



Review

Beyond the Norm: The emerging interplay of complement system and extracellular matrix in the tumor microenvironment

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ABSTRACT

Ground-breaking awareness has been reached about the intricate and dynamic connection between developing tumors and the host immune system. Being a powerful arm of innate immunity and a functional bridge with adaptive immunity, the complement system (C) has also emerged as a pivotal player in the tumor microenvironment (TME). Its "double-edged sword" role in cancer can find an explanation in the controversial relationship between C capability to mediate tumor cell cytolysis or, conversely, to sustain chronic inflammation and tumor progression by enhancing cell invasion, angiogenesis, and metastasis to distant organs. However, comprehensive knowledge about the actual role of C in cancer progression is impaired by several limitations of the currently available studies.

In the current review, we aim to bring a fresh eye to the controversial role of C in cancer by analyzing the interplay between C and extracellular matrix (ECM) components as potential orchestrators of the TME. The interaction of C components with specific ECM components can determine C activation or inhibition and promote specific non-canonical functions, which can, in the tumor context, favor or limit progression based on the cancer setting. An in-depth and tumor-specific characterization of TME composition in terms of C components and ECM proteins could be essential to determine their potential interactions and become a key element for improving drug development, prognosis, and therapy response prediction in solid tumors.

1. Introduction

The complement system (C) was discovered in the late 19th century as a heat-labile component of plasma able to 'complement' the activity of antibodies during the recognition and elimination of foreign pathogens. C is a complex network comprising over 50 fluid-phase and membrane-bound proteins, which are predominantly synthesized by hepatocytes but may also be produced by tissue-resident and infiltrating cells. C results as a set of inactive components, sequentially linked to each other, and activated in a cascading manner. All these components are strictly organized and regulated to participate in three independent but interactive activation pathways: classical (CP), lectin (LP), and alternative (AP), converging on a common terminal pathway (Fig. 1) [1, 2].

As a dominant contributor to innate immune responses against foreign and altered host cells, C carries out its actions prior to generation of the adaptive immune pathogen-specific responses [3] (Fig. 1A). Thus, C can cooperate in bacteria opsonization to enhance phagocytosis, in

quick recognition and clearance of immune complexes and apoptotic cells and in the lysis of target cell *via* membrane attack complex (MAC) formation. C can also contribute to inflammation and tissue damage by producing anaphylatoxins which serve as pro-inflammatory and immunoregulatory humoral mediators.

In addition to the well-known classical functions of C, the current view extends C roles far beyond the mere targeting of intruders, depicting a diverse array of non-canonical C functions, independently of cascade activation (Fig. 1B). First, non-immune C functions associated with angiogenesis and wound healing have been described [4–6]. Current evidence indicates that specific C components are directly involved in neurobiological processes related to brain development (*i.e.*, neurogenesis, neuronal migration, synaptic remodeling, and response to prenatal or early postnatal brain insults) [7], and in placental development (*i.e.*, implantation, fetal growth, and labor) [8,9]. Moreover, many C components, primarily C1q, C3a, C5a, and C5b-9, contribute to hematopoietic stem-progenitor cell mobilization and homing [10]. C contributes to directing the type and extent of the adaptive response by

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regulating antigen-presenting cells and directly activating the core adaptive immune cells (*i.e.*, B and T lymphocytes) [11]. Mediating the clearance of apoptotic cells is considered an additional non-canonical function; defective removal of these cells is primarily implicated in autoimmune diseases (*e.g.*, systemic lupus erythematosus) [12].

Novel identified functions include a pivotal contribution of C in shaping metabolic reprogramming and cell homeostasis, underlying T cell effector differentiation, and a role as a nexus for interactions with other effector systems, particularly the inflammasome and Notch transcription factor networks [13]. Emerging insights into C biology have also identified a cell-autonomous and intracellularly active C, known as “complosome”, which serves non-classical roles as a regulator of physiological processes [14].

This review will delve into the extracellular non-canonical functions of the C and the ambivalent role of C in cancer, mainly focusing on the emerging interplay between C and extracellular matrix (ECM) components in the tumor microenvironment (TME).

2. Alternative functions of the complement system: a double-edged sword in tumor progression

In the past decade, mounting evidence has shed light on the involvement of C components in cancer. These proteins can be locally produced by stromal, tumor, and immune cells within the TME; however, the concentration of C components results from an intricate balance between systemic compartment, local production, on-site activation, and further deposition.

Cancer is maybe the most striking example of a condition where C exerts a “double-edged sword” role. Thus, it acts as a key player in

cancer immunoeediting *via* direct cytolytic effects as exploited by antibody-based immunotherapeutic treatments against tumor cells. Still, it can also exert protumoral functions by sustaining long-lasting inflammation, immunosuppression, and further malignant transformation. This ambivalent behavior depends on the tumor model and disease stage, the large variety of C components, and the diverse functions related or not to C cascade activation they can serve [15].

The first and most straightforward way to understand whether anti-tumoral or protumoral functions will tip the scales in a given cancer type is to explore the co-expression of C genes and their prognostic significance. Gene expression profiling of C components unveiled constant patterns in human solid tumors (*i.e.*, high expression of CP, AP, and regulatory components; low expression of LP and terminal pathway components). Remarkably, four distinct tumor groups were identified based on the prognostic significance of C gene expression: protective C, protective C3, aggressive C, or uncertain significance of C [15]. Unsurprisingly, relevant heterogeneity can exist among patients with the same cancer type. The complex and multifaceted coexistence between the pro-tumorigenic and tumor-suppressor roles of C underscores the need for further exploration in the field. It should be noted that it is impossible to formulate a single decision for all tumors as the impact is context-dependent and varies depending on tumor type, subtype, and disease stage.

2.1. Activation of the complement system in the tumor microenvironment: anti-tumor effects

As part of an immune surveillance system typically perceiving and targeting neoplastic cells as noxious and non-self elements [16,17], C

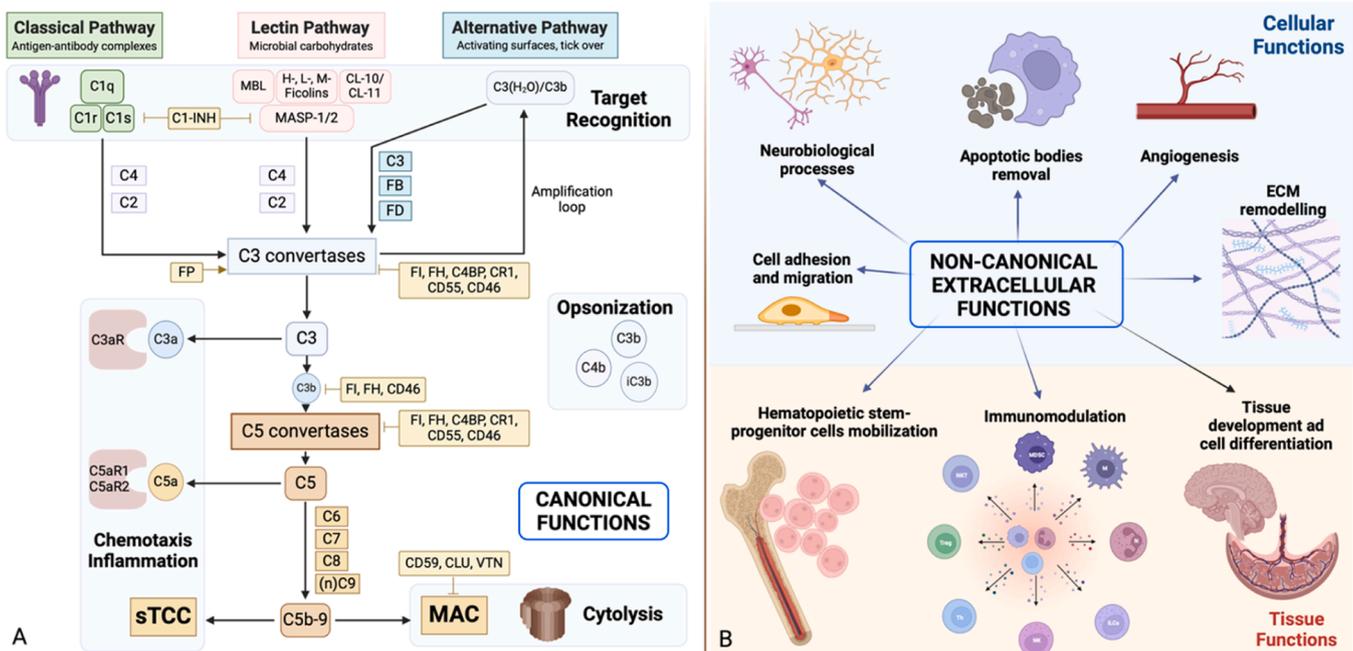


Fig. 1. The canonical and non-canonical functions of the complement system. (A) The canonical functions of the complement system (C) are directly connected to the cascade activation. The activation can occur *via* three pathways: the classical (green), the lectin (pink), and the alternative (cyan) pathway. All three pathways converge to the common component C3 and proceed with the terminal pathway (light brown). This generates the main effector molecules of the C system: the opsonins (*i.e.*, C3b, C4b, and iC3b) to improve the phagocytosis of the target, the anaphylatoxins (*i.e.*, C3a and C5a) as potent pro-inflammatory and chemotactic molecules, and the Membrane Attack Complex (MAC) for the cell lysis. The soluble form of MAC, namely the soluble Terminal Complement Complex (sTCC), has also been shown to have pro-inflammatory properties. A fine tuning of C activation is ensured by several regulators (yellow). (B) In addition to its classical functions, C is involved in various non-canonical roles, such as the regulation of cell differentiation, chemotaxis, adhesion, and migration. C also mediates the clearance of apoptotic cells and extracellular matrix (ECM) remodeling. These functions contribute to complex homeostatic processes, such as tissue development (*e.g.*, placenta and brain development) and immune system regulation. Non-immune functions of C include angiogenesis and wound healing. Recent evidence also shows that C is involved in neurobiological processes, including neurogenesis and the response to brain injury. Many C components (*e.g.*, C1q, C3a, C5a, and C5b-9) contribute to hematopoietic stem-progenitor cell mobilization and homing. Abbreviations: C1-INH, C1-inhibitor; C4BP, C4-binding protein; CLU, clusterin; CR1, complement receptor 1, FB, factor B; FD, factor D; FH, factor H; FI, factor I; FP, properdin; MBL, mannose-binding lectin; VTN, vitronectin. Image created with BioRender.com.

can also be counted among the potential players in cancer immunoe-diting. Specific tumor-associated molecules are expressed on cells un-dergoing malignant transformation and they can trigger C activation by all three pathways, leading to lytic or sub-lytic Membrane Attack Complex (MAC) formation at the tumor cell surface. Accordingly, the detection of C deposits in solid tumors confirmed C activation at the tumor site or in its proximity [18], potentially participating in immu-nosurveillance *via* C-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) [19,20]. C split products can also indirectly prompt cancer cell eradication by promot-ing inflammatory cell recruitment (e.g., M1-like macrophages and nat-ural killer cells), opsonization, and phagocytosis [21]. Local C activation can be coupled with systemic diffusion of C components, as confirmed in biological fluids of patients affected by lung cancer, astrocytoma, lym-phoma, leukemia, thyroid cancer, and oropharyngeal cancer [22–24].

While there is limited evidence of a significant cytotoxic activity towards malignant cells, primarily due to their evasion mechanisms and the increased expression of membrane-bound C regulators, recent in-sights from tumor models have revealed a more intricate role of C in cancer pathophysiology. It is now hypothesized that C may play a pro-protective role in the early stages of cancer development by restraining tumor expansion. Although the initial response to transformed cells eliminates the C-sensitive cells, it gradually enriches the cancer cell population with C-resistant tumor cells [20]. However, the role of C as an effector player of cytotoxic responses against tumor cells remains of utmost relevance in antibody-mediated immunotherapy, paving the way for novel therapeutic strategies [25].

2.2. Role of the complement system in tumor progression

As the involvement of C proteins in many hallmarks of cancer has recently emerged, perspectives have shifted from dissecting anti-tumor C activities to ever-growing evidence that optimal levels of C activa-tion are essential in developing tumors to foster local T cell immuno-suppression, chronic inflammation, angiogenesis, anti-apoptotic and metastatic cancer cell signaling. In 2008, the study of Markiewski and colleagues represented a significant milestone in cancer immunology as it provided the first concrete evidence of C contributing to tumor pro-gression [26]. Specifically, C5a generated in the TME was demonstrated to suppress the anti-tumor CD8⁺ T cell-mediated response by recruiting myeloid-derived suppressor cells and regulating their production of reactive oxygen and nitrogen species. Further confirmation was pro-vided by the blockade of C5a *via* C5a receptor (C5aR) antagonist or C5aR knockout animals, resulting in increased CD8⁺ lymphocytes at the tumor site [26].

Our group has also demonstrated that C1q, which is mainly syn-thesized by macrophages, dendritic cells, and cancer-associated fibro-blasts (CAFs) in the TME, promotes tumor development and pro-liferation, as well as angiogenesis and metastasis [27].

C components can be directly produced by tumor, stromal, and im-mune cells in the TME, or they can derive from the circulation and enter the tumor stroma through the vasculature. C3a and C5a, along with their cognate receptors (i.e., C3aR, C5aR1, and C5aR2), are universally recognized as the key modulators of cancer-promoting responses [23]. In various tumor models, the C3a/C3aR axis is implicated in cell migration, pro-tumorigenic modulation of macrophages, inhibition of neutrophil and CD4⁺ T cell response, promotion of epi-thelial-to-mesenchymal transition (EMT), and metastasis. The C5a/C5aR axis enhances proliferation, pro-angiogenetic properties, motility, and invasiveness of cancer cells [28]. Mouse modeling for ovarian cancer provided evidence about the activation of C3aR and C5aR leading to sustained cancer cell proliferation *via* PI3K/AKT pathway [29]. In colorectal cancer, C5a/C5aR signaling was demon-strated to promote the production of matrix metalloproteinase (MMP)-1 and MMP-9 as crucial players in ECM remodeling and tumor metastasis [30]. Therefore, C activation within the TME may enhance

pro-metastatic properties by supporting EMT. In ovarian cancer cells, the transcription factor TWIST1 can upregulate C3 gene expression, increasing C3a. In turn, C3a/C3aR signaling enhances EMT [31].

Aberrant expression of C components is typically associated with adverse prognosis and reduced patient survival. Moreover, genetic mutations, particularly driver mutations, occur in C genes at a relatively high frequency across at least 32 cancer types, including lung, pancre-atic, and haematological malignancies [32]. A common CD55 variant was associated with an increased susceptibility to non-small cell lung cancer [33]. Similarly, a promoter polymorphism of CD55 was shown to enhance the risk of esophageal cancer [34]. Analysis of the derived peptides revealed that many of these driver C mutations can induce neoantigen generation and are associated with altered immune infil-tration profiles and changes in overall survival, being potentially exploitable for enhancing T cell-mediated reactivity [32].

2.3. Pitfalls in the study of complement system in tumor: paving the way to a “Complement Score”

Publicly available tools for survival-based bioinformatics analysis can offer potent resources and additional opportunities to identify po-tential prognostic and predictive biomarkers in tumors. These web sources usually retrieve expression data from The Cancer Genome Atlas (TCGA) datasets, which comprise highly heterogeneous patient cohorts according to histology, age at diagnosis, molecular profiling, surgery, and chemotherapeutic treatments [35]. Considering that C can exert a multifaceted role based on the tumor type, model, and stage of the disease, it seems reasonable that cohort heterogeneity may lead to un-certain or improper results. Thus, a direct analysis of bulk RNA-Sequencing data, selecting specific histotypes or stages of the dis-ease, should be encouraged when dealing with C-related bioinformatics studies in cancer.

In the field of C, many studies have focused on the prognostic sig-nificance of individual C components in specific human cancers. Mainly, promising implications of C1q in prognosis have been assessed in car-cinomas, gliomas, osteosarcoma, and skin-cutaneous melanoma [36–40]. While informative, these studies are usually limited to pre-liminary *in silico* analysis, and their actual potential can only be realized through more comprehensive studies involving protein detection and functional assays. It is crucial to note that evaluating the expression of C genes may not reflect the protein amount of C components present at the tumor site. There is still a lack of clarity regarding the different contri-butions of systemic C proteins, and local activation or production of C components in the TME. Bioinformatics analysis can only provide in-formation about C components produced at the tumor site, neglecting the contribution of systemic C components and split products due to local activation.

Li *et al.* paved the way for a more comprehensive analysis of a “CScore” in clear cell renal cell carcinoma (ccRCC) as a C prognostic model that identifies two distinct gene expression C-associated clusters, including *C1S* and *VSIG4* as risk factors and *MASPI1* as a protective factor. Moreover, the authors provided potential explanations for their impact on prognosis: *C1S* may influence tumor progression by modu-lating fibroblast biological activity, and *VSIG4* by inhibiting C pathway or T-cell activation and inducing T-cell differentiation, while *MASPI1* may participate in C activation [41]. Investigations about gene expres-sion of C1q and CP components were innovatively coupled with protein staining by Roumenina *et al.* in ccRCC, where a high density of C1q-producing cells was associated with unfavorable prognosis in advanced stages of the disease [42]. Therefore, C was proposed as a prognostic biomarker and potential therapeutic target in RCC after an extensive evaluation combining gene expression, protein levels, plasma biomarkers, prognosis, and animal models [43]. Another promising approach is represented by humoral complementomics in ccRCC: a comprehensive assessment of C proteins, activation fragments, and au-toantibodies against C proteins in plasma was successfully correlated

with intratumoral C activation and local production [44]. A step forward was moved in malignant pleural mesothelioma (MPM), where *in silico* analyses were combined with the protein expression levels of several C components via immunohistochemistry and patients' survival to reach a more comprehensive and tumor-specific C overview. Interestingly, the authors reported that C1q^{HIGH} and C1-INH^{HIGH} patients displayed significantly increased survival [45].

The prognostic impact of C proteins derived from gene expression may reflect a multifaceted combination of actions, which can be fully understood at the protein level only through an extensive insight into both canonical and non-canonical modes of action in a tumor-specific

manner. The assessment of a tumor-specific "Complement Score" based on protein evidence should be combined with the already validated immune scores for predicting prognosis and determining therapeutic approaches in cancer.

3. Complement system and extracellular matrix: connected players in tumor shaping

A fundamental issue that has been scarcely debated by C-related literature is the emerging interplay between C and ECM as potential orchestrators of the TME. The interaction of C components with specific

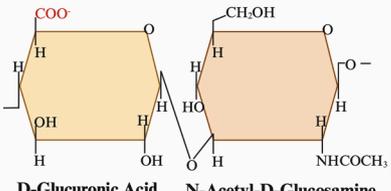
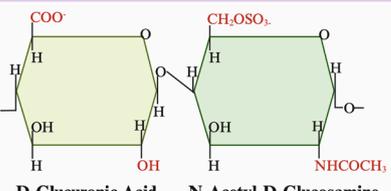
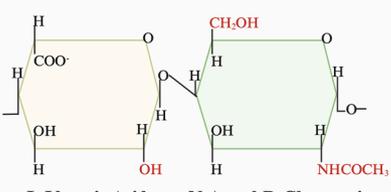
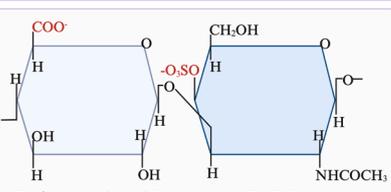
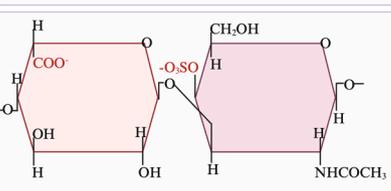
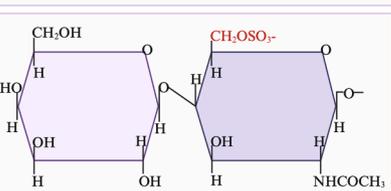
| GAG | Disaccharide Structure | Major location | Presence and functions in TME | Interaction and effects on complement system | Ref. |
|----------------------------|---|--|--|--|---------------|
| Hyaluronic acid |  | Synovial fluid; vitreous humor; ECM of loose connective tissue. | Cancer cell motility, invasion, adhesion, metastasis, neo-angiogenesis, multidrug resistance. | C1q | (55, 107-109) |
| Heparan sulfate |  | Basement membranes; component of cell surfaces. | Cancer cell proliferation, terminal differentiation, angiogenesis, metastasis; often associated in HS-proteoglycans. | C1q FH, FHL-1 (inhibition) Properdin (activation) | (58, 64-66) |
| Heparin |  | Intracellular granules of mast cells; lining the arteries of lungs, liver, and skin. | Not observed. | C1q (inhibition); C1-INH (inhibition); FB (inhibition); C2, C4, C4BP, FD, FH, properdin, C6, C8, C9, VTN. | (56-63) |
| Chondroitin sulfate |  | Cartilage; bone; heart valves. | Mainly associated in CS-proteoglycans and CS-glycoproteins in the TME. | C1q Properdin (activation) | (58,66) |
| Dermatan sulfate |  | Skin; blood vessels; heart valves; tendons; lungs; intestinal mucosa. | Mainly associated in DS-proteoglycans and DS-glycoproteins in the TME. | C1q FH, FHL-1 (inhibition) | (58, 64,65) |
| Keratan sulfate |  | Cornea; bone; cartilage; central nervous system. | Mainly associated in KS-proteoglycans and KS-glycoproteins in the TME. | | |

Fig. 2. Classification of glycosaminoglycans. Glycosaminoglycans (GAGs) are listed based on their disaccharide structure, major location, presence and functions in the tumor microenvironment (TME). Interactions with complement components and their effects on complement activation are also reported, when characterized. Abbreviations: C1-INH, C1-inhibitor; C4BP, C4-binding protein; CS, chondroitin sulfate; DS, dermatan sulfate; ECM, extracellular matrix; FB, Factor B; FD, Factor D; FH, Factor H; FHL-1, FH-like protein 1; HS, heparan sulfate; KS, keratan sulfate; VTN, vitronectin. Image created with BioRender.com.

ECM components can determine C activation or inhibition and promote specific non-canonical functions, which may favor or limit cancer progression based on the tumor model. First, proteolytic remodeling of ECM upon pathological conditions may induce the release of ECM macromolecules with the capacity to trigger C. Conversely, ECM molecules can also inhibit C activation by generating anti-inflammatory signals to maintain a counterbalance.

The ECM is an intricate set of extracellular macromolecules that provide biomechanical and biochemical support to the neighboring cells. The ECM mainly includes glycosaminoglycans (GAGs), proteoglycans (PGs), adhesion proteins (e.g., fibronectin and laminin), and structural proteins (e.g., collagen and elastin). Tumor progression strictly depends on the ECM in which the malignant cells are located [46]. Interestingly, ECM composition is a non-intrinsic factor that can significantly influence the response to chemotherapeutic agents, mainly by reducing drug diffusion [47]. Our group has also recently demonstrated that the ECM plays a fundamental role in influencing the response of tumor cells to chemotherapeutic treatments: in ovarian cancer primary cells, the supplementation of fibronectin as a culture substrate increased tumor cell sensitivity to platinum-based treatments, while hyaluronic acid (HA) reinforced cell resistance to chemotherapy [48].

3.1. Glycosaminoglycans

GAGs, or mucopolysaccharides, are unbranched anionic compounds composed of repeating disaccharide units. Based on their core disaccharide units and presence or absence of sulfation, they are classified into four groups: HA, heparin and heparan sulfate (HS), chondroitin (CS)/dermatan sulfate (DS), and keratan sulfate (KS) [49], as summarized in Fig. 2.

HA is characterized by a linear structure and the absence of sulfate groups. In contrast to other GAGs, HA is not covalently bound to proteins in the form of PGs, even though four PGs possess homologous globular G1 domains that interact with HA (versican, neurocan, brevican, and aggrecan) [50]. Thus, HA acts as a scaffold on which these PGs aggregate in structures adapted to diverse tissue functions. HA is one of the most widespread ECM components and contributes to the development and progression of cancer. Its molecular mass determines the pro- or anti-tumorigenic functions of the molecule: thus, high molecular weight HA is involved in anti-inflammatory, immunosuppressive, and anti-angiogenic processes, whereas low molecular weight HA fragments can trigger signaling pathways related to angiogenesis, inflammation, and impaired immune surveillance [51,52]. Interestingly, many HA activities depend on binding proteins present on the cell surface and/or secreted into the ECM [51,53,54].

GAGs can promote local C activation or inhibition when exposed to C components deriving from body fluids or produced *in situ*. Native HA is reported to be a weak anti-complementary agent [55], but also naturally occurring polysulfated GAGs (e.g., heparin) are widely known for limiting C activation *via* both the CP and AP. The anti-complementary activity of heparin was first suggested by Ecker and Gross in 1929 [56]. Further studies confirmed that heparin could inhibit C by interacting with the collagenous region of C1q (cC1q) [57,58] and significantly reduce C4 and C2 consumption [59]. In addition, most other polysulfated GAGs (*i.e.*, chondroitin-4-sulfate, chondroitin-6-sulfate, HS, and DS) also possess ionic binding specificity for C1q [58]. Heparin can also prevent the formation of AP C3 convertase by inhibiting the binding of Factor B (FB) to C3b [60]. By heparin-agarose affinity chromatography, Sahu and Pangburn demonstrated that C1q, C2, C4, C4-binding protein (C4BP), C1-inhibitor (C1-INH), FB, Factor D (FD), Factor H (FH), properdin, C6, C8, C9, and vitronectin were able to bind heparin, but not C1r, C1s, C3, Factor I (FI), C5, C7, C3b, Ba, and Bb [61]. Similar findings were confirmed by surface plasmon resonance [62]. Moreover, heparin was reported to enhance C1-INH activity, being proposed as a potential therapy for hereditary angioedema [63]. Overall, these studies

led to the development of compounds able to inhibit or control C component interactions with potential biological and therapeutic significance.

Interestingly, FH and FH-like protein 1 (FHL-1) prevent inappropriate AP activation and amplification in host tissue by binding to HS and DS on cell surfaces and within the surrounding matrix. Abnormalities in FH binding to GAGs can result in atypical hemolytic uremic syndrome (aHUS) or in age-related macular degeneration (AMD), based on the preferentially involved surface-recognition region of FH [64,65]. The contribution to host surveillance conferred by the two GAG-binding regions of FH depends on the tissue context, thus allowing organ-specific therapeutic approaches to readdress the balance of immune regulation in several diseases characterized by C dysregulation.

Properdin can recognize and bind to HS and CS on apoptotic T cells, thereby favoring properdin-cell contact and apoptotic cell clearance *via* C-mediated opsonization to avoid harmful inflammatory and autoimmune reactions [66].

3.2. Proteoglycans

PGs are complex molecules of the ECM, structurally characterized by GAG chains covalently bound to a multidomain protein core. PGs are pivotal components of the tumor stroma and are crucially involved in tumor shaping. They serve as key constituents of the ECM structure and are essential receptors for growth factors, cytokines, and chemokines [67]. They are usually classified based on their location as intracellular, cell surface, pericellular, and extracellular (Fig. 3).

Heparan sulfate PGs (HSPGs) are one of the major components of the ECM. They can maintain stromal structure stability and drive cell behavior by binding and releasing many signaling molecules [e.g., IL-8, fibroblast growth factors, and VEGF] after heparinase cleavage [68]. They can act as co-receptors for signaling receptors regulating cell proliferation, adhesion, migration, inflammation, and angiogenesis [69]. The small leucine-rich PGs (SLRPs) form the most prominent protein family of HSPGs, being ubiquitously and abundantly expressed in the ECM. All SLRPs share common biological functions and the capability to interact with various cell surface receptors (e.g., receptor tyrosine kinases and Toll-like receptors), thus regulating vital cellular pathways and cancer-associated processes, including inflammation and autophagy [70].

Extracellular PGs, particularly SLRPs, display diverse functions in regulating C activation in ECM-related diseases (e.g., rheumatoid arthritis, atherosclerosis, osteoarthritis, and chronic obstructive lung disease) [71]. Decorin and biglycan interact with cC1q and, to a lesser extent, with MBL, inhibiting CP and LP activation [72,73]. These findings were confirmed by Sjöberg *et al.*, highlighting a similar pattern also for lumican [71]. Conversely, fibromodulin, chondroadherin, and osteoadherin act as C1-activating SLRPs through their interaction with the globular heads of C1q (gC1q), also by capturing FH and C4BP and restricting C activation to the early steps of the CP [71,74,75]. Therefore, C activation can be potentiated or negatively regulated depending on the SLRP composition of the ECM. This property may result deleterious in inflammatory diseases where the integrity of the ECM is compromised by proteolytic degradation; thus, intact proteins or fragments can become exposed to C proteins, fueling cascade activation, opsonization of self-tissues, anaphylaxis, and sustained inflammation.

Thus, the binding of soluble C inhibitors to the ECM is also important to prevent excessive local C activation. Proline/arginine-rich end leucine-rich repeat protein (PRELP), in addition to binding C4BP and FH, can directly inhibit the AP C3 convertase and MAC formation [71, 75,76]. The C-type lectin domain (CTLD) of aggrecan, the major PG in the articular cartilage, activates the CP and, to a lesser extent, the AP *via* binding of C1q and C3, thus sustaining joint inflammation; conversely, the adjacent C control protein (CCP) domain did not affect C initiation. Interestingly, C activation by aggrecan was mitigated upon FH binding to CTLD and CCP domains [77]. It is worth mentioning that FH-related

| Location | Classification | | GAG chains | Interaction with complement components | Effect on complement system | Ref. |
|----------------|------------------------|----------------|------------|--|-----------------------------|---------------|
| Intracellular | Secretory granules | Serglycin | HS/CS/DS | C1q, MBL | Inhibitory | (110,111) |
| Cell surface | Transmembrane | Syndecan, 1-4 | HS/CS | Properdin | Activatory | (82-84) |
| | | NG2 | CS | | | |
| | | Betaglycan | CS/HS | | | |
| | GPI-anchored | Glypican, 1-6 | HS | | | |
| Pericellular | Basement membrane zone | Perlecan | HS | FH | Not characterized | (80) |
| | | Agrin | HS | FH | Not characterized | (81) |
| | | Collagen XVIII | HS | | | |
| | | Collagen XV | CS/HS | Vitronectin | Not characterized | (115) |
| Extracellular | Hyalactan lectican | Aggrecan | CS/KS | C1q, C3, FH | Activatory/Inhibitory | (77) |
| | | Versican | CS | | | |
| | | Neurocan | CS | | | |
| | | Brevican | CS | | | |
| | SLRPs - class I | Biglycan | CS | C1q, MBL | Inhibitory | (72,73) |
| | | Decorin | DS | C1q, MBL | Inhibitory | (72,73) |
| | | Asporin | | | | |
| | | ECM2 | | | | |
| | SLRPs - class II | Fibromodulin | KS | C1q, C4BP, FH, FHR1/5 | Activatory | (71,74,75,78) |
| | | Lumican | KS | C1q | Inhibitory | (71) |
| | | PRELP | | FH, C4BP, C3, C9, FHR1/5 | Inhibitory | (71,75,76,78) |
| | | Keratocan | KS | | | |
| | | Osteoadherin | KS | C1q, C4BP, FH, FHR1/5 | Activatory | (71,74,75,78) |
| | SLRPs - class III | Epiphycan | DS/CS | | | |
| | | Opticin | | | | |
| | | Osteoglycin | | | | |
| | SLRPs - class IV | Chondroadherin | | C1q, C4BP, FH | Activatory | (71,74,75) |
| | | Nyctalopin | | | | |
| | | Tsukushi | | | | |
| | SLRPs - class V | Podocan | | | | |
| Podocan-like 1 | | | | | | |
| SPOCK | Testican, 1-3 | HS | C1q | Not characterized | (85) | |

Fig. 3. Classification of proteoglycans and their interaction with complement system. Proteoglycans are classified based on their location as intracellular, cell surface, pericellular, and extracellular. Interactions with complement components and their effects on complement activation are also reported, when characterized. Abbreviations: C4BP, C4b-binding protein; CS, chondroitin sulfate; DS, dermatan sulfate; FH, factor H; FHR, factor H-related protein; GAG, glycosaminoglycan; GPI, glycosylphosphatidylinositol; HS, heparan sulfate; KS, keratan sulfate; MBL, mannose-binding lectin; NG2, neural/glial antigen 2; PRELP, proline/arginine-rich end leucine-rich repeat protein; SLRPs, small leucine-rich proteoglycans. Image created with BioRender.com.

protein (FHR)-1 and FHR-5 may compete with FH binding and interact with the ECM, thereby reducing the regulatory activity of FH and enhancing C activation [78]. In the glomerular glycocalyx, the relative balance between FH and FHR-1/FHR-5 in patients with mutations in HS-PG genes may lead to a “permissive” environment that promotes binding of FH-Rs over FH, impairing ligand selectivity and AP regulation [79]. Perlecan and agrin, two PGs of the basement membrane zone, were also proven to interact with FH [80,81], even though the effects of their interaction on C activation have not been characterized up to now. FH-agrin binding in the Alzheimer’s disease brain is mediated by the core protein of agrin and is not HS dependent [81].

As regards cell surface PGs, syndecan-1 was demonstrated as a ligand

for properdin in proximal tubular epithelial cells (PTECs), serving as a docking platform for properdin-mediated AP activation [82,83]. Interestingly, the reduction of syndecan-1 expression in PTECs by nano-complexes significantly reduced properdin binding and AP activation, suggesting novel therapeutic venues in renal diseases [84].

In a research paper investigating the expression, purification, and characterization of human testican-2 and its potential interacting partners, the three polypeptide chains of C1q (*i.e.*, A, B, and C) were identified as interactors after immobilization of testican-2 on sepharose beads, even though it was impossible to ascertain with which chain(s) testican-2 actually interacts [85]. This evidence suggests that interactions of the C1q subcomponent with testican-2 may be relevant

| Classification | | Interaction with complement components | Effect on complement system | Ref. |
|-------------------------------------|---|--|-----------------------------|------------|
| Collagens | Fibril-forming collagens (I, II, III, V, XI, XXIV, XXVII) | C1q (I, II, III) | Not characterized | (88,89) |
| | Fibril-associated collagens (IX, XII, XIV, XVI, XIX, XX, XXI, XXII) | MAC, C4BP, FH (IX) | Inhibitory | (90) |
| | Network-forming collagens (IV, VIII, X) | C1-INH (IV) | Inhibitory | (99) |
| | Beaded filaments and anchoring fibrils (VI, VII, XXVI, XXVIII) | | | |
| | Membrane-anchored collagens (XIII, XVII, XXIII, XXV) | | | |
| | Multiplexing collagens (XV, XVIII) | Vitronectin (XV) | Not characterized | (115) |
| Elastin and microfibrillar proteins | Elastin | | | |
| | Fibrillins | | | |
| | MAGPs | | | |
| | Fibulins | FH | Not characterized | (91) |
| | EMILIN-1 | | | |
| Glycoproteins | Fibrinogen | C1q, C3 | Not characterized | (92,93) |
| | Fibronectin | C1q, C3, C3c, C3d | Activatory ? | (94,96,97) |
| | Laminins | C1-INH, C1q, C3, C3d, FHR5 | Variable | (94,97-99) |
| | Vitronectin | C3 | Activatory | (94) |
| | Nidogens/Entactins | C1-INH | Inhibitory | (99) |
| Matricellular (glyco)proteins | COMP | C1q, MBL, C3, properdin | Activatory/Inhibitory | (105,106) |
| | Osteopontin | FH | Inhibitory | (113) |
| | Periostin | | | |
| | R-spondins | | | |
| | SPARC/Osteonectin | | | |
| | Thrombospondins | FH, FB, C3, C5, C8 | Inhibitory | (101-104) |
| | Tenascins | | | |

Fig. 4. Classification of structural proteins and glycoproteins and their interaction with complement system. Interactions with complement components and their effects on complement activation are also reported, when characterized. Abbreviations: C1-INH, C1-inhibitor; C4BP, C4b-binding protein; COMP, cartilage oligomeric matrix protein; EMILIN-1, elastin microfibril interfacier-1; FH, factor H; FHR, factor H-related protein; MAGPs, microfibril-associated glycoproteins; MBL, mannose-binding lectin; SPARC, secreted protein acidic and rich in cysteine. Image created with BioRender.com.

during several C-mediated inflammatory processes in nervous tissues, lung, and testis, where significant levels of testican-2 mRNA were found [86].

3.3. Glycoproteins

The ECM also comprises structural proteins (mainly collagens and elastin) and glycoproteins, as listed in Fig. 4.

Collagen is the most abundant protein in mammals and the main constituent of many connective tissues. Takahashi *et al.* first demonstrated the interaction and inactivation of C by 10 out of 12 different collagens in 1972 [87]. Collagens can interact directly with the C1 complex and, to a lesser extent, with C2, thus inactivating their functionality. C1q, *via* its cC1q, can form stable complexes with the native type I, II, and III collagens in solution, impairing C activation [88]. C1q interaction with type I, II, III collagen, and to a lesser extent type V and VI, was also confirmed by Agostinis *et al.* [89]. More recent studies have shown that the NC4 domain of cartilage-specific type IX collagen acts as a novel C inhibitor by directly impairing MAC formation and increasing the cofactor activity of C4BP and FH [90], downregulating local C activity in the joint and influencing inflammatory processes. Interestingly, NC4 interactions with fibromodulin and osteoadherin inhibited binding to C1q and C activation by these proteins [90].

In the context of age-related macular degeneration (AMD), Wyatt and colleagues provided compelling evidence for the interaction between fibulin 3 and FH in solution. Authors suggested that the binding of fibulin 3 in soft drusen in AMD could block FH function, thereby enhancing local C activation [91].

Glycoproteins are organic compounds consisting of oligosaccharide chains covalently attached to proteins and are mainly involved in cell-to-cell recognition and signaling.

Fibrinogen and fibrin can be bound by C1q with high-affinity interactions *via* cC1q and gC1q to sequester these complexes in tumor areas or wound sites [92]. Thus, macrophages, fibroblasts, and endothelial cells carrying C1q receptors would have an additional signal to localize them at the wound or tumor sites. It is, therefore, possible that the C1q-fibrin(ogen) interaction may provide one of several positive influences in inflammation, wound healing, fibrosis, and angiogenesis. Interestingly, fibrinogen was also reported to bind to C3, compromising fibrin clot lysis and thereby enhancing thrombosis risk; thus, the role of C3-fibrinogen interaction was investigated as a potential novel therapeutic target to reduce thrombosis risk in high-risk individuals *via* abolishment of C3-induced prolongation of clot lysis [93]. C3 deposition was also demonstrated as high on vitronectin, intermediate on fibronectin, and absent on laminin along areas of exposed sub-endothelial ECM in conditions associated with endothelial disruption, inducing C fixation and contributing to inflammation and vascular damage [94]. C3d was demonstrated to interact with laminin binding to basement membranes of glomerulus and trophoblast in the absence of evidence for ongoing local C activation; likely, retention of C3d at these sites is due to binding affinities between C3 and basement membrane molecules, playing pivotal roles in C-related pathological processes of the glomerulus [95]. Plasma fibronectin was reported to interact with native C3 purified from human sera, and C3c and C3d fragments as well [96]. Laminin and fibronectin can bind to C1q *via* cC1q without mediating C activation [97]. Laminin can also be bound by FHR-5 *via* a separate binding site than C3b [98]. The binding of FHR-5 to laminin as the major constituent of the glomerular basement membrane provides a new link for FHR-5 function in the kidney.

C1-INH binds to type IV collagen, laminin, and entactin, leading to localization of C1-INH in the ECM at high local concentrations; mainly type IV collagen caused an increase in stoichiometry of C1s inhibition by C1-INH [99]. Interestingly, the ECM could be a rich source of C1-INH due to local production (*e.g.*, by endothelial cells) and sequestration from the plasma.

Matricellular glycoproteins have been defined as a subset of non-

structural ECM proteins that modulate cell-matrix interactions and cell regulatory functions through different mechanisms and in a context-dependent manner [100]. The binding of FH to thrombospondin-1 (TSP-1) was reported to have high affinity, thereby increasing FH binding to platelets [101,102]. TSP-1 can strongly inhibit AP in normal human and aHUS patients' serum. This inhibition is only partly dependent on FH since TSP-1 binds to several central C proteins of the AP (*i.e.*, FB, C3, C5, and C8). TSP-1 does not display intrinsic cofactor or decay acceleration activities, but it inhibits the cleavage of FB and C3. TSP-1 prevents MAC formation as well [103]. Interestingly, sublytic C5b-9 induces the expression of TSP-1 in rat glomerular mesangial cells [104].

Lastly, the cartilage oligomeric matrix protein (COMP) has a dual effect on C modulation, as it can bind to C1q and MBL, thereby inhibiting the activities of the CP and LP, as well as activate the AP by binding to C3 and properdin [105]. Evidence of COMP-induced C activation *in vivo* is given by the formation of complexes between COMP and C3b, which can be found in the bloodstream of patients with rheumatoid arthritis and other rheumatic diseases [106].

3.4. Interaction between complement and extracellular matrix components in the tumor microenvironment

Even though the interaction between C and ECM components has been described in several physiological and pathological conditions, little evidence is available about their interplay in cancer.

High expression levels of C1q were detected in the TME of MPM, where it was demonstrated to strongly bind to HA through its gC1q. HA-bound C1q induced pro-tumorigenic behaviors of MPM cells by enhancing tumor cell adhesion and proliferation *via* enhancement of ERK1/2, SAPK/JNK, and p38 phosphorylation. C1q binding to HA did not activate C, confirming a non-canonical role of C1q in tumors [27, 107]. Additionally, the involvement of HA and C1q interaction in regulating HA metabolism in MPM, increasing the local production of LMW-HA fragments *via* HAS3 upregulation [108] and HA fragmentation *via* HYAL2 upregulation [109], added another layer of complexity to this intriguing relationship. Similar to its contribution to trophoblast invasion and placental development [89], C1q exerts its pro-tumorigenic functions only upon binding to the ECM, suggesting that conformational changes occur to unmask binding sites for cell interactions.

Another ECM component involved in C-associated tumor-promoting activities is serglycin. Serglycin, an intracellular PG, is secreted by aggressive breast cancer cells and inhibits both the CP and the LP by directly binding to cC1q and MBL [110], as previously observed for serglycin secreted by myeloma cells [111]. This binding results into protecting tumor cells from C attack and supporting cancer cell survival and progression. C1q binding is mediated through the GAG moieties, whereas MBL binds additionally to the protein core. Moreover, Skliris and colleagues demonstrated that serglycin can inhibit the deposition of C3b and C9 on the cell surface of three different myeloma cell lines (U266, J2N3, and CAG) [111]. Similar characteristics may help tumor cells to evade immune clearance during early disease stages and in immunotherapeutic treatments [112].

Similarly, tumor cell C evasion is also promoted by two glycoproteins, namely osteopontin and bone sialoprotein, which were demonstrated to form rapid and tight complexes with FH. The expression of these ECM proteins in tumor cells provides a selective advantage for survival *via* initial binding to $\alpha\beta3$ integrin or CD44 (osteopontin only) on the cell surface, followed by sequestration of FH to the cell surface and inhibition of C-mediated cell lysis [113]. Fedarko and coworkers reported that whether the complex between these glycoproteins and FH occurs before binding to their cell surface receptors, their ability to protect from C-mediated attack is lost [113]. This evidence suggests that the protective activity of osteopontin and bone sialoprotein is limited to autocrine or paracrine distances from their sites of secretion.

Conversely, collagen XV can bind to vitronectin, inhibiting the adhesion of HT1080 fibrosarcoma cells [114]. Collagen XV, when used

as a coating material, has been shown to inhibit the adhesion of cells to fibronectin and vitronectin and reduce cell migration. Previous studies indicate that collagen XV expression in human cervical carcinoma cells can decrease their tumorigenicity. This likely occurs by altering the ECM, which interacts differently with the tumor cells [115]

The findings reported so far highlighted a direct interaction between C and ECM components, determining an overall promotion of tumor progression and inhibition of C-associated cytotoxicity. However, an indirect regulatory effect on ECM can also be exerted by C components. The latter is the case of cutaneous squamous cell carcinoma (cSCC), where the upregulation of the serine proteases C1r and C1s in tumor cells, particularly the role of C1r in upregulating the production of matrix metalloproteinase (MMP)-13, is a crucial factor in promoting tumor invasion [116]. It has been observed that *C1R* knockout in cSCC cells resulted in a significant decrease in their proliferation, migration, and invasion through collagen type I. *C1R* deficiency suppressed the growth and vascularization of cSCC xenograft tumors and promoted apoptosis of tumor cells *in vivo*. Furthermore, RNA-sequencing analysis of *C1R*-silenced vs control cSCC cell lines revealed significantly regulated signature regarding cell-matrix adhesion, ECM components, basement membrane, and metalloendopeptidase activity. Similarly to C1r, CFI overexpression, leading to the upregulation of MMP-13 and MMP-2, is a potential marker for invasion and metastatic behaviors in cSCC [117].

It is self-evident that scientific literature is still poor in studies regarding the role of the direct interaction between C proteins and ECM in the TME. It remains to be investigated, for instance, how C may influence the synthesis of the ECM in both quantitative and qualitative aspects and how the C and the C activation could act on the cells responsible for the ECM production, such as CAFs. Concerning this point, evidence is available in the rheumatology field, where few studies reported the capability of C to modulate the metabolism of fibroblasts [118,119].

4. Conclusions and perspectives

Based on the vast array of aforementioned studies, C and ECM are both crucial players in determining the fate of cancer progression. Understanding their interactions in physiological conditions as well as in cancer is essential for developing targeted therapies and advancing anti-tumor strategies. It should be noted that C and ECM interplay may be relevant to C-related or C-independent mechanisms, leading to different outcomes.

Interestingly, one can premise that several ECM proteins share structural similarities with C components, particularly with C1q, suggesting an overlap in functional domains and receptors [120–122]. gC1q domain is common to a wide range of proteins due to its ancestral evolutionary history [123], while cC1q exhibits structural similarities with collagen VIII and X; additionally, the collagen receptors $\alpha 2\beta 1$, LAIR-1, and DDR1 have been reported to be also C1q receptors [124, 125]. Also, the CTLD, typically found in human collectins (*i.e.*, MBL, collectin liver 1, surfactant proteins A and D), occurs in several PGs that lack a transmembrane domain and reside in the ECM, comprising aggrecan, brevican, versican, and neurocan [126].

Partly due to their structural analogies and functional overlaps, C and ECM appear to be linked by a two-way interaction: in physiological and pathological contexts, ECM can determine C activation or inhibition, while C can change the physical properties of ECM. In particular, alterations in the ECM stiffness play a critical role in promoting carcinogenesis and progression by regulating the malignant behavior of cancer cells [127]. It can be speculated that the interaction of C with the ECM may lead to changes in ECM stiffness, affecting cellular mechanotransduction and biology (*e.g.*, metabolic reprogramming, tumor cell proliferation, EMT, metastasis, angiogenesis, immune surveillance, and resistance to therapeutics) [107,116,117]. This may influence tumor progression, shift to a more malignant state, and blockage of immune

infiltration specifically due to the formation of a denser structural barrier. It is even feasible to suppose that some C components could be trapped in the ECM as part of the “immune trapping” phenomenon, thus determining local C modulation in the TME. In addition to cancer progression, changes in stiffness can also affect the efficacy of immunotherapy.

As mentioned, C factors can also interact with specific ECM components, driving C activation or inhibition. Thus, the outcomes of this interplay may be multifaceted. The most prominent example is C1q interacting with SLRPs, where the different interaction sites (*i.e.*, gC1q or cC1q) may determine a positive or negative outcome of CP propagation. Conversely, soluble C inhibitors (*i.e.*, FH and C4BP) binding to the ECM can successfully prevent excessive local C activation, even though FHRs competition with FH binding can reduce the regulatory activity of FH and restore C activation. Interestingly, the interaction between C and ECM components can mediate some non-canonical C functions in tumors, as we observed for HA and C1q interplay in MPM [107–109].

It is noteworthy that not only their direct interaction but also the indirect reciprocal regulatory mechanisms among them can influence the TME properties. The latter is the case of C1r, C1s, and CFI in cSCC, whose overexpression is responsible for the upregulation of MMPs [116, 117]; the extent of changes in the expression level of MMPs in the TME represents the malignant degree of the tumor.

As already mentioned, the different composition of the ECM and, therefore, the different affinity between these proteins/glycoproteins and the C components can modulate the expression and deposition pattern of specific C proteins at the tumor site, thus influencing the “Complement Score”. To this purpose, the fulfillment of an effective tumor-specific “Complement Score”, based on bioinformatics analysis, protein expression, and functional evidence, should be encouraged to gain a complete overview of C contribution to tumor progression. This could be integrated with the established immune scores to effectively predict prognosis and guide therapeutic strategies in cancer treatment.

In conclusion, further research is essential for an in-depth and tumor-specific understanding of TME composition in terms of C components and ECM proteins, opening novel scenarios of potential interactions in cancer and disease outcomes. Therapeutic intervention should be the definitive goal, as already proven for specific heparinoids able to inhibit the properdin binding to HS on renal PTECs, without affecting FH and thereby controlling C activation [128]. Similar approaches could impair the binding of C to specific ECM structures, thereby correcting C dysregulation in cancer. This knowledge could be transformative for improving drug development, prognosis, and therapy response prediction in solid tumors.

Author contributions

AB, CA, and RB reviewed the literature and wrote sections of the review article. AB created the figures. All authors contributed to the article and approved the submitted version.

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CRedit authorship contribution statement

Roberta Bulla: Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Chiara Agostinis:** Writing – review & editing, Writing – original draft, Funding acquisition. **Andrea Balduit:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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