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Review

Protein engineering to target complement evasion in cancer

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A B S T R A C T

The complement system is composed of soluble factors in plasma that enhance or "complement" immune-mediated killing through innate and adaptive mechanisms. Activation of complement causes recruitment of immune cells; opsonization of coated cells; and direct killing of affected cells through a membrane attack complex (MAC). Tumor cells up-regulate complement inhibitory factors – one of several strategies to evade the immune system. In many cases as the tumor progresses, dramatic increases in complement inhibitory factors are found on these cells. This review focuses on the classic complement pathway and the role of major complement inhibitory factors in cancer immune evasion as well as on how current protein engineering efforts are being employed to increase complement fixing or to reverse complement resistance leading to better therapeutic outcomes in oncology. Strategies discussed include engineering of antibodies to enhance complement fixation, antibodies that neutralize complement inhibitory proteins as well as engineered constructs that specifically target inhibition of the complement system.

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1. The complement system and its regulation

For over a hundred years, the interaction of adaptive immunity with a heat-labile serum component that "complements" cytotoxic activity has been recognized [1]. In what is now defined as the "classical pathway", antibody is bound to the surface of a cell and recruits serum components that lead to cell killing and clearance of pathogens [2]. Two other branches of complement are now recognized: the "lectin pathway", in which signaling is initiated by binding to certain polymeric molecules and carbohydrates; and the "alternate pathway", where cells that are not host specific are destroyed due to the lack of inhibitory factors. This pathway initially was thought to be constitutive, but recent research suggests it is also triggered through specific binding interactions [3,4]. The separation into these three cascades is somewhat artificial: in response to various signals complement activation is orchestrated by a network of interactions allowing elegant distinction of healthy host cells from debris, foreign intruders, and apoptotic cells. A description of these specific interactions is beyond the scope of this review and the reader is referred to other excellent articles on the complement system [5,6].

Central to the activation of the complement system is the activity of C3 which is cleaved into active forms C3a and C3b by C3 convertases. Deposition of C3b on cell surfaces and its association with either factor B or the C4bC2a complex leads to further activation of complement through C3 conversion as well as initiation of the terminal complement cascade and formation of the membrane attack complex [7–9]. In concert, the other products of C3 and C5 cleavage, the anaphylatoxins C3a and C5a, have numerous other signaling activities. They generate pro-inflammatory signals, increase vascular permeability, and stimulate phagocytosis [3,6]. Through complement receptors, inflammatory cytokines, and in conjunction with TLR pathways, the products of C3 and C5 cleavage influence B cell maturation, antigen presenting cell activation, and T cell influx providing a bridge to adaptive immunity [10–14].

To prevent uncontrolled amplification of the effects of complement there are a variety of complement regulatory proteins (CRPs). These include soluble factors like C1 inhibitor, factor H, factor I, and vitronectin as well as membrane-bound complement regulatory proteins (mCRPs) like CD35, CD46, CD55, and CD59. Tables 1A and 1B summarize major complement regulators and their functions. Because of the high levels of serum complement proteins that range into the high hundreds of milligrams per liter [15], it is unlikely that cancerous growths could influence the soluble complement protein balance. Tumors that evade complement's action therefore appear to do so by modulating the levels of the membrane bound components. In addition to direct inhibition of the complement system, these inhibitor can also influence cellular and humoral immune responses [16,17] and eliminating this inhibition can enhance cellular immunity, with key implications for cancer immune therapy [18].

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Table 1A
Soluble complement regulatory factors.

Regulator	Function
C1 inhibitor	Serine protease that targets the C1s/C1r, inhibiting activation of C4 and C2 [19]
Factor H	Modulates C3b formation by acting as a co-factor for Factor I and by accelerating the decay of the C3 convertases [20]
Factor I (C3b/C4b inactivator)	Decreases complement activation by cleaving C3b and C4b when complexed with co-factors such as CD46 [21,22]
C4 Binding Protein (C4BP)	A co-factor for Factor I, binds C4b increasing proteolytic accessibility [23]
Vitronectin (S40)	Inhibits the terminal cascade and formation of the MAC; may have other roles in regulation of disease responses [24–26]
Clusterin (Apo J)	Similar to vitronectin, inhibits the formation of the MAC and may have other functions [26]

Table 1B
Membrane-bound complement regulatory factors.

Regulator	Function
CD35 (complement receptor 1, CR1)	Decay accelerating factor for C3/C5 convertases, facilitates phagocytosis of cells with complement activated, co-factor for Factor I, fixes complement immune complexes on erythrocytes, has limited tissue distribution in humans [27]
CD46 (membrane cofactor protein, MCP)	Cofactor for factor I, regulator of T-cell differentiation and apoptosis, widely expressed in humans [16,28,29]
CD55 (decay accelerating factor, DAF)	Inhibits formation and accelerates decay of C3 convertases [30–32]
CD59 (MAC inhibitory protein, MAC-IP, 20 kDa homologous restriction factor, HRF20)	Inhibits formation of the MAC by binding C5b/8 complex and interfering with insertion of C9 [33,34]

2. Cancers and complement regulatory proteins

In the development of immune therapies for cancer the role of complement has often been neglected, but as more insight is gained into the mechanisms of action of monoclonal antibodies new approaches to improve specific antibody functions are emerging. Monoclonal antibodies have contributed substantially to progress in treating many types of cancers, but tumors evasion mechanisms lead to low complete response rates for many of these therapies [35–38]. For example, rituximab is a humanized IgG1 antibody against the surface protein CD20 which is expressed on the surface of normal B-lymphocytes and B-cell malignancies but not on hematopoietic stem cells and plasma cells. It is currently used for the treatment of B-cell non-Hodgkin lymphoma, mantle cell lymphoma, hairy cell leukemia, and chronic lymphocytic leukemia. Survival rates for B cell non-Hodgkin's lymphoma have increased signifi-

cantly since the introduction of rituximab, but only about half of the patients suffering from this disease survive ten years after diagnosis. The cancer recurs and patients often become resistant to rituximab therapy. One of the mechanisms of action of rituximab involves binding to malignant B-cells with subsequent activation of the complement system [39–43]. By inhibiting the action of complement, cancer cells could be able to evade killing by rituximab.

Overexpression of complement inhibitory proteins is a well-documented phenomenon in cancer cells and has been proposed as an escape mechanism from monoclonal antibody therapy [44–46]. This up-regulation blocks complement signaling and allows cells with bound antibody to evade killing by the complement system. The pattern of complement inhibition is diverse for many different types of tumors, stages of tumor and can exceed many orders of magnitude of overexpression versus primary, normal tissue. That being said, the levels of complement inhibitory proteins do not necessarily corre-

Table 2
Complement regulatory proteins and documented increases in expression.[a]

Tissue type	CD46	CD55	CD59	Cit.
Lung cancers	Consistent high levels found	Low levels on few tumors	Variable levels detected on majority of cancers	[50]
Breast cancer	Expressed in all breast carcinoma and normal tissue examined, increase associated with poor prognosis	No staining on cancer cells in ductal carcinomas	Trend towards increase in staining versus normal tissues; variable on some patient samples	[50–52]
Colorectal cancer	Strong increase in staining found on most samples	None found	Increase in staining, variable for some samples	[51]
Prostate cancer	Expressed but does not increase	Increase in cancer and further with malignancy	Expressed, but does not increase	[53]
Bladder cancer	Upregulation up to around 10 fold in 77% of samples tested	Upregulation up to around 10 fold in 55% of samples tested	Upregulation up to around 10 fold in 59% of samples tested	[54]
Malignant endometrial tissue	2.5 fold rise in optical density on stained image	2.2 fold rise in optical density on stained image	1.7 fold rise in optical density on stained image	[55]
Head and neck cancer	Highly expressed in all forms, low to no staining in normal surrounding tissue	Highly expressed in all forms, low to no staining in normal surrounding tissue	Highly expressed in all forms, low to no staining in normal surrounding tissue	[56]
Esophageal cancer	Dramatic increase in staining	Pronounced decrease in staining	Uniform between normal and cancer tissue	[57]
Non-Hodgkin's lymphoma[b]	High level expression with possible correlation to outcome	High level expression with possible correlation to outcome	High level expression with possible correlation to outcome	[58,59]
Renal cell cancers	Low, scattered staining	No staining detected	High levels on most tumors tested	[50]
Primary gynecologic carcinosarcoma	High level expression	High level expression	High level expression	[60]
Ovarian cancer	Robust expression on the surface of cells	Robust expression on the surface of cells	Robust expression on the surface of cells	[61]

[a] No information is usually found on CD35 so this mCRP is not presented.
[b] The conclusions in the two papers contradict as to correlation with outcome; high levels were found in both studies.