

Complement in cancer: untangling an intricate relationship

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Abstract | In tumour immunology, complement has traditionally been considered as an adjunctive component that enhances the cytolytic effects of antibody-based immunotherapies, such as rituximab. Remarkably, research in the past decade has uncovered novel molecular mechanisms linking imbalanced complement activation in the tumour microenvironment with inflammation and suppression of antitumour immune responses. These findings have prompted new interest in manipulating the complement system for cancer therapy. This Review summarizes our current understanding of complement-mediated effector functions in the tumour microenvironment, focusing on how complement activation can act as a negative or positive regulator of tumorigenesis. It also offers insight into clinical aspects, including the feasibility of using complement biomarkers for cancer diagnosis and the use of complement inhibitors during cancer treatment.

An enormous body of data produced by converging disciplines has provided unprecedented insight into the intricate and dynamic relationship that exists between developing tumours and the host immune system^{1–3}. It is widely accepted that neoplastic transformation is a multifactorial process that the immune system can detect through genetic and epigenetic changes that alter the antigenic signatures of tumour cells. Control of tumour growth relies on both innate and adaptive mechanisms of immunosurveillance and is subject to the selective pressure exerted by distinct immunoregulatory pathways in the tumour microenvironment^{4–7}. In this regard, cancer treatment has been revolutionized by new immunomodulatory approaches that elicit durable clinical responses in cancer patients by restoring antitumour immunity and potentiating standard therapeutic regimens, such as tumour radiotherapy^{5,8}.

Elements of the innate immune response are integral components of antitumour effector mechanisms^{3,9}. In many ways, tumour cells are perceived by the innate immune system as noxious, ‘non-self’ matter that must be disposed of¹⁰. Therefore, potent antitumour cytotoxic responses are elicited by innate immune cells, followed by elimination of opsonized tumour cells through the concerted action of tumour-directed antibodies and complement^{9,11,12}. Complement is a phylogenetically conserved branch of the innate immune response that has traditionally been perceived as a network of proteins that rapidly respond to microbial intruders, triggering the release of inflammatory mediators, phagocytic responses and cell lysis¹³ (BOX 1). Growing evidence has

indicated, however, that this versatile innate immune effector system mediates key homeostatic functions in processes ranging from early vertebrate development and tissue morphogenesis to tissue regeneration, central nervous system synaptic pruning, host–microbiota symbiosis and adaptive immune regulation^{14–17}. In the context of cancer immunotherapy, complement can be readily triggered into action by damage-associated molecular patterns (DAMPs) exposed on the surface of tumour cells^{3,6}. Although the role of complement as an effector mechanism that potentiates antibody-dependent tumour cytotoxicity has been long appreciated, clinical challenges, such as the upregulation of a wide spectrum of complement regulatory proteins, remain to be addressed^{12,18,19}.

Clearly, the role of complement as an effector of tumour cytotoxic responses remains relevant to antibody-mediated immunotherapy, guiding new therapeutic options. Intriguingly, however, accumulating evidence from studies published in the past decade has pointed to a fascinating paradigm shift: the realization that complement activation within the tumour microenvironment can serve a tumour-promoting role by perpetuating local T cell immunosuppression and chronic inflammation that promotes tumour immune escape, outgrowth and metastasis^{20–23}. Diverse complement-derived effectors and downstream signalling partners have been implicated in processes ranging from tumour cell anchorage and proliferation to tumour-associated angiogenesis, matrix remodeling, migration, tissue invasiveness and metastasis^{6,19}.

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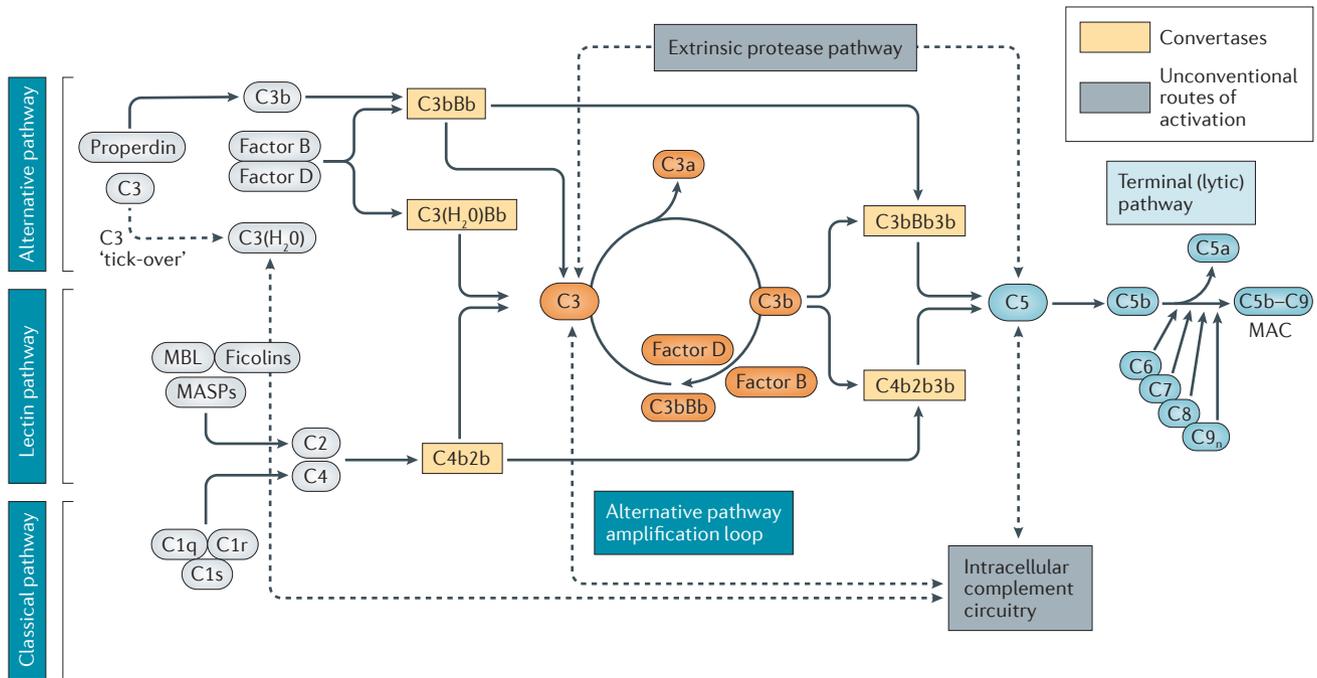
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Box 1 | An overview of the complement system

The complement system comprises an extensive network of fluid-phase and membrane-bound glycoproteins, cofactors, receptors and regulatory proteins that engage in innate immune recognition, adaptive cell stimulation and pro-inflammatory effector responses¹²⁶. Being a crucial mediator of tissue immunosurveillance, complement responds rapidly to molecular stress signals through a cascade of sequential proteolytic reactions initiated by the binding of pattern recognition molecules (for example, C1q, mannose-binding lectin (MBL), MBL-associated serine proteases (MASPs), ficolins and properdin (also known as Factor P)) to distinct structures on damaged cells, biomaterial surfaces or microbial intruders¹⁷. Whereas three ‘canonical’ pathways of activation have been described to date (that is, the classical, alternative and lectin pathways), mounting evidence indicates that complement can be activated via multiple routes, depending on the initiating triggers and the distinct microenvironment or pathophysiological context¹²⁶. The classical pathway is initiated by binding to circulating or surface-bound immune complexes, while the lectin and alternative pathways are triggered by pathogen-associated molecular patterns (PAMPs) or aberrant carbohydrate structures on damaged or necrotic cells. All activation pathways converge at the cleavage of C3, an abundant plasma protein that undergoes elaborate conformational changes upon activation, thereby exposing multiple sites

of interaction with diverse immune effectors¹²⁷. Notably, the spontaneous low-level hydrolysis of C3 keeps the alternative pathway in ‘standby’ mode to allow for rapid amplification upon microbial challenge¹²⁸. Complement activation culminates in the assembly of short-lived multiprotein complexes with enzymatic activity termed ‘convertases’. These enzymes are responsible for the proteolytic activation of the central components C3 and C5 and the release of their respective bioactive fragments, C3a and C3b, and C5a and C5b. The rapid amplification of C3b deposition via the alternative pathway is a process known to underlie several clinical disorders associated with genetic or acquired complement dysregulation (for example, C3 glomerulopathy)^{17,129}. Cleavage of C5 leads to the release of the potent inflammatory mediator C5a and initiates a sequence of protein–protein interactions that induces assembly of the membrane attack complex (MAC, C5b–C9), a multiprotein ‘pore’ with cell activating and cytolytic properties. Unconventional routes of complement activation include the deployment of proteolytic enzymes of the coagulation and fibrinolytic systems that can efficiently cleave both C3 and C5 into their bioactive fragments¹³⁰. These non-canonical routes of activation, in conjunction with the intracellular complement circuitry^{38,131}, define a broader pathophysiological base of triggering cues that can initiate homeostatic or disease-tailored complement responses.



With new insights being gained from a variety of tumour models, it is becoming clear that the contribution of complement to cancer pathophysiology is far more complex than originally thought and appears to be largely contextual, depending on several factors, such as the cellular origin of the tumour in question, its inherent capacity to produce autologous complement proteins, the nature of the tumour microenvironment and the magnitude of complement activation.

In this Review, we discuss current and emerging aspects of complement’s contribution to cancer elimination and progression and suggest therapeutic targets with clinical potential. We also place emphasis on clinical challenges faced in translating these findings to efficacious tumour cytotoxic modalities or combination

immunotherapies and interrogate the feasibility of using complement-based biomarkers for cancer diagnosis, tumour staging and prognosis.

Complementing cancer immunotherapy

There is a growing appreciation that durable clinical responses in patients with cancer are more likely to be achieved when targeted therapies that ablate key oncogenic signalling pathways are combined with approaches that effectively reverse tumour-instigated immunosuppression and augment antitumour immunity — an example has been the success of immune checkpoint blockade^{5,8}. Among the currently explored targeted therapies, monoclonal antibody (mAb)–based cancer immunotherapy has gained considerable traction as a

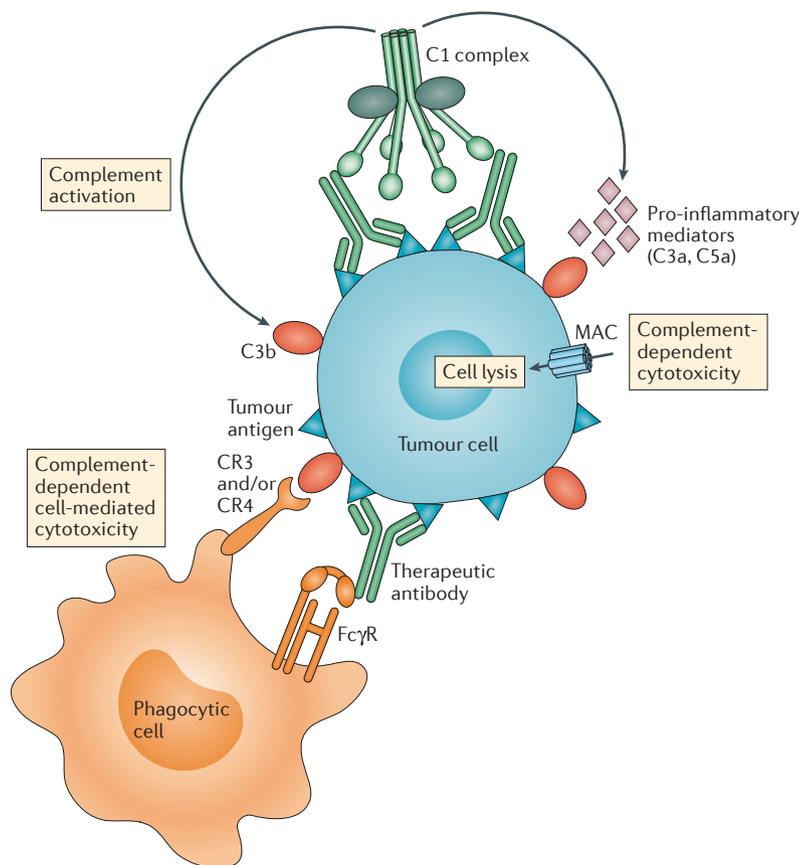


Figure 1 | Complement mediates tumour cytotoxicity in the context of antibody-based immunotherapy. Schematic illustration of the basic humoral and cellular elements that evoke tumour cytotoxicity in the context of monoclonal antibody (mAb)-based cancer immunotherapy. Direct tumour cell elimination is achieved by (i) complement-dependent cytotoxicity (CDC) and (ii) antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent phagocytosis elicited upon targeting of tumour antigens by clinically available therapeutic mAbs. Tumour-targeted mAbs known to elicit complement-mediated cytolytic responses include the anti-CD20 mAbs rituximab and ofatumumab in B cell lymphomas and chronic lymphocytic leukaemia. Targeting of tumour cells by therapeutic mAbs triggers complement activation via the classical pathway. Binding of C1q to the Fc portion of these antibodies leads to the assembly of the active C1 complex (C1q, C1r and C1s), which acquires proteolytic activity over complement components, initiating the cascade. Complement activation leads to tumour cell opsonization by C3-derived opsonins (C3b, iC3b and C3dg) and the generation of potent pro-inflammatory mediators (C3a and C5a), which in turn recruit and activate immune cells with phagocytic properties (neutrophils and macrophages). Downstream activation of terminal complement components results in the assembly of the pore-forming membrane attack complex (MAC), or C5b–C9, on the tumour cell membrane. The anaphylatoxin C5a is known to upregulate activating Fcγ receptors (FcγRs) on phagocytic cells, priming them for enhanced phagocytosis and increasing the magnitude of the tumour cytolytic response. C3-derived fragments (C3b, iC3b and C3dg) on tumour cells bind to CR3 and/or CR4 complement receptors on phagocytes, thus augmenting the FcγR-dependent phagocytic uptake of opsonized tumour cells.

viable therapeutic modality and has shown promising results, particularly in the treatment of certain haematological malignancies, such as B cell lymphomas, multiple myeloma and chronic lymphocytic leukaemia^{12,24–27}.

How mAbs harness the complement system. The clinical benefit of mAb-based immunotherapies is attributed partially to their ability to evoke complement-dependent cytotoxicity (CDC) that culminates in tumour cell

elimination^{12,18}. Indeed, complement has long been considered a potent cytolytic ‘payload’ for tumour-specific antibodies, and seminal studies in the early 1970s laid the groundwork for what would later become a prevalent concept in the field^{28,29}. Notably, various cancer immunotherapeutic protocols used today in the clinic rely on the two-pronged capacity of mAbs to halt oncogenic signalling and tumour cell growth and to simultaneously fix complement on the surface of the targeted tumour cells, thereby eliciting CDC^{18,24,25}.

Despite modest attempts to harness the cytolytic capacity of complement in mAb-based cancer immunotherapy over the years, full realization of its tumoricidal potential came only with the US Food and Drug Administration (FDA) approval of rituximab, a chimeric anti-CD20 mAb developed for the treatment of B cell lymphomas^{12,24}. A wide array of *in vitro* studies using cancer cell lines, primary tumour cells, mouse tumour models and blood profiling of patients with chronic lymphocytic leukaemia after mAb infusion have provided evidence for the auxiliary, tumour-destructive role of complement in the context of anti-CD20 immunotherapy¹². mAbs targeting surface antigens such as CD20, CD38 and CD52 that are highly expressed on B cell- or T cell-derived tumours are among the most well-studied tumour-specific mAbs in terms of their ability to elicit CDC^{18,24,30}.

The binding of mAbs to tumour antigens triggers the concerted activation of multiple effector pathways that operate in parallel to elicit cytolytic responses. These include CDC elicited via the classical pathway of complement activation and assembly of the membrane attack complex (MAC) (BOX 1); antibody-dependent cell-mediated cytotoxicity (ADCC), operating primarily through the engagement of Fc receptors on natural killer (NK) cells; and antibody-mediated phagocytosis¹² (FIG. 1). Depending on the magnitude of the complement response on targeted cells, tumour cell elimination can occur either through MAC assembly and direct cell lysis or via recruitment of macrophages that take up opsonized tumour cells through the recognition of opsonic fragments (for example, C3b, iC3b and C3dg) by complement receptors CR1, CR3, CR4 or CR1g¹² (BOX 2). Concomitant recognition of the Fc portion of tumour-specific mAbs by Fcγ receptors (FcγRs) on macrophages allows for synergistic clearance of tumour cells via complement CR3- and FcγR-dependent uptake (FIG. 1). Notably, the release of complement-derived inflammatory mediators, such as C5a (BOX 1), further potentiates the cytolytic activity of mAbs by promoting the recruitment of phagocytic cells and skewing the relative expression of activating and inhibitory FcγRs on neutrophils and macrophages³¹.

It should be noted, however, that a wide array of tumour cells, especially in solid tumours, have evolved diverse mechanisms by which they can limit CDC on their surfaces. Overexpression of membrane-bound complement regulatory proteins (that is, CD55 (also known as complement decay-accelerating factor (DAF)), CD46 (also known as membrane cofactor protein (MCP)), and CD59) or sequestering of fluid-phase regulators

Box 2 | Complement: numerous proteins, plenty of functions

Soluble complement proteins are primarily produced in the liver (the exceptions being C1q and Factor D, which are secreted mainly by macrophages and adipocytes, respectively)⁵⁵. Whereas the major 'pool' of complement is located in the blood, a variety of cell types, including fibroblasts, adipocytes and endothelial and immune cells, secrete complement proteins. A boost in complement production is often observed during inflammatory and pathologic conditions, so that virtually any tissue can be surrounded by complement activation products¹³. Such a notion of local complement is key to perceiving how imbalanced complement activation affects pathology in distinct tissues, from the skin to the kidneys, from the lung to the brain, and influences the fate of an assortment of tumour cells^{3,17,126}. Essentially, the presence of a trigger or improper regulation results in the production of activation fragments (BOX 1) that can bind to their respective receptors on cell surfaces and elicit biological responses. Upon complement activation, C3 fragments (that is, C3b, iC3b and C3dg) that are associated with antigens bind to the complement receptors CR1 (CD35), CR3 (CD11b and CD18), CR4 (CD11c and CD18) and/or CR1g, inducing phagocytosis and modulating the function of antigen-presenting cells¹³. In turn, triggering of CR2 by iC3b and C3dg is associated with the activation of B cells³⁵. Furthermore, signalling through the G protein-coupled anaphylatoxin receptors C3aR, in the case of C3a, or C5aR1 (also known as CD88), in the case of C5a and desarginated C5a (C5a-desArg), regulates cell activation, degranulation, proliferation and cytokine responses, which are essential for initiating and maintaining inflammatory responses³⁶. In addition, adaptive immune responses are modulated when membrane cofactor protein (MCP, also known as CD46) is triggered on T cells¹³². Such an array of interactions between complement proteins and cell-surface receptors not only guarantees immune surveillance but also determines the outcome of immune responses, metabolic pathways and developmental processes. Proper control of the activation signals described above is required to prevent excessive activation that can lead to tissue damage and is achieved by an assortment of complement regulators (TABLE 1) that restrain certain steps of the complement cascade¹³³.

(for example, Factor H or C4-binding protein (C4BP, also known as C4BPA)) (TABLE 1) by tumour cells has been shown to prevent CDC by limiting C3 opsonization to levels well below the threshold required for terminal pathway activation and MAC assembly^{32–34}. In this regard, several strategies have been devised to overcome this impediment in mAb-based immunotherapy (discussed below).

Beyond cytotoxicity. Growing evidence indicates that complement, in addition to providing an adjunctive mechanism for mAb-mediated tumour cytolysis, mediates a plethora of immunomodulatory functions that are pertinent to tumour immunosurveillance and anti-tumour immunity^{35,36}. Fundamental processes such as tumour antigen processing and presentation by antigen-presenting cells (APCs), as well as B cell activation and T helper and effector T cell survival and differentiation, appear to be influenced by the intricate crosstalk of complement effector proteins with key cellular pathways that drive B cell and T cell responses^{37,38}. Dendritic cells (DCs), for instance, express a wide array of complement proteins and receptors³⁹. In this regard, C3 deficiency has been associated with a reduced antigen-presenting capacity of APCs (lower expression of MHC class II molecules and impaired co-stimulation via CD80 and CD86), with important implications for autoimmune conditions and anti-tumour immunity⁴⁰. Notably, the absence of C3aR or C5aR1 stimulation at the APC–T cell interface has been shown to negatively affect the survival of CD4⁺ T cells⁴¹. Moreover, genetic or pharmacological ablation of C3aR or C5aR1 signalling has also been linked to the induction

of an immunosuppressive phenotype in CD4⁺ T cells in a manner that could diminish the magnitude of antitumour effector T cell responses⁴².

Further insight into the fundamental role of complement in immune cell homeostasis has been provided by studies describing an intracellular complement circuitry in CD4⁺ T cells that modulates cell differentiation through its crosstalk with the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome and the generation of reactive oxygen species (ROS)^{38,43}. Taken together, these studies support the presence of a constitutively active complement circuitry within lymphocytes that has a role in the maintenance of the T cell pool in the periphery and may also potentiate effector T cell responses in the context of tumour immunotherapy.

Complement and cancer vaccines. Cancer vaccines represent another promising modality in cancer immunotherapy, and several studies have indicated that complement activation may contribute to vaccine efficacy through enhanced antigen uptake and co-stimulatory signalling⁴⁴. Indeed, C5a has been shown to potentiate antigen processing and presentation by human DCs^{45,46}. In this context, a C5a-derived C5aR1 agonist fused to a peptide-based cancer vaccine has been tested in a preclinical model of melanoma, with encouraging results⁴⁷. These studies point to a different therapeutic perspective whereby harnessing complement activation and triggering C5aR1-dependent signalling on tumour antigen-loaded DCs may help patients elicit potent anti-tumour immune responses. From a different standpoint, the function of the opsonin C3dg as a molecular adjuvant that lowers the activation threshold of B cells and the role of C3-derived fragments in promoting antigen retention by follicular DCs and boosting memory responses have long been appreciated as mechanisms bridging innate and adaptive immune responses⁴⁸. However, this fundamental facet of complement biology has not been rigorously investigated in the context of cancer immunotherapy. These key immunoregulatory activities of complement may have important implications for tumour immunotherapy and may be worth exploring during the design of antitumour vaccines.

Complement and radiotherapy. Alongside surgical resection and chemotherapy, radiotherapy is also recognized as a clinical mainstay of cancer treatment, especially in the context of very aggressive tumours with poor prognosis⁴⁹. Recent evidence supports a causative association between radiation-induced tumour cell damage, the tumour-derived pro-inflammatory milieu and the potentiation of antitumour immunity^{50,51}. Interestingly, radiotherapy-induced cell death was recently shown to induce transient complement activation within solid tumours, with both tumour cells and infiltrating immune cells serving as sources of complement protein production⁵². More importantly, radiotherapy was found to upregulate the release of C3a and C5a within the tumour microenvironment, inducing potent DC responses and subsequently leading to pronounced infiltration of CD8⁺ effector T cells into the irradiated tumours⁵². Notably, an

Table 1 | Regulators of complement activation

Regulators	Gene	Main expression	Function	Connection with disease	Refs
C1 inhibitor (SERPING1)	<i>SERPING1</i>	Liver	Regulates the CP and LP. Acts as an inhibitor for various proteases involved in the complement (C1r, C1s and MASPs), kinin, fibrinolytic and coagulation systems.	Hereditary angioedema.	134
sMAP (MAp19)	<i>MASP2</i>	Liver	Form complexes with MBL. Reported to compete with MASP2, but physiologic function still controversial.	ND	135
MAP1 (MAp44)	<i>MASP1</i>	Liver	Form complexes with MBL and ficolins. Reported to inhibit deposition of C4.	Mutations are associated with 3MC syndrome.	136,137
C4b-binding protein (C4BP)	<i>C4BPA</i> , <i>C4BPB</i>	Liver	Accelerates decay of CP and LP convertases. Cofactor for FI. Reported to bind apoptotic and necrotic cells and amyloid protein, preventing inflammation.	Mutations associated with aHUS and recurrent pregnancy loss.	138,139
CR1 (CD35)	<i>CR1</i>	Kidney, bone marrow-derived cells, excluding platelets	Accelerates decay of AP, CP and LP convertases. Cofactor for FI.	Variants associated with susceptibility to SLE and resistance to malaria. Deficiency associated with glomerulonephritis. Low levels of CR1 on erythrocytes associated with poor outcome in PNH patients.	140–143
Factor H (FH)	<i>CFH</i>	Liver	Main regulator of the AP. Accelerates decay of AP convertases. Cofactor for FI. Recognizes self-surfaces, enabling local regulation of complement activation in cells and tissues.	Gene variants associated with aHUS, AMD and C3G. Deficiency associated with aHUS and increased susceptibility to infections.	144–149
FHL1 (Reconnectin)	<i>CFH</i>	Liver	Accelerates convertase decay. Cofactor for FI.	Gene variants associated with aHUS, AMD and C3G.	150,151
Factor I (FI)	<i>CFI</i>	Liver	Protease that mediates degradation of C3b and C4b fragments, interrupting complement cascade activation.	Gene variants associated with aHUS and AMD. Deficiency associated with increased susceptibility to infections.	152–154
MCP (CD46)	<i>CD46</i>	All nucleated cells	Cofactor for FI.	Gene variants associated with aHUS. Deficiency associated with male infertility.	152,155
DAF (CD55)	<i>CD55</i>	Wide expression	Accelerates convertase decay.	Deficiency associated with PNH or Inab phenotype.	156
CD59 (Protectin)	<i>CD59</i>	Wide expression	Binds to C8 and C9; prevents assembly of TCC.	Deficiency associated with PNH and neurological symptoms.	157,158
Vitronectin (S-protein, S40)	<i>VTN</i>	Liver	Binds to C5b–C9; prevents assembly of TCC. Also implicated in cell adhesion via binding to integrins.	ND	159
Clusterin (Apolipoprotein J, SP-40)	<i>CLU</i>	Reproductive organs	Binds to C7–C9; prevents assembly of TCC. Additionally, is a stress-activated chaperone associated with various physiologic processes.	ND	160
Carboxypeptidase N (Polypeptide 1)	<i>CPN1</i>	Liver	Degrades C3a and C5a to their desArg forms.	Angioedema, chronic urticaria, asthma.	161,162

Abbreviations: 3MC syndrome, a rare developmental disorder that unifies the overlapping autosomal recessive disorders previously known as Mingarelli, Malpuech, Michels and Carnevale syndromes; aHUS, atypical haemolytic uraemic syndrome; AMD, age-related macular degeneration; AP, alternative pathway; CP, classical pathway; CR1, complement receptor 1; DAF, decay accelerator factor; FH, Factor H; FI, Factor I; LP, lectin pathway; MBL, mannose-binding lectin; MCP, membrane cofactor protein; MASP, MBL-associated serine protease; ND, not determined; PNH, paroxysmal nocturnal haemoglobinuria; SLE, systemic lupus erythematosus; TCC, terminal complement complex.^{134–162}

apparent discordance between these findings and those of a recent report showing that C3 inhibition potentiates antitumour immunity after fractionated radiotherapy⁵³ could be attributed to the different radiotherapeutic regimens applied in each case and to the variable extent of tumour-associated inflammation elicited by these models. While these findings await corroboration in different tumour models, they provide fertile ground for considering complement activation as a beneficial component of radiotherapeutic protocols in various types of cancer.

Feeding inflammation and tumorigenesis

Undeniably, in the context of immunotherapy, complement potentiates tumour killing via its immune adjuvant properties and induction of cytolysis (BOX 2). Extensive investigation of the tumour microenvironment, however, has uncovered molecular mechanisms linking imbalanced complement activation and cancer progression. The tumour microenvironment is essentially composed of genetically abnormal cells surrounded by blood vessels, fibroblasts, immune cells, stem cells and extracellular

matrix (ECM), and a dynamic crosstalk among these various components ultimately determines the fate of the tumour⁵⁴ (FIG. 2). It is fascinating that tumours emerge from a single cell or a small number of cells bearing mutations in tumour suppressor genes and/or oncogenes. To become cancerous, however, these cells must acquire several mutations in specific genes that allow proliferation beyond the regular rate, as well as mutations that facilitate cell migration, survival in the circulation, invasion of tissues and adaptation to new tissue environments. In this context, it is particularly interesting that the past decade has uncovered dysregulated complement activation in the tumour microenvironment as a key link between inflammation, the suppression of antitumour immune responses and the promotion of tumorigenesis³.

Production and activation of complement in the tumour microenvironment. The local production of complement proteins by various tissues is well recognized^{55,56}

(BOX 2). Furthermore, both tumour and stromal cells produce complement proteins, and evidence for local and systemic activation of complement is often observed in cancer settings^{57,58}. In line with the notion that initiators of complement activation can sense DAMPs⁵⁹, complement activation appears to occur in response to tumour-associated antigens, and increased deposition of complement activation fragments is commonly detected in tumour tissues. Indeed, the presence of C1q along the tumour vasculature has been detected in a rodent model of cervical cancer, and C1q has also been shown to bind directly (independent of antibodies) to phospholipids in lung tumour cell lines^{22,60}. Evidence of classical pathway activation in cancer, such as increased plasma concentrations of the C4d fragment, has been obtained from patients with lung cancer, thyroid cancer, astrocytoma, lymphoma, leukaemia, or oropharyngeal cancer^{3,60}. Moreover, signs of a deregulated alternative pathway of complement activation have been reported

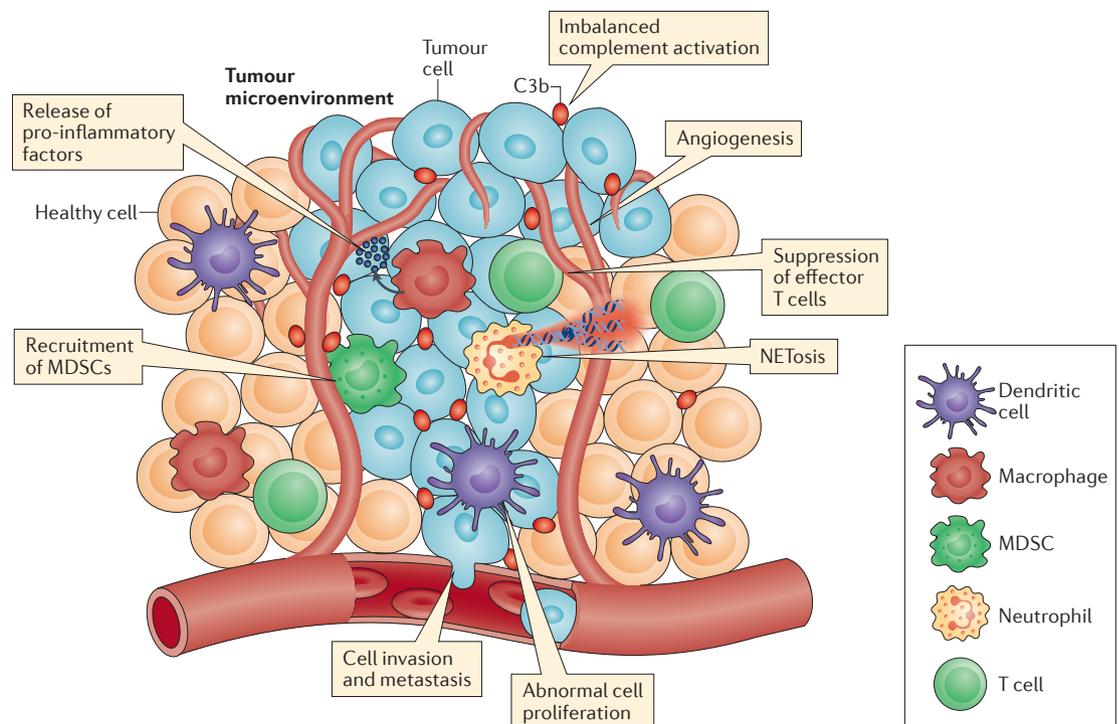


Figure 2 | Complement activation in the tumour microenvironment promotes tumorigenesis. A schematic illustration of the fine interplay between complement effectors, tumour cells and innate and adaptive immune cells in the tumour microenvironment, highlighting the many means by which complement contributes to tumour progression. Complement proteins can be secreted by tumour cells or tumour-infiltrating immune cells or can enter the tumour stroma through the vasculature. Local complement imbalance and complement activation within the tumour vasculature can lead to the release of pro-inflammatory factors by both tumour cells and tumour-infiltrating myeloid cells (for example, macrophages and dendritic cells (DCs)). Deregulated complement activation perpetuates macrophage-sustained tumour inflammation, creating a favourable environment for tumour growth. Local release of distinct complement activation fragments, such as C5a, recruits myeloid-derived suppressor cells (MDSCs) into the solid tumour. MDSCs exert potent immunosuppressive effects by ablating effector CD8⁺ T cell responses and facilitating tumour growth. Complement proteins have also been shown to modulate CD4⁺ T helper cell responses within the tumour microenvironment, shifting the inflammatory milieu towards a pro-tumorigenic phenotype. Complement activation products have been shown to promote tumour-associated angiogenesis, enhancing cell invasion into adjacent tissues and tumour metastasis to distant organs. Moreover, complement activation has been associated with the induction of a neutrophil-driven hypercoagulable state in certain solid tumours that further promotes tumour progression. In this respect, complement activation triggers the accumulation of pro-tumorigenic neutrophils within solid tumours, potentiating their procoagulant responses (for example, NETosis) and thus establishing a link between complement-driven coagulation, inflammation and tumour progression.

in patients with acute lymphoblastic leukaemia, Burkitt lymphoma or multiple myeloma, and a deregulated lectin pathway has been found in patients with T cell leukaemia⁶¹. Although evidence associating specific tumour antigens with differential activation of complement pathways is still scant, it is tempting to speculate that modified glycoproteins and glycolipids and aberrantly expressed mucins in transformed tumour cells can all act as DAMPs that are able to activate complement. Identification of such triggers would be of great value should anti-complement therapy become a reality in the adjunct treatment of cancer.

While tumour and stromal cells are active in producing complement proteins, and the local microenvironment favours complement activation, complement regulates the activation status of the local cells. In the past decade, a wealth of research has provided compelling evidence regarding the role of complement in the modulation of immune cells (discussed above and BOX 2). In addition, *in vitro* data indicate that complement is linked to the differentiation of myeloid-derived suppressor cells (MDSCs), which are seemingly involved in immune responses to cancer^{22,62}. Given that cancer development is strongly influenced by immune responses, one may assume that complement is a linking factor determining the quality and magnitude of the local antitumour immune response.

Does complement activation by infections affect tumorigenesis? Our current understanding suggests a dual role of immunity in cancer, in which pro-inflammatory signals lead to DC activation, antigen presentation and consequent activation of effector T cells, while chronic inflammation, by contrast, promotes an immunosuppressive environment that supports tumour progression and metastasis⁶³. Indeed, epidemiological data clearly show that tumour-associated inflammation is a hallmark of cancer, with close to 20% of the cancers worldwide being driven by microbial infection with organisms such as *Helicobacter pylori*, human papilloma virus (HPV), hepatitis virus, Epstein–Barr virus (EBV) and HIV. Although some of these infectious agents (for example, HPV and EBV) induce cell transformation directly, others promote tumorigenesis via local tissue inflammation^{64,65}. Whether these infections trigger complement activation that fuels the inflammatory response in cancer is an interesting topic for future investigation.

Complement modulates immune responses in the tumour microenvironment. Animal models have contributed immensely to increasing our understanding of the participation of complement in the promotion of tumorigenesis (TABLE 2). Whereas a pioneering study demonstrated that the activation fragment C5a (BOX 1) recruits MDSCs to the tumour microenvironment, resulting in suppressed antitumour immune responses²², numerous other investigations have corroborated the view of imbalanced complement activation as a promoter of a pro-inflammatory environment that sustains tumorigenesis. MDSCs, for instance, are increasingly recognized to suppress effector T cells through deprivation of amino acids,

production of nitric oxide (NO) and ROS, upregulated expression of programmed cell death 1 ligand 1 (PDL1) and secretion of angiogenic factors⁶⁶. C5a-mediated activation of MDSCs has been associated with the induction of immunomodulators such as arginase 1 (ARG1), cytotoxic T lymphocyte antigen 4 (CTLA4), IL-6, IL-10, lymphocyte activation gene 3 protein (LAG3) and PDL1 in a murine model of lung cancer⁶⁷. Interestingly, generation of C5a in this model appears to be independent of complement activation. Consistent with this observation, *in vitro* studies have indicated that C5a can be generated by the cleavage of C5 by serine proteases expressed on the surface of tumour cells⁶⁸. In addition, a model of lung cancer has provided evidence to suggest that tissue factor-induced coagulation leads to C5 activation and tumour promotion, further supporting the notion of local complement activation by coagulation-related proteases in the tumour microenvironment⁶⁹ (BOX 1). C5a has also been shown to participate in tumorigenesis by upregulating the expression of molecules that suppress local immune responses such as PDL1, transforming growth factor- β (TGF β) and IL-10 (REFS 58,70).

Findings from two independent groups, using models of melanoma and lung cancer, have uncovered a therapeutic potential for combined blockade of the immune checkpoint molecule PDL1 and complement^{71,72}. Anti-PDL1 mAbs are currently used in the clinic for the treatment of melanoma and lung cancer. By binding to its receptor PD1 (also known as PDCD1) on activated T cells, PDL1 dampens T cell receptor (TCR)-mediated production of IL-2 and subsequent T cell proliferation. Therefore, instead of targeting tumour cells, these mAbs block inhibitory pathways that prevent effective antitumour responses⁷³. Of note, mAbs that target immune checkpoints are designed to induce limited complement activation, CDC and ADCC. Combined ablation of C5a- and PDL1-induced signalling appears to be more efficient in restraining tumour growth than anti-PDL1 therapy alone^{71,72}, thus indicating that complement inhibition increases the clinical benefit of immune checkpoint blockade. Additionally, the long pentraxin PTX3 has been shown to act as a tumour suppressor in skin, epithelial and mesenchymal carcinogenesis²¹. Evidence suggests that PTX3 is induced during carcinogenesis by IL-1 and that it regulates complement-derived chemokine production via recruitment of Factor H²¹ (TABLE 1). Most importantly, in three independent studies, the PTX3 gene was found to be methylated and silenced in selected human tumours⁷⁴.

The immunosuppressive role of C5a in cancer settings is somewhat paradoxical, considering that it induces the maturation of DCs and the differentiation of T helper 1 (T_H1) cells^{42,75}. Such apparent contradictions were addressed in a study comparing tumour progression in mice injected with distinct murine lymphoma cell lines that secrete either low (RMA-1474 cells) or high (RMA-3CF4 cells) amounts of C5a. Notably, high production of C5a was associated with more prominent tumorigenesis accompanied by decreased numbers of interferon- γ (IFN γ)-producing CD8⁺ and CD4⁺ cells in the spleens and lymph nodes

Table 2 | Animal models targeting complement in cancer research

Cancer model	Target protein	Observations	Refs
Cervical: syngeneic model (TC-1 cells)	C3, C5aR1	C3 is deposited on engrafted tumours. Impaired tumour growth in C3 ^{-/-} and C5aR1 ^{-/-} mice. Impaired tumour growth upon pharmacological inhibition of C5aR1. C5aR1-dependent migration of MDSCs to tumours. C5aR1-dependent production of ROS and RNS by MDSCs.	22
Ovarian: xenograft model (RMA-3CF4 cells) Lymphoma: syngeneic model (RMA-1474 cells)	C5aR1	Tumour burden is associated with increased expression of C5a. C5a-induced inflammation correlates with decreased numbers of effector T cells.	76
Ovarian: genetic model (TgMISIIR-Tag)	C3, C5aR1	Attenuated tumorigenesis in TgMISIIR-Tag mice that have a full or partial deficiency in C3 or C5aR1. C3-dependent production of pro-inflammatory cytokines. C5a-dependent production of pro-angiogenic factors. Impaired tumour growth upon pharmacological inhibition of C5aR1.	85
Ovarian: syngeneic model (ID8-VEGF) Ovarian: xenograft model (SKOV3ip1 cells)	C3, C5aR1	Tumour cells secrete complement proteins that promote tumour growth in an autocrine fashion via induction of the PI3K-AKT pathway. Impaired tumour growth in C5aR1 ^{-/-} mice.	57
Breast: syngeneic, metastatic (4T1 cells)	C5aR1	C5aR1-dependent recruitment of immature myeloid cells to the lung and consequent inhibition of effector T cells via production of TGFβ and IL-10. Pharmacologic or genetic ablation of C5aR1 attenuated lung metastasis.	23
Melanoma: syngeneic model (B16F10 cells transfected with gp33)	C3	Robust antitumour response in C3-depleted mice (using CVF) via NK-mediated activation of gp33-specific CD8 ⁺ T cells.	104
Colon: syngeneic model (MC38 cells)	C3	Robust antitumour response in C3-depleted mice (using CVF) via increased expression of CCL5, CXCL10 and CXCL11 and migration of CD8 ⁺ T cells.	134
Colon: syngeneic, metastatic (SL4, CT26 cells) Colon: xenograft model (HCT116, SW116 cells)	C5a	Increased production of C5a by colon cancer cell lines. Decreased infiltration of inflammatory cells and number of metastases in the liver of C5aR1 ^{-/-} mice. C5a-dependent production of CCL2 by macrophages via the AKT pathway.	58
Sarcoma: induced by carcinogen (3-MCA)	PTX3 C5a	PTX3 regulates complement activation by interaction with C1q and FH. Increased complement activation in Ptx3 ^{-/-} mice. Ptx3 ^{-/-} mice are more susceptible to cancer development. Increased generation of C5a, CCL2 production and recruitment of tumour-promoting macrophages in Ptx3 ^{-/-} mice	21
Colitis-induced colorectal cancer (azoxymethane plus dextran sulfate sodium)	C3, C5, C5aR1	Decreased tumorigenesis in C3 ^{-/-} , C5 ^{-/-} or C5aR1 ^{-/-} mice. C5a signals on epithelial cells do not contribute to tumorigenesis. C5a signalling on neutrophils promotes IL-1β production. Complement inhibition results in decreased production of IL-1β and IL-17.	79
Melanoma: syngeneic model (B16F10 cells)	C3, C3aR, C5aR1	Decreased tumorigenesis in C3 ^{-/-} , C3aR ^{-/-} or C5aR1 ^{-/-} mice. Expression of C3aR and C5aR1 by 20% of melanoma TILs. C5a promotes secretion of IL-10 by effector CD8 ⁺ T cells. Complement inhibits antitumour immunity via a PDL1-independent pathway. Combined blockage of PDL1 and complement results in enhanced antitumour response.	71
Melanoma: syngeneic model (B16F10 cells)	C3aR	Decreased tumorigenesis in C3aR ^{-/-} mice. Increased numbers of neutrophils and CD4 ⁺ T cell subsets and decreased numbers of macrophages in the tumours of C3aR ^{-/-} mice.	77
Intestine: genetic model (Apc ^{Min/+})	C3aR	Decreased systemic levels of C3a in aged Apc ^{Min/+} mice. Reduced tumorigenesis in Apc ^{Min/+} C3aR ^{-/-} mice. C3a-induced hypercoagulation and NET formation leading to polarization of low-density neutrophils with a pro-tumorigenic phenotype.	78
Intestine: genetic model (Apc ^{Min/+}); diet-induced	C3, C5aR1	Decreased tumorigenesis in C3 ^{-/-} and C5aR1 ^{-/-} mice. Impaired tumour growth upon pharmacological inhibition of C5aR1. Tumorigenesis associated with increased levels of C5a and inflammation. Feeding with HFD using corn or coconut oil as a source of fat results in C5a generation, inflammation and tumorigenesis. Olive oil used as a source of fat does not promote complement activation or tumorigenesis.	82
Melanoma: syngeneic model (B16F10 cells)	C1q	C1q is expressed in the stroma and endothelium of various malignant tumours. Decreased tumorigenesis and metastasis in C1q-deficient mice. C1q promotes tumour growth independent of complement activation by inducing angiogenesis, cell adhesion and proliferation.	86
Squamous cells: xenograft model (cSCCIS cells)	Factor B, C3	Decreased tumorigenesis in mice injected with cancer cells that had the genes encoding C3 or Factor B knocked down using siRNA.	90
Leptomeningeal metastasis (various breast cancer cell lines)	C3aR	C3 is upregulated in the cerebrospinal fluid in metastatic settings. C3 expression is correlated with poor disease outcome. C3aR is activated in the choroid plexus epithelium, resulting in the disruption of the blood-cerebrospinal fluid barrier. Ablation of C3aR signalling is beneficial in attenuating leptomeningeal metastasis.	91
Lung: syngeneic model (393P or LLC cells) in Kras ^{LSL-G12D/+} mice	C5aR1	Combined inhibition of C5a- and PD1-induced signalling pathways results in decreased tumorigenesis and metastasis via decreased numbers of MDSCs and increased numbers of effector T cells in the tumour.	72

Abbreviations: APC, adenomatous polyposis coli; CCL, CC-chemokine ligand; CVF, cobra venom factor; CXCL, CXC-chemokine ligand; HFD, high-fat diet; MDSCs, myeloid-derived suppressor cells; NET, neutrophil extracellular traps; NK, natural killer; PD1, programmed cell death protein 1; PDL1, programmed cell death 1 ligand 1; PTX, pentraxin; RNS, reactive nitrogen species; ROS, reactive oxygen species; siRNA, small interfering RNA; TGFβ, transforming growth factor-β; TgMISIIR-Tag, transgenic mice that express the transforming region of Simian virus 40 under the control of the Mullerian inhibiting substance type II receptor and are prone to developing ovarian tumours; TILs, tumour-infiltrating lymphocytes.^{104,163}

of the injected mice⁷⁶. Consistent with these findings, *in vitro* polarization of CD4⁺ T cells in the presence of increasing amounts of C5a revealed a concentration-dependent polarization of T cells towards either the T_H1 cell or regulatory T (T_{reg}) cell lineage; high levels of C5a (500 ng/ml) favoured the polarization of CD4⁺ T cells to a T_{reg} cell phenotype instead of a T_H1 cell phenotype⁷⁶. These observations nicely illustrate the dual role of complement in modulating immune responses. Whereas physiological concentrations of activated complement molecules can promote anti-tumour immune responses, imbalanced chronic complement activation, usually preceding pathologic conditions, is associated with tumour progression.

Supporting a role for complement in tumour promotion, the activation fragment C3a has also been implicated in tumorigenesis. Improved disease outcome in *C3aR*^{-/-} mice has been observed in a model of melanoma; tumorigenesis was associated with increased numbers of neutrophils and a pro-inflammatory tumour microenvironment⁷⁷. The deleterious role of neutrophils in cancer has been further confirmed in a model of spontaneous intestinal tumorigenesis in mice with a mutation in the adenomatous polyposis coli (*Apc*) gene (that is, *Apc*^{Min/+} mice). In this model, tumour growth was correlated with the accumulation of neutrophils having a pro-tumorigenic phenotype and the formation of neutrophil extracellular traps (NETs). Mechanistically, circulating lipopolysaccharide, likely leaking from the intestine, appears to induce complement activation and upregulate the expression levels of C3aR on neutrophils. C3aR-mediated signalling then triggers NET release, coagulation pathways and polarization of neutrophils towards a pro-tumorigenic phenotype⁷⁸.

Complement in intestinal cancers. The role of complement in tumorigenesis is particularly interesting in the settings of intestinal and colorectal cancer because of its strong association with inflammatory bowel disease. Indeed, complement activation in a colitis-associated cancer model induced by combined administration of azoxymethane and dextran sulfate sodium has been shown to result in the induction of the pro-inflammatory cytokine IL-1 β , mainly by infiltrating neutrophils and consequent production of IL-17, a major inducer of intestinal disease⁷⁹. Supporting the correlation between imbalanced complement activation, inflammation and intestinal tumorigenesis, an elegant study using a model of diet-induced intestinal tumorigenesis in genetically susceptible *Apc*^{Min/+} mice has demonstrated that the source of dietary fat, and not obesity itself, triggers complement activation, inflammation, expression of oncogenes and intestinal polyposis; obesity and its associated metabolic changes had previously been associated with imbalanced complement activation, inflammation and cancer^{80,81}. However, the *Apc*^{Min/+} study dissociated the direct link between obesity and cancer by showing that whereas obese mice showed increased tumorigenesis when fed a high-fat diet containing fat derived from corn and coconut oil, intestinal polyposis was not observed in obese mice who were fed a high-fat diet consisting of fat from

olive oil⁸². In this model, tumour development was associated with increased amounts of C5a, both systemically and in the intestine. As no complement activation was observed in the mice fed the olive oil-based diet, these findings pointed to a novel concept of diet-induced complement activation and raised questions as to whether and how different sources of fat or other 'trigger' foods, such as sugar, dairy and gluten, trigger an imbalanced complement activation in the gastrointestinal tract that could be connected with pathological conditions.

Effects of complement on tumour cell proliferation and metastasis. In addition to fuelling inflammation, C3aR and C5aR1 expressed in tumour cell lines appear to signal in an autocrine manner, triggering the phosphoinositide 3-kinase (PI3K)–AKT pathway involved in tumour cell proliferation⁵⁷. Similarly, sublytic levels of C5b–C9 deposited on cancer cells induce cell cycle progression by activating signal transduction pathways in cancer cells^{83,84}. Furthermore, complement components C5a and C1q have been implicated in promoting angiogenesis in models of ovarian cancer and melanoma^{85,86}. Interestingly, triggering of cell proliferation pathways by C3a and C5a is not necessarily deleterious and has been implicated in physiological processes such as liver and retina regeneration^{87,88}.

Metastatic pathways are also triggered by imbalanced complement activation and inflammation. Various models of tumorigenesis, including colon cancer, pancreatic cancer, squamous cell carcinoma, lung cancer and breast cancer models, support this contention by showing increased production of complement proteins in metastatic settings, followed by modulation of immune responses and establishment of an environment that suppresses effector T cells^{23,58,89,90}. C3aR has been further implicated in the dissemination of cancer to the nervous system by disrupting the blood–cerebrospinal fluid (CSF) barrier and permitting the access of plasma components and other mitogens to the CSF⁹¹.

Alongside the promotion of proliferation, angiogenesis and metastasis, hyperactivation of complement is associated with cancer cell motility and invasiveness. Aberrant expression of C5aR1 has been detected in squamous cell carcinoma, adenocarcinoma and transitional cell carcinoma from various organs, and vascular invasion appears to be correlated with the expression of C5aR1 (REFS 67,92,93). Cell invasiveness has been shown to occur in response to C5a-induced secretion of metalloproteinases from C5aR1-expressing cancer cells and degradation of ECM⁶⁸. Interestingly, the basic helix–loop–helix transcription factor twist-related protein 1 (TWIST1), which is involved in epithelial–mesenchymal transition (EMT), binds to the promoter region of the *C3* gene. *C3* expression is then correlated with increased cell invasiveness through downregulation of E-cadherin on the surface of cancer cells and promotion of EMT⁹⁴. In addition, leukaemia cell lines as well as clonogenic blasts derived from patients with chronic myeloid leukaemia or acute myeloid leukaemia show increased cell adhesion in response to C3a and C5a fragments⁹⁵. In a model of gastric cancer, C5a promoted the invasiveness of human

gastric cancer cell lines (the adenosquamous carcinoma cell line MKN1 and the tubular adenocarcinoma cell line MKN7) via conversion of RhoA-guanosine diphosphate (RhoA-GDP) to RhoA-guanosine triphosphate (RhoA-GTP), resulting in morphological cell changes and increased expression of stress fibres and filopodia⁹².

Collectively, the research described above points towards local production and increased activation of complement in the tumour microenvironment that is associated with modulation of antitumour immune responses and promotion of cancer development.

Challenges of translational research

The findings discussed above might seem controversial and unlikely to be incorporated into clinical practice, especially considering that rodent models of tumorigenesis do not fully recapitulate the diversity and course of human disease. Moreover, current clinical practices that rely on personalized protocols using immunotherapy, checkpoint inhibitors and vaccines are achieving favourable outcomes without considering complement responses. These concerns notwithstanding, it is remarkable how consistent the involvement of complement is in such a large variety of cancer models.

The apparent discrepancy between the 'benign' complement activation that potentiates immunotherapy and the 'harmful' complement activity that sustains inflammation and tumorigenesis is likely the consequence of a fine balance between physiological levels of and imbalanced complement activation. As mentioned above, differential concentrations of C5a in the tumour microenvironment seem to regulate the quality of immune responses and determine the fate of a tumour⁷⁶. Furthermore, increased tumorigenesis, accompanied by early onset of lung metastasis and increased frequency of T_{reg} cells and Her2 expression in the tumour microenvironment, has been observed in C3^{-/-} mice transgenic for the *Her2/neu* oncogene *neuT* when compared with their C3-sufficient counterparts. Of particular interest is the observation that the transplantation of *neuT-C3^{-/-}* malignant cells into syngeneic immunocompetent hosts results in milder pathology than does transplantation of tumour cells from *neuT-C3^{+/+}* tumours, suggesting that complement is a key factor in immunosurveillance mechanisms in the early stages of tumorigenesis and is also involved in the immunoeediting of Her2-driven autochthonous mammary tumours⁹⁶. Given that substantial evidence associates complement with both CDC and inflammation-mediated tumorigenesis, manipulation of the complement system as a means of supporting current anticancer therapies should be further explored.

Harnessing complement to enhance mAb-based immunotherapy. Immunotherapy based on mAbs, though a mainstream therapeutic option for patients with cancer, would further benefit from improvement in mAb-mediated effector functions and prevention of drug resistance. To this end, antibodies with increased propensity to form hexamers (HexaBodies) upon antigen (CD20 or CD38) binding have been engineered⁹⁷.

These HexaBodies have been shown to induce strong complement activation and CDC of primary chronic lymphocytic leukaemia cells, even in the presence of limiting amounts of C9 (REF. 98). Additionally, dose titration of the therapeutic mAb appears to be critical for optimal CDC. Evaluation of samples from patients under treatment with the anti-CD20 mAb ofatumumab indicates that doses currently used in the clinic may be excessive, leading to a rapid depletion of systemic complement and consequent exhaustion of complement-dependent effector functions. On the other hand, a lower mAb concentration in a more frequent dosing scheme has instead been accompanied by continuous deposition of C3b on malignant B cells and robust CDC^{99,100}.

Importantly, the major mechanism responsible for the anti-CD20 response in patients, that is, the individual contributions of CDC, ADCC and phagocytosis, remains in debate¹⁰¹. In some therapeutic anti-CD20 mAbs (for example, obinutuzumab) ADCC appears to be dominant over CDC when compared with rituximab or other antibodies. Preclinical and *in vitro* data indicate that complement activation, and in particular C3b deposition, inhibits rituximab-induced NK cell activation and ADCC¹⁰²⁻¹⁰⁴. Further, anti-CD20 mAbs have been implicated in the induction of ROS by monocytes with consequent inhibition of NK cell-mediated cytotoxicity¹⁰⁵. In light of these findings, a better understanding of the precise mechanisms triggered by therapeutic mAbs in the context of different tumours is imperative to determining the optimal strategy for clinical manipulation of complement in cancer patients.

Targeting complement regulators. In line with the idea of optimizing mAb effector functions, extensive research indicates that increased resistance to CDC results in part from the expression of complement regulators (TABLE 1) by tumour cells. Indeed, increased expression of complement regulators is often observed in cancer settings¹⁰⁶⁻¹¹¹, possibly representing a selective adaptation by tumours to escape from host immune responses. Notably, local inflammation has also been associated with an upregulation of the regulators CD55 and CD59 in hepatomas, again supporting a role for inflammation in tumour promotion¹⁰⁶. It has been reported that although there is no direct correlation between lysis of tumour cells and expression of complement regulators, the ratio between the levels of CD20 and complement regulators on the membrane of mantle-cell lymphoma cells is indicative of their sensitivity to CDC^{112,113}. Therefore, dual targeting of tumour antigen and complement regulators has been well investigated in *in vitro* models¹¹⁴⁻¹¹⁹.

Although a rational approach in theory, inhibition of complement regulators can be challenging because of the broad expression of regulators by normal host cells and the consequent pathology associated with the lack of proper regulation of complement on cell surfaces (TABLE 1). Nevertheless, a novel strain of NOD-*scid* *IL2ry^{null}* (NSG)-*Hc1* mice with an intact complement system has been generated for use as a platform for testing mechanisms underlying CDC *in vivo*¹²⁰. The parental

strain, NSG, allows for engraftment of primary tumours from patients and investigation of 'personalized' therapy options but is deficient in the C5 component, making it impractical for *in vivo* evaluation of CDC. Collectively, these studies have taught us that the clinical efficacy of CDC in immunotherapeutic protocols largely depends on multiple factors, including tumour antigen expression, the overall tumour burden, the distinct expression profile of complement regulators and the surface density and tertiary conformation of tumour-deposited mAbs¹². Issues related to the potential exhaustion of complement stores during the administration of high doses of therapeutic mAbs and the precise contribution of each individual effector mechanism to tumour cell killing warrant further consideration when mAb-based treatment regimens are being designed.

How is complement activated in tumours? With the exception of mAb-induced complement activation, initiators of the complement cascade in the tumour microenvironment are still elusive. Whereas complement activation in response to natural IgM antibodies against tumour antigens as well as direct cleavage of C3 and C5 by tumour-derived proteases have been detected in cancer settings¹²¹, novel insights into the mechanisms driving complement activation by specific cancer types are essential for rationalizing therapeutic approaches aimed at manipulating the complement system in cancer. A variety of complement inhibitors that target distinct stages and pathways of the complement cascade are under development. These compounds are in different phases of clinical evaluation and can be potentially employed in case imbalanced complement activation in the tumour environment results from classical (for example, C1 inhibitor or anti-C1s antibody),

alternative (for example, C3 inhibitors, mini-Factor H or Factor B or Factor D inhibitors) or lectin (for example, anti-MASP antibodies) pathway activation or by targeting specific effector functions (for example, C5aR1 antagonists)^{122,123}.

Complement proteins as biomarkers for cancer.

Whatever the initiation route, evidence of excessive complement activation has been detected in a variety of clinical samples. In fact, several studies have suggested the use of complement proteins or activation fragment as biomarkers for disease diagnosis and prognosis (TABLE 3). By the use of proteomic techniques, increased levels of C3 fragments have been identified in the serum of patients with colorectal cancer, breast cancer, pancreatic cancer, lymphoma, prostate cancer, leukaemia, lung cancer and oesophageal cancer (TABLE 3). It is still questionable, however, whether complement biomarkers have clinical value and can discriminate between benign and malignant tumours or if they are reliable markers of disease prognosis. Increased levels of C3 fragments, for instance, are detected in a wide variety of clinical conditions, and biomarkers identified by surface enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry often represent abundant serum proteins (C3 is found at 1–2 mg/ml in the serum). Interesting findings based on samples from patients with ovarian cancer, however, have associated the expression of intra-tumoural C3 with disease prognosis¹²⁴. In addition, increased levels of a previously uncharacterized C3 fragment have been detected in prostatic fluid from cancer patients. Of note, biochemical evaluation of this C3 cleavage fragment revealed a putative prostate-specific antigen (PSA) cleavage site. Indeed, purified PSA was able to cleave C3 and C5 *in vitro*, suggesting that PSA acts as an immunoregulatory protease that contributes to a pro-tumorigenic environment through the proteolysis of complement proteins¹²⁵.

Thus, whereas complement biomarkers confirm the occurrence of increased complement activation in cancer settings, the use of such markers to guide clinical decisions is still improbable. Further insights into tumour antigens that are able to activate complement and mAb-mediated complement effector functions are highly anticipated to influence current cancer therapy practices.

Conclusion

Given the multiple factors that shape the intricate interaction between complement, tumour cells and the tumour stroma, and the dynamic nature of this relationship throughout the various stages of cancer immunosurveillance and promotion (that is, tumour seeding at the primary site, immune escape, progression and metastasis), therapeutic manipulation of the complement system in cancer settings has great potential. Aside from further improvements in mAb-induced complement cytotoxicity, special consideration should be given to exploring approaches combining the targeting of complement and immune checkpoints.

Table 3 | Complement markers as prognostic and diagnostic tools

Cancer type	Markers	Refs
Acute leukaemia	C3f-desArg	164
B cell lymphocytic lymphoma	C3a, C4a	165
Bladder	C3f	166
Breast	C3 fragments, C4a, C5aR1	166–170
Colorectal	C3a-desArg	171,172
Renal	C5a, C5aR1	173,174
Oesophageal	C3a, C4a	175
Familial adenomatous polyposis	C3 and C4 fragments	176
Gastric	DAF	177
Glioblastoma	MBL	178
Non-Hodgkin lymphoma	C1RL	179
Lung	C3, CD55	180,181
Ovarian	MBL, C4BP	182,183
Pancreatic	C3 fragments, Factor B	184–186
Prostate	C3 fragments	125,187
Thyroid	C3f	188

Abbreviations: C1RL, complement C1r subcomponent-like; C4BP, C4b-binding protein; C5aR1, C5a receptor (CD88); DAF, decay accelerator factor; MBL, mannose-binding lectin.^{164–188}

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Author contributions

E.S.R. researched the literature and wrote and edited the manuscript. D.C.M. researched the literature and wrote and edited the manuscript. D.R. edited the manuscript and contributed to discussions of the content. A.M. edited the manuscript and contributed to discussions of the content. J.D.L. wrote and edited the manuscript and contributed to discussions of the content. E.S.R. and D.C.M. contributed equally to writing the manuscript and to reviewing and editing the manuscript before submission.

Competing interests statement

The authors declare competing interests: see Web version for details.

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