Complement and Cancer Immunity

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Abstract

The complement system is considered as a crucial mediator of the innate immune mechanisms, its three major activation pathways contribute to cellular homeostasis, and protect the host from exogenous pathogens and self-derived components. The complement pathways are tightly regulated and monitored by surface bound molecules and soluble proteins, and avoid over activation and pathological

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inflammatory responses. However, tumor cells have evolved several strategies to escape from complement-dependent elimination and create a conducive tumor microenvironment and promote metastasis. The excessive expression of complement proteins in malignant tumors and avoidance of membrane attack complex formation on its surface have been observed in almost all types of cancers. Complement cleavage products are found extensively deposited along the tumor vasculature, influencing the immunosuppressive tumor microenvironment. This chapter discusses the role of complement effectors and regulators in cancer immunity.

Keywords

Cancer · Complement · Tumor microenvironment · Angiogenesis · Metastasis · Anaphylatoxins · Complement regulator

Introduction

The complement proteins are abundant throughout the body especially in blood and are the central pillar of the innate immunity. The complement system is composed of over 50 blood and lymph circulating, secreted, and membrane proteins which interact with each other to provide an effective anti-microbial defense system, clear immune complex, and control damage to self. The key complement components also act as pattern recognition receptors (PRRs) that are capable of recognizing a wide array of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). The complement activation generates anaphylatoxins that recruit an array of immune cells which further secrete several mediators such as cytokines and chemokines mounting anti-microbial response. In cancer, such aggressive complement activation often leads to tumor-promoting inflammation (Dunkelberger and Song 2010). This chapter elaborates the role of complement system in tumor, its interaction with the tumor microenvironment (TME), and the different regulatory mechanism that modulate the TME.

Complement System

The complement system is a tightly regulated cascade of serine proteases, present in plasma, other biological fluids, and on cells as surface-bound regulators (Sjöberg et al. 2009). There are three interlinked complement pathways. Classical pathway lectin pathways.

Complement-fixing antibodies, IgM or IgG bound to its antigen either on a pathogen or host cell membrane, can trigger classical pathway by binding to C1q of the C1 complex with its Fc region. C1q has a multimeric structure and can also recognize C-reactive protein (CRP), pentraxin 3 (PTX3), or apoptotic cells. C1r and C1s are the proteolytic units of the C1 complex. Once C1q binds to the Fc region of

the antibody, C1r is activated which cleaves C1s, generating the active C1s protease. The active C1s cleaves C4 and C2 generating C4a and C4b and C2a and C2b, respectively. Carbohydrate patterns such as N-acetyl glucosamine and mannose which are abundant on the surface of viruses, bacteria, and fungi that can trigger lectin pathway by binding to MBL and ficolins. MBL-associated serine proteases-2 (MASP-2) complexed with MBL or ficolin can capture C4 and C2 cleaves them, similar to the classical pathway. C4b and C2a together then form the C3 convertase (C4b2a). The alternative pathway functions in two interlinked ways: it gets triggered either by bacterial surface components or by spontaneous fluid phase hydrolysis of C3 thioester and carries on with the amplification loop to continue complement activation. Factor B (FB) binds to form C3Bb. FB is cleaved by factor D, a plasma serine protease, releasing fragment Bb. This C3Bb complex then cleaves C3, forming C3a and C3b. C3b then binds with Bb forming the C3 convertase (C3bBb). Activation of the C3 convertase results in amplification of the complement cascade.

The classical, the lectin, and the alternative pathways all lead to the formation of C3 convertases and the activation of C3, which is the central component of the complement pathway. Propagation of complement activation by C3 convertase results in the generation of the C5 convertase complex (C4b2a3b/C3bBb3b) on the cell surface. C5 convertase then cleaves C5 to C5a and C5b, leading to the terminal pathway which joins with four other plasma proteins, C6, C7, C8, and C9, forming an amphipathic membrane-inserted complex, the membrane attack complex (MAC) or C5b-9 complex.

There are complement regulators which control the activation cascade by inhibiting the protease activities, decaying convertase, or inhibiting MAC formation (Revel et al. 2020). These regulators are present both on host cell membranes and in the fluid phase. Membrane-bound inhibitors are complement receptor 1 (CD35), membrane cofactor protein (MCP or CD46), and decay accelerating factor (DAF or CD55). CD55 accelerates decay of convertase by dissociating C4b2a/C3bBb and C5 convertase. CD46 binds with membrane-associated C4b and C3b inhibiting the further complement activation. CD35 regulates C4b2a through decay and can bind with C4b to catalyze its cleavage by factor I. The soluble factors such as factor H and factor I can inactivate C3b fragment (Ricklin et al. 2010; Rutkowski et al. 2010) (Fig. 1).

Role of Tumor Cell-Derived Complement Components Expressed in TME

TME is a highly heterogeneous milieu which is continuously evolving to counteract the immune surveillance mechanism of both innate and adaptive immunity. The composition of the immune infiltrate and the nature of the inflammatory response finally decide the future of the TME, i.e., whether the tumor cells will be eliminated by the immune system, or it escapes immune surveillance and continues to grow. Usually, at the onset of tumorigenesis, anti-tumor immunity predominates, where

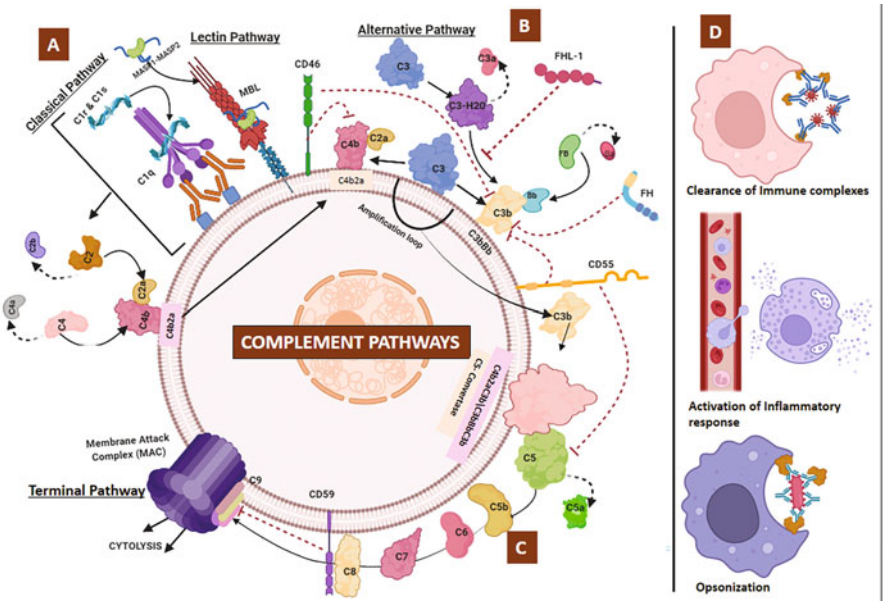

Fig. 1 Complement system comprises of three pathways: the classical, lectin, and alternative. (A) The classical pathway is activated upon binding of C1 complex to the Fc portion of immunoglobulins bound to antigen. In lectin pathway, MBL binds to surfaces bearing mannose groups or other pathogen-associated molecular patterns (PAMPs). Both pathways subsequently cleave C2 and C4 into C4a, C4b, and C2a, C2b, and generate C4b2a (C3 convertase of classical and lectin pathways). (B) In alternative pathway, hydrolyzed C3 aided by factor B cleaves C3 into C3a and C3b, where C3b is the highly active form generating C3bBb (C3 convertase of the alternative pathway). Complement regulator CD46 acts as a cofactor for factor I-mediated cleavage of C3b and C4b, whereas CD55 binds to C3b and C4b and accelerates the decay of C3 and C5 convertases; FH and FHL-1 accelerate decay of C3bBb. (C) All complement pathways lead to a common end pathway that cleaves C3 into C3a and C3b. Then, C3b joins with C4b2a/C3bBb and forms the C5 convertases (C4b2aC3b/C3bBbC3b). C5 convertase cleaves C5 into C5a and C5b, where C5b assembles with C6, C7, C8, and multiple C9 molecules, generating the membrane attack complex (C5b-9; MAC). CD59 binds to C8 and C9 and prevents MAC formation. (D) The split components (C3a and C5a especially) can act as potent anaphylatoxins and mediate several functions such as clearing of immune complexes, activating inflammatory response, and enhancing opsonization

cytotoxic T (Tc) cells, Helper T (Th) cells, natural killer (NK) cells, neutrophils, proinflammatory macrophages (M1), dendritic cells, and B cells play important roles. However, as the tumor continues to develop, the TME gradually becomes tumor-promoting with the predominant role of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs). Tumor cells create a niche by inducing profound phenotypic changes in non-immune stromal components and by modulating secretion of various cytokines, chemokines, and soluble factors such as complement, which foster tumor growth and metastasis (Hinshaw and Shevde 2019; Fig. 2). Recently, much interest has been generated towards understanding the role of

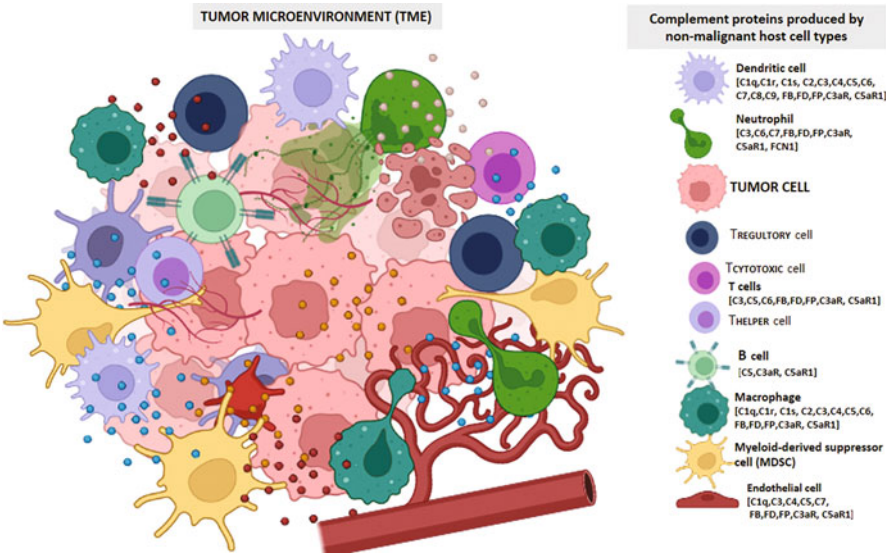

Fig. 2 Role of different non-malignant host cells in the tumor microenvironment (TME): Different host cell types present in the TME produce complement proteins along with the tumor cells. Macrophages, dendritic cells, neutrophils, myeloid-derived suppressor cells (MDSCs), T cells, and B cells participate in the local production of complement proteins, membrane receptors, and regulators creating a complement-rich TME. Both stromal and tumor cells express high levels of complement receptors and regulators

complement in regulating the immunosuppressive mechanisms in TME (Kolev and Markiewski 2018). The complement system is an important component of the inflammatory response, and its expression increases at various stages of tumorigenesis and cancer progression in almost all types of cancers (Zhang et al. 2019).

In TME, host and tumor cells are capable of synthesizing complement proteins (Cho et al. 2014). Certain tumor cells also contain intracellular pools of C3 and C5 (Cho et al. 2014, 2016; Xi et al. 2016). Chao et al. showed that C3 gene knock-down using siRNA inhibited proliferation and invasion of SKOV3ip1 ovarian cancer cells *in vitro*. C3 knock-down also inhibited tumor growth in hC3 siRNA in tumor-bearing mice, where 70% of tumor reduction was observed after 4 weeks compared to control (scrambled siRNA) (Cho et al. 2014). Tumor cells were found to secrete C3, and its cleavage products were found extensively deposited along the tumor vasculature in a murine model of cervical cancer (Markiewski et al. 2008).

Complement proteins and their degradation products (C3d, C4d, C5a, C3a, and C5b-9) are readily detectable in various types of cancer, such as ovarian cancer, melanoma, cervical cancer, colorectal cancer, pancreatic ductal adenocarcinoma, glioblastoma, cervical intraepithelial neoplasia III, and non-small cell lung cancer. It is possible that different cancer types use distinct mechanisms to take advantage of complement activation, promoting tumor growth (Afshar-Kharghan 2017; Zhang et al. 2019; Vadrevu et al. 2014).

Several tumor intracellular signaling pathways were found to be activated with binding of C3a and C5a to their receptors C3aR and C5aR (Rutkowski et al. 2010). C3aR-dependent neutrophil extracellular traps (NETs) could also induce coagulation in intestinal tumors (Guglietta et al. 2016) (Fig. 2).

Roumenina et al. showed that clear-cell renal cell carcinoma (ccRCC) expressed high amount of complement components related to classical pathway such as C1q and C4, suggesting classical pathway being the key inflammatory mechanism in this type of cancer (Roumenina et al. 2019a, b). Caki-1, A498 and ccRCC cell lines showed high expression of C1r, C1s, C4, and C3 mRNAs, as well as proteins. Formation of C1q complex was observed with the addition of C1r- and C1s-containing supernatants of these two ccRCC cell lines. IgG deposits bound with C1q were found to be localized on ccRCC tumor cells. Activation of classical pathway was also ascertained with C1q partially co-localized with C4d (Roumenina et al. 2019a, at the same time C4 fragment deposition and C2 cleavage were detected in the ccRCC cell line supernatant. Cytoplasm of the infiltrating cells in ccRCC tissue was C1q+, some of these infiltrating cells were CD31+ vascular endothelial cells, and majority (80%) were macrophages expressing both CD68 and CD163. These C1q+ tumor-associated macrophages (TAMs) expressed high mRNA levels for cell cycle negative regulators such as PD-1, Lag-3, PD-L1, and PD-L2; this is possibly to dysregulate T-cell-mediated immune response by creating an immuno-suppressive microenvironment (Roumenina et al. 2019a, b.

Another study showed that the C3 and CFB mRNAs expression levels were significantly higher in cSCC cells, as compared to normal human epidermal keratinocytes (NHEKs). When compared between the primary and metastatic cSCC cell lines, C3 mRNA expression did not change, whereas FB mRNA expression was higher in primary cSCC cell lines than in metastatic ones. C3 was localised within cytoplasm and on the cell surface in tissue samples at different stages of epidermal carcinogenesis: premalignant precursor lesion, cutaneous squamous cell carcinoma in situ (cSCCIS), cutaneous squamous cell carcinoma (cSCCs), and recessive dystrophic epidermolysis bullosa squamous cell carcinoma (RDEBSCC). Strong C3 staining was observed in tumor cells in RDEBSCC samples (Riihilä et al. 2017).

The migration and proliferation of cSCC cells and growth of human cSCC xenograft tumors in vivo were inhibited with knockdown of C3 and FB expression. This led to a strong inhibition of extracellular signal-regulated kinase 1/2 activation (Riihilä et al. 2017). A study conducted by Bonavita et al. showed that C3-deficient mice could reduce susceptibility to epithelial skin carcinogenesis (Bonavita et al. 2015). PTX3-deficient tumor mice are characterized by increased tumor-promoting macrophage recruitment which was found to be completely abolished in C3-deficient or C3/PTX3-double-knock-out mice. PTX3 acts as an extrinsic tumour suppressor gene in mouse and human by regulating complement activation, and thus its deficiency probably leads to complement-dependent tumor-promoting inflammation in mesenchymal (3-MCA) and epithelial carcinogenesis (Bonavita et al. 2015). C3 and FB were expressed in several tumor cells such as in rhabdomyosarcoma, cutaneous squamous cell carcinoma (cSCC), pancreatic ductal

adenocarcinoma, and glioma (Brideau et al. 2007; Legoedec et al. 1995; Gasque et al. 1992; Lee et al. 2014).

In gastric cancer tissue C3 mRNA ~~expression~~ and protein ~~levels~~ (together with C3a) were ~~found~~ higher compared to non-tumorous tissues. TCGA cohort analysis also showed upregulation of the C3 mRNA levels compared to normal gastric tissues or adjacent normal tissues (Yuan et al. 2020). Expression of C3 and C3a, but not of C5, was also highly upregulated in SGC-7901 and MGC-803 gastric cancer cell lines compared with gastric mucosa (GES-1) cell lines. Exogenous treatment of SGC-7901 cells by C3 induced high expression of p-STAT3 and p-JAK2; however, administration of CR1, which has the capability to block exogenous activation of C3, downregulated the expression of p-STAT3 and IL-6 compared with AG490-treated cancer cells. This suggests the important role of C3 in promoting tumor growth and metastasis by activating JAK2/STAT3 signaling (Yuan et al. 2020). C3 and C5b-9 have been found in the cervical tumor cells and the surrounding stroma, which was significantly associated with low expression of CD46 (Gelderman et al. 2002).

Tumor cell-derived C5a can induce the recruitment and differentiation of myeloid-derived suppressor cells (MDSCs) within the TME, which in turn, suppress effector T cells resulting in tumor progression (Markiewski et al. 2008). C5a contributes to the accumulation of $CD11b^{+}Gr\text{-}1^{+}$ MDSCs in peripheral lymphoid organs and migration into tumors. The deposition of C3 cleavage products, along the tumor vasculature, also indicates generation of C5a in tumor tissue and nearby blood vessels (Markiewski et al. 2008). Cancer cell membrane-bound serine proteases, possibly belonging to the kexin subfamily, can cleave C5 and generate C5a without complement activation.

Expression of complement regulators (CRP, CD55, CD59, CD46, factor H, and factor H-like proteins) is increased in several cancer types that can limit the cytotoxic effects of complement activation (Fishelson et al. 2003; Roumenina et al. 2019a, b; Gancz and Fishelson 2009 (Fig. 3).

Several membrane regulators have been found to be released in the form of tumor-derived vesicles. These extracellular vesicles are important constituents of TME; they act as bioactive cargo carrying several proteins and deliver them to the target cells, and thus, mediating effective signaling between tumor and non-tumor cells within the TME (Yuan et al. 2020).

Hakulinen et al. showed that CD46 becomes proteolytically modified on cell membranes. Functionally active forms of CD46 (60–65 kDa) are released from the tumor cell membranes in extracellular vesicles (200 nm diameter) and also in soluble forms (Hakulinen et al. 2004). Soluble CD46 (55–60 kDa) was generated via proteolytic cleavage by metalloproteinase containing the glycosylated STP-region, lacking the hydrophobic transmembrane and cytoplasmic domains (Hakulinen et al. 2004). The soluble as well as vesicule-contained CD46 were also found to act as a cofactor for factor I-mediated C3b cleavage. Thus, the incorporation of CD46 in the extracellular vesicles is meant to inhibit complement activation across TME (Hakulinen et al. 2004). Shedding of CD46 and CD59 was also observed in several

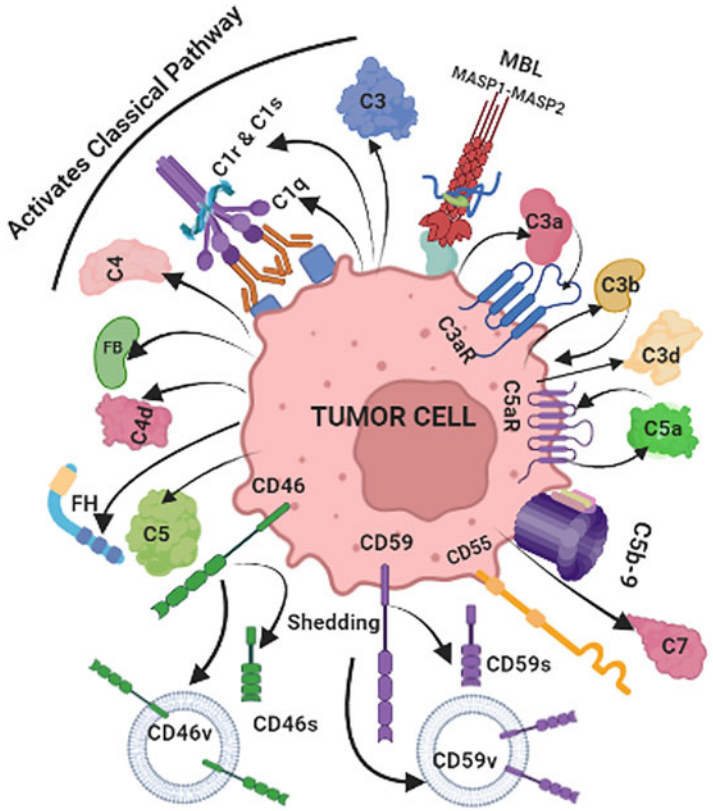

Fig. 3 Tumor cell-derived complement components: In cancer, expression of complement proteins and its receptors is heightened. C1q, C3, C3a, C4, C5, C5a, and the membrane attack complex (MAC/C5b-9) play important roles in creating the inflammatory tumor microenvironment. Tumor cells contain intracellular pools of C3 and C5 and other complement proteins such as C1q and C5b-9; the complement cleaved products C3a, C3d, C4d, and C5a are easily detectable in various types of cancer. Tumor cells can also synthesize C1r, C1s, C4, and C3, where C1r and C1s reconstitute with C1q produced by TAMs. Several human malignant hematopoietic cell lines of various lineages express C3aR and C5aR. Complement cleavage products are found extensively deposited along the tumor vasculature influencing the immunosuppressive TME. Cancer cells evade complement attack by expressing membrane-bound regulators (CD21, CD35, CD46, CD55, CD59), or soluble regulators (FH, FHL-1, FHR). Tumor cells also shed complement membrane receptors. CD46 and CD59 can be released via microvesicles (CD46v and CD59v), or in soluble forms (CD46s and CD59s) that lack the transmembrane and cytoplasmic regions

tumor cell lines, such as breast cancer (T47D), fibrosarcoma (HT-1080), glioma (H2), urinary bladder carcinoma (CAK1), melanoma (G361), and ovarian adenocarcinoma (SW626) (Fig. 3). Thus, overexpression of complement regulators by cancer cells limits immune surveillance by the complement system.

The following section of the chapter explains the role of complement protein in tumorigenesis and its regulation.

Role of Complement Proteins and ~~its~~ Split Products

Complement components and split products such as C1q, C3, C3a, C4, C5, C5a, and the membrane attack complex (MAC) play an important role in modulating the TME. Expression of complement proteins and ~~its~~ receptors are heightened in cancer cells. This section elaborates the role of these complement components and ~~its~~ split products in tumorigenesis.

C1q

C1 is the first component of the classical pathway, containing one C1q and two copies of C1r and C1s proteins. C1q is the recognition unit made up of six copies of three polypeptide chains (A, B, and C), each with a collagenous stalk and C-terminal globular head (gC1q) (Kishore et al. 2004). Fc regions presented within immune complexes containing IgG or IgM antibodies are the primary targets for the six globular heads of C1q (Reid 2018). C1r and C1s are homologous serine proteases held together in a calcium-dependent manner. C1q is a charged pattern recognition molecule of the innate immunity where the exposed head modules mediate the ligand binding and is capable of functioning both in a complement-dependent and complement-independent manner. C1q is a very efficient scavenging molecule, mostly modulating pro-inflammatory responses, either promoting cancer or protecting it. It is expressed locally in TME that can influence cell adhesion, migration, and proliferation.

Circulating immune complexes can modulate cell-mediated immune responses against tumor. Thus, C1q binding to antigen-antibody complexes in the serum can help predict the residual disease, its stages, and prognosis. Serum C1q binding activity has been found to be higher in cancer patients than the healthy subjects. This pattern was observed in over 80% of cancer patients with lung carcinoma, malignant melanoma, breast cancer, colon carcinoma, leukemia, and lymphoma. Cancer patients with progressively growing tumor and with residual tumor after surgical therapy had high C1q binding activity, whereas Clq binding activity is less in patients with no evidence of residual tumor (Rossen et al. 1977).

There are several immunomodulatory functions of C1q that are independent of complement activation. The presence of C1q was examined in different invasive malignant neoplasm tissues (colon adenocarcinoma, melanoma, lung adenocarcinoma, breast adenocarcinoma, and pancreatic adenocarcinoma), in view of the fact that C1q acts like an extracellular matrix protein, favoring tumor growth and metastasis. C1q was strongly detected in all the tumor tissues; however, the presence of other complement components such as C1s, C3, and C4 was not consistent, suggesting that C1q deposition was independent of complement activation (Bulla et al. 2016). C1q-deficient ($C1qa^{-/-}$) mice with syngeneic B16 melanoma had prolonged survival and reduced tumor growth with delay in C1q deposition compared to the wild type and with lesser vascular density and lower number of lung metastases. However, C3- or C4-deficient mice did not exhibit similar activities. The

tumor-promoting activity of C1q was independent of circulating C1q levels (Bulla et al. 2016).

In malignant pleural mesothelioma (MPM), which is an aggressive malignancy, hyaluronic acid (HA) is produced in high levels. HA is a connective tissue glycosaminoglycan (GAG) that acts as an enhancer of tumor invasion. Heat-treated or freeze-dried HA can selectively interact with C1q, C1r, and C1s, to exhibit complement inhibitory activity (Hong et al. 2007). C1q could be detected in epithelioid tissue, where monocytoid cells were possibly its main source in MPMs. C1q concentration was found to be two- or threefold lower in MPM subjects than the control serum. C1q bound to HA *via* its globular domain and impacted cell adhesion and proliferation of mesothelioma cells in a manner similar to IgG (Agostinis et al. 2010). C1q could also bind to high molecular weight HA via its gC1q domain independent of complement activation (Agostinis et al. 2017). C1q bound to HA was able to induce adhesion and proliferation of mesothelioma cells (MES) *via* enhancement of ERK1/2, SAPK/JNK, and p38 phosphorylation; however, it did not activate the complement cascade. C1q seemed to be locally produced by non-tumor cells and could interact differentially to the extracellular matrix components present in the TME (Agostinis et al. 2017).

Dembitzer et al. (2012) and Kaur et al. (2016) showed that C1q treatment could induce apoptosis in an ovarian cancer cell line, SKOV3. Along with human C1q, recombinant globular head modules (ghA, ghB, and ghC) could also induce apoptosis. Exogenous treatment of C1q and its globular heads could significantly upregulate TNF-α and NF-κB expression, compared to control. C1q also upregulated mRNA expression of proapoptotic markers, such as Bax and Fas, and downregulated mTOR, RICTOR, and RAPTOR, which are cell cycle and cell division regulators (Kaur et al. 2016). Thus, serum complement activation can be an important strategy to mount anti-tumor response. C1q showed antitumor response by activating WOX1 to induce apoptosis and inhibited the growth of DU145 cells. C1q treatment caused cell cycle arrest (Hong et al. 2009). The *N*-terminal WW domain of WOX1 seemed responsible for mediating apoptosis of cancer cells. C1q suppressed DNA fragmentation in the p53/WOX1-expressing cells, suggesting that C1q treatment did not induce p53-mediated apoptosis. It was also observed that in the absence of C1q, HA induced STAT3 activation in DU145 cells (Hong et al. 2009).

Based on Oncomine database and survival analysis platform Kaplan-Meier plotter, Mangogna et al. (2019a) showed that C1q can be a prognostic biomarker for various carcinoma, such as breast, renal, and lung adenocarcinoma. C1q seemed to have a negative prognostic value in lung adenocarcinoma (Mangogna et al. 2019a). In another study involving in silico parameters along with immunohistochemistry and fluorescence microscopy, the expression of the genes encoding C1qA, C1qB, and C1qC was assessed as a potential prognostic marker for gliomas (Mangogna et al. 2019b). Single-cell sequencing (scRNA-seq) revealed $C1q^{+}$ tumor-associated macrophages as an important component in the TME. The study also suggested that C1q was co-expressed in healthy and tumor macrophages with HLA-DR, *apolipoprotein E*, and *mannose receptor* C-type 1 (Margot et al. 2022).

Involvement of C2, C3, C3b, C4, C5, C5a, and MAC

As mentioned earlier, C3 is the central molecule for complement activation cascades. In response to TNF-α, the internal synthesis and secretion of C3 were observed in various gastric cancer cell lines (MKN28, MKN45, and MKN74) (Kitano and Kitamura 1993).

Human malignant hematopoietic cell lines of various lineages, including myeloid (HEL, THP-1, U937, K-562, KG-1a, HL-60, DAMI) and lymphoid (DAUDI, Jurkat, RAJI, NALM-6, MOLT4) cell lines, expressed mRNA for both C3aR and C5aR receptors; however, K-562 did not express C3aR, and DAMI and Jurkat cells did not express C5aR. All the cell lines examined also expressed a second non-signaling receptor, C5a-like receptor 2 (C5L2) (Abdelbaset-Ismail et al. 2017). When U937 and KG-1a cells were activated with C3a and C5a, the expression of heme oxygenase 1 (HO-1) was downregulated, both at protein and mRNA level in leukemic cells. HO-1 is a negative regulator of complement cascade-mediated trafficking (Abdelbaset-Ismail et al. 2017).

In case of human lung cancer, silencing of C5aR1 caused reduction in skeletal metastatic burden and osteolysis, dampening their tumor-associated osteoclastogenic activity (Ajona et al. 2018).

Human ovarian carcinoma SKOV-3 cells, transfected for secreting murine C5a, were grown in SCID mice. C5a tumors induced infiltration of $DX5^{+}CD11b^{+}$ NK cells and $F4/80^{+}CD11b^{+}$ subsets of macrophages. C5a tumors showed significantly lower mRNA levels for VEGF, arginase, and TNF-α. VEGF expression was low in tumor cells, whereas iNOS mRNA level was considerably lower in C5a-expressing $CD11b^{+}$-infiltrating leukocytes. Non-adherent leukocytes from naïve SCID mice were capable of mounting cytotoxic response against SKOV-3 tumor cells, whereas $Gr\text{-}1^{+}CD11b^{+}$ cells from C5a tumor inhibited cytotoxic response toward tumor cells and were less suppressive than SKOV-3 tumors (Gunn et al. 2012). Blocking C5aR in a murine syngeneic lung cancer model significantly reduced MDSCs and other factors as arginase-1, CTLA-4, IL-6, IL-10, LAG3, and PD-L1 (Corrales et al. 2012). Non-small cell lung cancer (NSCLC) patients showed high level of C5a in plasma, which further enhanced invasion of C5aR-expressing cancer cells (Nitta et al. 2014).

Role of Complement Regulators

To protect the host cells from complement-mediated damage and to regulate the complement activation, most cells express complement regulatory proteins on their cell surface. Cancer cells have the capability to evade complement attack by expressing membrane-bound regulators (CD21, CD35, CD46, CD55, CD59) or secreting soluble regulators (FH, FHL-1, FHR) that hinder complement-mediated cytotoxicity and anti-tumor response.

Membrane-Bound Complement Regulatory Proteins

Several studies have shown that tumor cells overexpress CD46, CD55, and CD59 (Gelderman et al. 2002). Both CD55 and CD46 expressions are found at higher levels in gastric carcinoma (Li et al. 2001; Kiso et al. 2002), ovarian cancer (Bjorge et al. 1997), and cervical cancer (Gao et al. 2009). In colorectal cancer, soluble CD55 (sCD55) is found in stool, released by protease activity (Kohno et al. 2005; Kawada et al. 2003). CD59 has been detected in ascitic fluid of ovarian cancer and also in erythroleukemic cells (Hakulinen et al. 2004; Jorge et al. 2005; Jurianz et al. 2001).

Anti-sense-based inhibition of CD46 and CD55 expression enhanced complement-dependent cytolysis of T47D (breast), A549 (lung), and PC3 (prostate carcinoma) cells. C3 opsonization of CD55/CD46-deficient tumor cells was also enhanced (Zell et al. 2007). In human cervical cancer, the expression of CD55 as well as CD46 increased significantly which correlated well with the decrease in C3b deposition on human cervical cancer tissues (Gao et al. 2009). Geis et al. (2010) used small interfering RNAs (siRNAs) for post-transcriptional gene knock down of CD46, CD55, and CD59 in prostate (DU145), breast (BT474), and erythroleukemia (K562) cells to sensitize them to complement attack (Geis et al. 2010).

CR1 (CD35) is rarely expressed on tumor cells. CR1 A(3650)G RsaI polymorphism was found to be linked with genetic susceptibility to gallbladder cancer in north Indian population (Srivastava and Mittal 2009).

Gene expression of CD46, CD55, and CD59 in 15 primary USC cell lines (USPC-ARK-1 to USPC-ARK-15) was found severalfold higher compared to normal control endometrial cells. Sensitivity of USPC-ARK-2 and USPC-ARK-3 cell lines to NK cell cytotoxicity and trastuzumab was evaluated after knockdown of CD46, CD55, and CD59 by siRNA. Downregulation of CD55- and CD59-induced NK-mediated killing was observed in both the cell lines, but not with CD46 (Bellone et al. 2012).

Soluble Complement Regulators

Human FH is the most prominent fluid-phase complement inhibitor that regulates alternative pathway activation in plasma and on host surfaces. Recent studies on several cancers (lung, colon, ovarian, bladder, and glioblastoma) showed that FH and factor H-like protein 1 (FHL-1) help them bypass complement-mediated cytotoxicity (Wilczek et al. 2008; Junnikkala et al. 2002; Cheng et al. 2005).

The mRNA expression of factor H and FHL-1 was found to be at high levels in many non-small cell lung cancer cell (NSCLC) lines; hese proteins were found to be secreted extracellularly. Blocking of FH/FHL-1 increased C3 deposition on a lung adenocarcinoma cell line (H1264), and enhanced the release of C5a (Ajona et al. 2004). NSCLC lines, H2228 and H226, were capable of binding to endogenous FH on their surface (Cui et al. 2011).

In colon adenocarcinoma, both primary and metastatic tumors and the presence of FH/FHL-IR were predominant, with a strong increase in extracellular FH/FHL-IR at

the invasion site toward normal liver, whereas the signal was weak for normal colon. The level of FH/FHL-IR in metastasis was two-fold higher than at the primary sites. FH mRNA expression was higher in SW620 cells, and its functional blocking inhibited C3b inactivation at the cell membrane (Wilczek et al. 2008).

The mRNA expressions of FH, FHL-1, and FI were markedly increased in cutaneous squamous cell carcinoma (cSCC) along with the expression of other complement components such as C1r, C1s, C3, and FB. The levels of FH and FHL-1 mRNA were low in immortalized nontumorigenic keratinocyte-derived cell line (HaCaT) lacking functional p53, whereas they were abundantly expressed in Ha-*ras*-transformed HaCaT cell lines. This seems to suggesting that inactivation of p53 is not sufficient for FH and FHL-1 expression, rather activation of ras signaling is also required. At the same time, FH/FHL-1 expression in cSCC cells was downregulated by inhibition of ERK1/2 signaling and p38 MAPK signaling, when cSCC cells were treated with inhibitor of MEK1/2 (PD98059), p38a and p38b mitogen-activated protein kinases (SB203580 and BIRB796). This confirms that FH and FHL-1 are targets for ERK1/2 and p38 MAPK signaling cascades in cSCC cells (Riihilä et al. 2014). In vivo study showed higher expression of FI in invasive sporadic cSCCs and recessive dystrophic epidermolysis bullosa-associated cSCCs compared to cSCC in situ (Bowen's disease), premalignant epidermal lesions (actinic keratoses), benign epidermal papillomas (seborrheic keratoses), and normal skin. Aggressive Ha-*ras*-transformed cell line (RT3) showed higher FI expression than less tumorigenic HaCaT cell lines (HaCaT, A5, and II-4). Knockdown of FI expression also inhibited proliferation and migration of cSCC and also down-regulated ERK 1/2 activation (Riihilä et al. 2015).

Glioblastoma (GBM) is the most aggressive primary brain tumor in adults, with a poor prognosis. Human H2 glioblastoma cell line was found exceptionally resistant to complement-mediated lysis; it secretes FH and FHL-1, which bind to the cell membrane, promoting the cleavage of C3b to iC3b more efficiently in U251 and EA. hy 926 cells (Junnikkala et al. 2000). DeCordova et al. showed that the primary GBM cells (B30, B31 and B33), obtained from GBM patients post-surgery, secreted complement factor H-related protein 5 (FHR5), but not FH (DeCordova et al. 2020). The primary GBM cells produced FHR5 constitutively without cytokine stimulation. The culture supernatant of the primary GBM cells did not show the presence of FH, FHL-1, and complement factor H-related 1–4 (CFHR1–4) proteins, whereas H2 cells secreted FH and FHL-1. FHR5, purified from primary GBM cells, inhibited complement lysis, acting as a co-factor for factor I-mediated cleavage of C3b, and accelerated the decay of C3 convertase (DeCordova et al. 2019).

FH can bind to anionic molecules, including glycosaminoglycans (GAGs), which are highly expressed on basement membranes, ECM, and sialic acid in cell membranes to control alternative pathway activation at these locations. Robust complement activation was observed in the liver sinusoids of $fH^{-/-}$ mice, where alternative pathway activation in male $fH^{-/-}$ mice was associated with hepatocellular inflammation, chronic liver damage, and gradual development to liver tumors. Deposition of C3b and iC3b/C3d was also detected in the liver sinusoids of $fH^{-/-}$ mice (Laskowski et al. 2020).

Properdin is a 50 kDa glycoprotein fluid-phase positive regulator of complement pathway. Properdin can also function as a PRR, binding to microbial targets; it can also modulate phagocytosis by macrophages and influence pro-inflammatory cytokine production (Al-Mozaini et al. 2018; Kouser et al. 2018). Properdin was found to suppress breast cancer cell growth in vitro and in vivo. Properdin induced upregulation of intracellular pro-apoptotic transcription factor DDIT3 (DNA damage-inducible transcript 3) in MCF7 cell lines (Block et al. 2019). A recent bioinformatics study was undertaken utilizing Oncomine and UALCAN to investigate whether properdin can serve as a potential prognostic marker for human lung adenocarcinoma (LUAD), liver hepatocellular carcinoma (LIHC), cervical squamous cell carcinoma (CESC), and pancreatic adenocarcinoma (PAAD/PDAC). Lower expression of properdin was observed in both LUAD and LIHC tissues compared to their corresponding healthy l tissues. A significant negative correlation was observed between the expression of properdin and tumor purity in both LIHC and LUAD patients. However, partial positive correlation was observed with levels of infiltrating $CD8^{+}$T cells, B cells, and dendritic cells, but not with $CD4^{+}$T cells, macrophages, and neutrophils. However, LUAD samples showed partial correlation with infiltrating B cells, $CD4^{+}$T cells, $CD8^{+}$T cells, macrophages, neutrophils, and dendritic cells (Mangogna et al. 2021).

Conclusion

Complement appears to have a key role in tumorigenesis. There is definitely a local synthesis of complement proteins in the TME. However, many tumor cells have evolved strategies to counter complement attack by expressing ~~on their surface~~ complement inhibitor r by secreting soluble complement inhibiting factors. Much focus has recently shifted to infiltrating immune cells in the TME. Thus, their ability to secrete complement components to modulate the immunosuppressive TME by polarizing macrophages and neutrophils is of considerable interest. It is well known that several key complement proteins have immunomodulatory properties independent of their roles in the complement activation pathways. There is a great need to investigate immune cell-complement nexus in the TME.

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