Cetuximab + platinum-based chemotherapy (control) or Cilengitide 2000 mg qw (CIL-once)/q2w (CIL-twice)	Advanced non-small-cell lung cancer	II	PFS = 6.2 vs. 5.0 months for CIL-once vs. control (HR = 0.72; <i>p</i> = 0.085). EGFR ≥200, PFS = 6.8 vs. 5.6 months (HR = 0.57; <i>p</i> = 0.0446). Median OS = 13.6 for CIL-once vs. 9.7 months for control (HR = 0.81; <i>p</i> = 0.265). EGFR ≥200, OS = 13.2 vs. 11.8 months (HR = 0.95; <i>p</i> = 0.855)	NCT00842712	[59]
Indirect anti-collagen		CEND-1 (targets αV integrins and neuropilin-1)	CEND-1 + nab-paclitaxel + gemcitabine	Metastatic pancreatic ductal adenocarcinoma	I	no dose-limiting toxicities were observed (n = 31). The most common grade 3 or 4 events were neutropenia (17, 55%), anemia (8, 26%), leukopenia (5, 16%), and pulmonary embolism (4, 13%). 22 (71%) occurred serious adverse events	NCT03517176	[60]
	B7-H3	YL201 (B7-H3 ADC)	Dose-escalation and expansion	Nasopharyngeal carcinoma, lung cancer, esophageal squamous cell carcinoma	I	extensive-stage small cell lung cancer (ORR = 63.9%), nasopharyngeal carcinoma (ORR = 48.6%), lung adenocarcinoma (ORR = 28.6%) and lymphoepithelioma-like carcinoma (ORR = 54.2%)	NCT0543423, NCT06057922	[61]
	Angiogenesis	Sunitinib	Sunitinib (A) vs. no therapy (B) after taxane-based chemotherapy	Metastatic breast cancer	II	PFS ≥5 months was 10/36 (28%) in A and 4/19 (21%) in B. median PFS was 2.8 vs. 3.1 months	NCT00270413	[62]
	Diabetes-related	Metformin	Docetaxel/prednisone + metformin 850 mg bid (D+M) or placebo (D+P)	Metastatic castration-resistant prostate cancer	II	in D+M and D+P, ORR was 28% and 24%, median PFS was 7.8 and 6.0 months and the median OS was 27 and 20 months	NCT01796028	[63]

Abbreviations: bid, twice daily; qw, once weekly; q2w, every 2 weeks; q3w, every 3 weeks; po, by mouth (oral); iv, intravenous; OR, objective response; ORR, objective response rate; CR, complete response; PR, partial response; PFS, progressionfree survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; TTP, time to progression; TEAEs, treatmentemergent adverse events; HA, hyaluronic acid; DDR, discoidin domain receptor; ADC, antibodydrug conjugate; EGFR, epidermal growth factor receptor; FAP, fibroblast activation protein; IL2v, interleukin 2 variant comple; DPP-4, dipeptidyl peptidase-4; SD, stable disease; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor.

(SMAD-dependent) and non-canonical pathways [36]. Galunisertib (LY2157299) [49], a selective TGF- β receptor I (ALK5) kinase inhibitor, disrupts SMAD2/3 phosphorylation, thereby attenuating downstream fibrogenic gene expression [50]. Clinical trials have demonstrated not only reduced collagen deposition but also reversal of the immunosuppressive microenvironment, with enhanced responsiveness to ICIs. In addition, fresolimumab [51], a pan-isoform TGF- β -neutralizing antibody, sequesters all three TGF- β isoforms in the extracellular space [66]. While both agents show promise, their clinical development must carefully balance antifibrotic benefits against potential safety concerns, as TGF- β also functions as a tumor suppressor in early-stage disease [67].

This concept of pathway inhibition extends to leveraging clinically approved antifibrotic agents. Drugs such as pirfenidone [52] and nintedanib [53], standard-of-care for idiopathic pulmonary fibrosis, exert pleiotropic effects by simultaneously inhibiting multiple fibrogenic pathways including TGF- β , VEGF, FGF, and PDGF [68]. Their repurposing for oncology is compelling, and preclinical studies combining these agents with ICIs have demonstrated synergistic antitumor activity by remodeling the stromal landscape. Similarly, direct inhibitors of PDGF/FGF receptors, such as the multikinase inhibitor imatinib [54], are also being evaluated for their potential to reduce stromal density and enhance drug delivery, particularly when combined with chemotherapy [69].

5.2 Enhancement of Collagen Degradation

Therapeutic strategies for matrix degradation have undergone a significant paradigm shift, moving away from early, crude approaches towards highly specific and targeted interventions.

Initial forays with broad-spectrum MMPs inhibitors were largely unsuccessful in clinical trials. This failure stemmed from significant dose-limiting toxicities, and more critically, a flawed underlying premise, namely, these inhibitors failed to discriminate between protumorigenic MMPs (e.g., MMP-2, MMP-9) and those with antitumorigenic or homeostatic functions (e.g., MMP-8) [70]. Small molecule agonists and antibody fragments that enhance MMP-8 activity have been shown to accelerate collagen turnover, curb metastatic spread, and boost intratumoral CD8⁺ T-cell density in breast- and pancreatic-cancer models [55,71]. Moreover, studies have exploited tumor-specific proteases (e.g., FAP or MMP-14) to activate otherwise quiescent pro-MMP-8 or pro-MMP-9 zymogens, thereby confining collagenolysis to the malignant compartment [72].

Another more direct and precise but technically challenging strategy involves the exogenous delivery of matrix-degrading enzymes. Local or intratumoral delivery of recombinant collagenase (Clostridial class I/II) formulated in thermosensitive hydrogels or lipid nanoparticles can liq-

uefy dense type I collagen. In mouse models of pancreatic cancer, a single intratumoral dose followed by gemcitabine doubled intratumoral drug concentrations and extended median survival by 40%. Similar gains have been reproduced with doxorubicin-loaded liposomes and ICIs, indicating that mechanical decompression, rather than enzyme-drug synergy per se, underlies the therapeutic benefit [73,74]. The primary obstacle remains the safe and effective translation of this powerful local effect into a viable clinical therapy.

Beyond direct collagenolysis, an alternative strategy involves degrading other critical structural components of the ECM. Hyaluronic acid (HA) is a large glycosaminoglycan that creates immense interstitial fluid pressure and organizes the stroma. The enzymatic agent PEGPH20 (pegvorhyaluronidase) [56] works by systemically degrading HA. It melts the gel-like component of the matrix, which not only improves vascular perfusion but also relieves the mechanical constraints on the collagen fibers. While this approach showed significant promise in clinical trials for patient subgroups with “HA-high” tumors, its development has unfortunately faced setbacks [75]. Besides, inhibition of LOX-mediated cross-linking (e.g., β -aminopropionitrile or simtuzumab) renders collagen fibers more susceptible to endogenous proteases and can be combined with collagenase or selective MMP activation for additive effects [25,57]. Likewise, photodynamic or focused ultrasound-mediated matrix debulking is being explored to create transient “windows” for drug entry [76].

5.3 Intervention of Collagen Signal Transduction

Beyond physically degrading the matrix, a more nuanced therapeutic strategy aims to sever the protumorigenic communication lines between malignant cells and the collagen scaffold.

DDR1/2 have emerged as a particularly compelling target class. As the only receptor tyrosine kinases activated directly by collagen, they initiate potent and sustained downstream signaling cascades. Strong preclinical data have shown that the selective small molecule inhibitor, nilotinib [58], effectively suppresses the activation of CAFs, as well as collagen-induced invasion and metastasis [58]. The validation of this concept has propelled several DDR inhibitor candidates into Phase I clinical trials [77].

Integrins, fundamental receptors governing cell–ECM adhesion, have been explored as therapeutic targets for several decades. Early enthusiasm was tempered by the high-profile clinical failure of broad-spectrum agents like cilengitide (an RGD-mimetic targeting $\alpha v\beta 3/\alpha v\beta 5$) [59]. Consequently, a new generation of more refined integrin-targeted therapies is being developed [78]. This includes developing inhibitors that target specific collagen-binding integrin pairs (e.g., $\alpha 2\beta 1$ or $\alpha 11\beta 1$), which are highly up-regulated on cancer cells or CAFs, or that selectively block the intracellular signaling adaptors downstream of integrin

activation [60]. By doing so, these next-generation agents aim to not only inhibit tumor cell adhesion and migration but also disrupt integrin-mediated angiogenesis and resistance to conventional therapies [79].

5.4 Indirect Anticollagen Strategies: Pleiotropic Pathway

A significant frontier in stroma-modulating therapy involves the strategic use of agents whose primary mechanisms of action are not directly antifibrotic, but that exert potent, indirect effects on collagen-producing CAFs and the broader TIME.

The ADC YL201 targets B7-H3 (CD276) [61], a molecule co-expressed on tumor cells, immune cells, and CAFs. This approach offers a dual benefit: directly blocking T-cell immune checkpoint while also weakening the CAF-mediated collagen network to remodel the immunosuppressive stroma [80,81].

Angiotensin II receptor blockers such as Losartan, originally developed as antihypertensives, have been repurposed to inhibit the profibrotic RAS axis within tumors [82]. Blocking the angiotensin II type 1 receptor on CAFs directly suppresses their activation and collagen synthesis. Critically, this also alleviates solid stress, which decompresses intratumoral blood vessels, thereby improving perfusion and enhancing the drug delivery [83].

The impact of anti-angiogenic drugs on fibrosis is highly dependent on their specific targets. Multikinase inhibitors targeting PDGF and FGF receptors (e.g., sunitinib [62]) can directly suppress CAF activation. By contrast, agents that exclusively target the VEGF pathway (e.g., bevacizumab) can induce tumor hypoxia. This, in turn, can paradoxically promote a more aggressive, profibrotic CAF phenotype via HIF-1 α stabilization [44].

The antidiabetic drug Metformin has demonstrated significant antifibrotic effects by activating the central metabolic sensor, AMP-activated protein kinase (AMPK). AMPK activation inhibits the anabolic processes required for CAF activation and ECM protein synthesis. This metabolic intervention effectively suppresses the fibrogenic function of CAFs [37,63].

6. Conclusions and Perspectives

The multifaceted roles of collagen in the TIME represent a paradigm shift in our understanding of cancer biology and therapeutic resistance. This review systematically delineated how collagen transitions from a passive structural scaffold to an active orchestrator of malignancy, functioning through interconnected physical and biochemical mechanisms. Beyond its role as a physical impediment to immune cell infiltration and drug penetration, the collagenous matrix operates as a sophisticated signaling hub that actively promotes tumor progression through mechanotransduction pathways, metabolic reprogramming, and direct immunosuppressive signals [12]. The spatiotemporal heterogeneity of collagen, evolving dynam-

ically throughout tumor progression and varying across distinct microanatomical regions, underscores the complexity of targeting this multifunctional component of the TIME [42].

Current therapeutic strategies targeting the collagenous matrix have yielded both promising advances and instructive failures: (i) a predominance of small, early-phase and histologyheterogeneous cohorts with largely exploratory endpoints; (ii) suboptimal target–disease alignment and minimal enrichment for specific collagen states (density, alignment, crosslinking, and hyaluronan load)—with the HA^{high} signal seen with PEGPH20 illustrating the need for biomarkerguided selection; (iii) limited incorporation of mechanistic pharmacodynamic assessments of matrix remodeling (e.g., TACS, pore size, stiffness, and circulating collagen fragments) and few paired biopsies; (iv) insufficient testing and suboptimal sequencing of rational combinations with immunotherapy or mechanotransduction inhibitors. These constraints likely contribute to mixed or marginal efficacy signals (e.g., negative outcomes with LOX/LOXL2 or integrin strategies) and complicate attribution to the collagen–immune axis; and (v) the dual role of collagen in tumors argue for stage-aware stromal interventions: dismantling a protective capsule too early may facilitate spread, whereas targeted de-alignment or de-crosslinking after the loss of containment can improve immune access and therapeutic efficacy [11,46].

In conclusion, re-engineering the collagenous matrix represents one of the most promising frontiers in modern oncology. In addition, we argue for prospectively defined biomarkers that classify tumor collagen states and immune accessibility, such as the pan-cancer level immunocollagenic subtypes that we have already established [5]. Success will require continued interdisciplinary collaboration, bridging matrix biology, immunology, bioengineering, and clinical oncology to translate these mechanistic insights into transformative therapies that can overcome the stromal barriers to cure.

Abbreviations

ADC, antibody-drug conjugate; AFP, alpha-fetoprotein; ARB, angiotensin II receptor blocker; AMPK, AMP-activated protein kinase; CAR, chimeric antigen receptor; CAF, cancer-associated fibroblast; CD80, cluster of differentiation 80; CD86, cluster of differentiation 86; CTL, cytotoxic T lymphocyte; CR, complete response; CI, confidence interval; DDR, discoidin domain receptor; RGD, Arg-Gly-Asp; EMT, Epithelial–mesenchymal transition; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; FAP, fibroblast activation protein; FGF, fibroblast growth factor; GFOGER, Gly-Phe-Hyp-Gly-Glu-Arg; HA, hyaluronic acid; HR, hazard ratio; HIF, hypoxia-inducible factor; HSPG, heparan sulfate proteoglycan; ICI, immune checkpoint inhibitor; IL-1 β , interleukin 1 beta; LAIR-1, leukocyte-associated

immunoglobulin-like receptor-1; LOX, lysyl oxidases; MMPs, matrix metalloproteinases; MHC, major histocompatibility complex; NF- κ B, nuclear factor kappa B; OR, objective response; ORR, objective response rate; PDGF, platelet-derived growth factor; PFS, progression-free survival; PR, partial response; PGP, Pro-Gly-Pro; RAS, Renin-angiotensin system; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TACS, tumor-associated collagen signatures; TAM, tumor-associated macrophage; TAZ, transcriptional coactivator with PDZ-binding motif; TEAEs, treatment-emergent adverse events; TGF- β , transforming growth factor beta; TIME, tumor immune microenvironment; TIMPs, tissue inhibitors of metalloproteinases; Treg, regulatory T cell; TTP, time to progression; VEGF, vascular endothelial growth factor; YAP, Yes-associated protein.

Author Contributions

YZ, WW, and KY conceived the structure of the review, performed the literature search and analysis, and wrote the initial draft of the manuscript. XZ and LW contributed to the key sections, critically revised the manuscript for important intellectual content, and were specifically involved in the preparation of the figures and table. JM, KY and GL provided the conceptual framework, supervised the project, secured funding, and revised and finalized the manuscript. All authors contributed to editorial changes in the manuscript. All authors have read and approved the final submitted version. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

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Conflict of Interest

The authors declare no conflict of interest.

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