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Review Article

Targeting complement regulatory proteins in tumor immunotherapy

Jun Wu[1,2], Qirui Liu[2,3], Chong Wang[1,2], Min Tao[1,2], Chenglin Liu[1], Feiteng Lu[1,2], Muying Ying[1]

[1]Department of Molecular Biology and Biochemistry, Basic Medical College of Nanchang University, Nanchang, PR China; [2]The Second Clinic Medical College of Nanchang University, Nanchang, PR China; [3]Queen Mary College of Nanchang University, Nanchang, PR China

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Abstract: The complement system is a vital part of the immune system, and its activation can eliminate malignant tumor cells. Complement regulatory proteins (CRPs) can regulate complement activation precisely. However, studies have reported that some cancer types upregulate CRPs, and the upregulated CRPs inhibit complement activation and membrane attack complex formation, and they restrict T cell activation, proliferation and differentiation; this helps tumor cells escape immune attacks and leads to the poor prognosis of patients with tumors. Furthermore, anti-tumor monoclonal antibodies (mAbs) can induce anti-tumor effects through complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity by activating the complement system. Upregulated CRPs on tumor cells limit the therapeutic efficacy of mAbs in tumor immunotherapy. Thus, targeting CRPs to treat patients with tumors is essential in tumor immunotherapy. Here, we present a review of the most recent studies that have investigated the role of CRPs in tumor immunity, T cell response and immunotherapy, and the mechanisms underlying CRPs' overexpression in tumor cells. We also discuss the approaches to targeting CRPs to improve the therapeutic efficacy of mAbs in tumor immunotherapy and emphasize the chief challenges of each approach for better applications in clinical settings.

Keywords: Complement regulatory proteins, tumor, T-cell response, anti-tumor monoclonal antibody, tumor immunotherapy, therapy strategies

Introduction

Activation of the complement system can eliminate malignant tumor cells by causing complement-dependent cytotoxicity (CDC) and enhancing antibody-dependent cell-mediated cytotoxicity (ADCC) [1, 2]. Complement regulatory proteins (CRPs), comprising soluble CRPs (sCRPs) and membrane-bound CRPs (mCRPs), can regulate complement activation in different stages of the classical, lectin, and alternative pathways.

sCRPs primarily comprise C1 inhibitors (C1INH), complement factor I (CFI), complement factor H (CFH), C4 binding protein (C4BP) and clusterin/vitronectin. C1INH is a serine protease inhibitor which can bind serine proteases C1r and C1s, isolate C1 complexes and block the initiation of the classical pathway. In addition, C1INH can bind the mannose-binding lectin-related serine proteases -1 and 2 to regulate the lectin pathway [3]. CFI is a common regulator of the three complement activation pathways and cleaves C3b and C4b by the cofactors CFH, C4BP, CD46, CR1, etc., thereby hindering the formation and activities of C3 and C5 convertases [4]. With the multiple binding sites of C3b, CFH impedes the formation of C3 convertase by acting as a CFI cofactor in the alternative pathway [5]; moreover, CFH can contribute to the decay-accelerating activity of C3 convertase of the classical pathway. Factor H-like protein 1 (FHL-1) exhibits similar complement regulatory functions as CFH and can bind to C3b, thereby hindering C3 convertase. C4BP can regulate the classical and lectin pathways by inhibiting their C3 convertase [6]. Clusterin/vitronectin can prevent the binding of sC5b67 to the cell membrane, thereby hindering the formation of MAC [7].

mCRPs primarily comprise CD46, CD55, CD59 and CD35; CD46 inhibits the formation of C3 convertase of the alternative and classical pathways by combining C3b. CD46 also exhibits the cofactor property of CFI [8]. CD55 is a glycosylphosphatidylinositol (GPI) glycoprotein; it exists in anchored form and prevents C4b2a and C3bBb formation, thereby inhibiting complement C3 activation [9]. CD59, a small GPI-anchored glycoprotein with a globular structure, can inhibit the formation of MAC by binding C8 and C9, thereby blocking complement-mediated cell lysis [10]. CD35 can recognize C3b and C4b, leading to a decay-accelerating activity toward the C3 and C5 convertases [11]. Furthermore, CUB and Sushi multiple domain 1 (CSMD1), a transmembrane protein, can inhibit complement activation by promoting CFI-mediated C4b/C3b degradation and inhibit the MAC assembly at the C7 level [12].

Unpredictably, the CRPs on tumor cells are regulated to protect themselves from complement attack, affecting the prognosis of patients with tumors and resisting mAb-mediated killing in immunotherapy. In addition, mCRPs suppress T cell anti-tumor responses. Hence, targeting CRPs is crucial for treating patients with tumors. This review focuses on the role of CRPs in tumor immunity, T cell response and immunotherapy and discusses the approaches to improve the efficacy of mAbs by targeting CRPs. This review also emphasizes the chief challenges of each approach for better applications in clinical settings in the future.

Role of sCRPs in tumor immunity

Inhibiting sCRPs could inhibit tumor growth. Both CFH and FHL-1 are upregulated in some tumor cells, including lung cancer cells [13], cutaneous squamous cell carcinoma (cSCC) cells [14] and liver cancer cells [15]. In lung cancer, upregulated CFH and FHL-1 can bind to the surface of non-small cell lung cancer (NSCLC) cells to avert C3b deposition in their cell membranes, thereby hindering complement activation [13]; this suggests that upregulated CHF in tumor cells renders the cells resistant to complement-mediated lysis. The *in vivo* growth of CFH-deficient cells in athymic mice was reportedly considerably declined and recovered when mice were depleted of the complement using cobra venom factor [16]. In addition, CFH overexpression in lung adenocarcinoma is associated with a poor prognosis [17]. In cSCC, the knockdown of CFH and FHL-1 expression repressed the proliferation and migration of cSCC cells [14]. In liver cancer, tumor-initiating cells upregulated CFH and C7, and their overexpression could upregulate the expression of stemness factors by inducing the expression of late SV40 factor. Besides, the knockdown of C7 and CFH expression abrogates tumorsphere formation and induces differentiation [15], demonstrating that CFH and C7 play vital roles in maintaining stemness in liver tumor-initiating cells. Riihilä et al. reported that the IFN-γ could upregulate the expression of CFH by cSCC cells, and basal CFH and FHL-1 expression was dependent on extracellular signal-regulated kinase (ERK) 1/2 and p38 signaling [14].

Other sCRPs are also crucial in helping tumor cells escape immune attack. In liver cancer, the knockdown of C4BPα noticeably augments the deposition of C5b-9 *in vitro* and *in vivo* [18]. Data obtained from a clinic scrutinizing patients with breast cancer revealed that CFI correlated with a considerably short cancer-specific survival [19]. In NSCLC cells, the tumor cells produce CFI and C4BP, thereby decreasing C3 deposition and CDC *in vitro* [20]. Furthermore, the knockdown of CFI expression potently inhibits the growth of human cSCC xenograft tumors *in vivo* [21].

The role of mCRPs in tumor immunity and their mechanisms of overexpression

Compared with sCRPs, the role of mCRPs in tumors has been investigated comprehensively. Almost all cancer types highly express at least one mCRP, and some cancer types express two or three, compared with normal tissues. mCRP overexpression in some tumors types indicates a poor prognosis in patients with breast cancer [22], colorectal cancer [23], ovarian cancer [24], and other cancers. A recent study reported that CD55 and CD59 are clinically relevant for the differentiation and TNM staging of colon cancer [25]. In lung cancer cells, CD59 is overexpressed and inhibits the formation of MAC. Further, the weight of nude mice tumor grafts markedly decreases, and the survival rate markedly increases, upon silencing CD59 [26]. In breast cancer, the knockdown of CD59 significantly inhibits MDA-MB-231-HM cell growth both *in vitro* and *in vivo* [22]. Furthermore, in NSCLC cancer cells, the declined expression of CD59 results in the

increased expression of caspase-3 and Fas and decreases the expression of Bcl-2 [26], indicating that CD59 could regulate the apoptosis of cancer cells. The overexpression of CD55 in colon cancer cells restricts their sensitivity to CDC triggered by the heterologous expression of α-gal xenoantigen [27]. In prostate cancer, the expression of CD55 is associated with poor patient survival and is found to be increased in epithelial cells. Downregulation of CD55 via small interfering RNA (siRNA) in prostate tumor epithelial cells significantly reduces the overall tumor burden *in vivo* in severe combined immunodeficiency (SCID) mice [28]. In addition, a retrospective study reported that the overexpression of CD55 or CD59 results in a higher relapse rate in patients with breast cancer [29]. In cervical carcinoma, a low expression of CD46 is significantly associated with the deposition of C3 [30].

Several studies have previously reported the mechanisms underlying mCRP overexpression on tumor cells. Tumor cells or tumor stromal cells can secrete vascular endothelial growth factor (VEGF), which induces the upregulation of mCRPs [31]. Cytokines, such as IL-6, IL-1β and TNF-α, can upregulate the expression of CD59 and CD55 on hepatocellular carcinoma cells [32]. Both epidermal growth factor and prostaglandin E2 can upregulate the expression of CD55 in colorectal cancer [33]. Du et al. reported that Sp1, the extensively expressed transcription factor, may regulate the constitutive expression of CD59, whereas CREB-binding protein (CBP)/p300 bridge NF-κB and CREB, which surprisingly function as enhancer-binding proteins that induce CD59 upregulation during lipopolysaccharide (LPS)-triggered complement activation [34]. Likewise, Cui et al. reported hepatitis B X-interacting protein could upregulate CD59, CD55, and CD46 through p-ERK1/2/NF-κB signaling to protect breast cancer cells from CDC [35]. In addition, signal transducers and activators of transcription 3 (STAT3) are activated in many tumor cell types [36], and their activation could induce the expression of CD46 and protect tumor cells from CDC [37]. Interestingly, two studies reported that microRNA (miRNA) participates in regulating the expression of mCRPs in tumors. In breast cancer cells, miR-520b and miR-520e could sensitize these cells to CDC by directly targeting 3'-UTR of CD46. Furthermore, the overexpression of miR-520b and miR-520e led to the increased deposition of C3b [38]. In K562 cells, the overexpression of miR-217 and -200 (b and c) could enhance the expression of CD55 and CD46 but not of CD59 [39]. Additionally, sublytic MAC, a complement activation product, could surprisingly promote tumor cell activation [40]; however, the underlying mechanism warrants further investigation. Besides, sublytic MAC and C5a can increase the expression of CD59 by activating the NF-κB and CREB signaling pathways [34]. Nonetheless, the underlying mechanisms that regulate the expression of CRPs require further comprehensive investigation to elucidate how tumor cells upregulate CRPs.

The role of CRPs in T cell anti-tumor immune responses

Several recent studies have reported that CRPs participate in T cell anti-tumor immune responses and that these CRP functions may be complement-dependent or beyond complement regulation. Mostly, these CRPs comprise mCRPs.

CD59 in T cell anti-tumor immune responses: CD59 is reportedly upregulated on human activated $CD4^+$ T cells. A CD59 blockade with CD59-specific antibodies can enhance the response of human $CD4^+$ T cells isolated from patients with colorectal cancer [41]. Likewise, Xie et al. reported that the activation and proliferation of $CD4^+$ and $CD8^+$ T cells were enhanced by the gene silencing of CD59 by siRNA. In addition, the mechanism by which CD59 suppresses the antigen-specific activation of human T cells involves binding its ligand on antigen-presenting cells (APCs) [42]. However, the proliferation of $CD4^+$ T cells was reportedly enhanced in CD59a knockout mice, whereas $CD8^+$ T cell responses remained unaffected [43]; a possible reason for this finding could be a difference in the function of CD59 between mice and humans. For example, it showed impaired T cell responses in patients with paroxysmal nocturnal hemoglobinuria [44]. CD59 may act via the kinase Lck to modulate T cell responses, and Lck could alter the intracellular calcium concentration and eventually alter critical gene expression for T cell activation and survival by phosphorylating the immunoreceptor tyrosine-based activation (ITAM) motifs of the T cell

receptor (TCR)/CD3 complex [45, 46]. Lipp et al. reported that CD59-mediated signaling is strongly dependent on the TCR/CD3 surface expression of Jurkat T cells and demonstrated that Lck is a key component for signal transduction from CD59 to the TCR/CD3 pathway [47]. However, the underlying mechanism of Lck-mediated signaling from CD59 to TCR/CD3 remains unclear.

CD55 in T cell anti-tumor immune responses: The role of CD55 in T cell responses depends on systemic complement activation and its function may span beyond complement regulation. In addition, CD55 controls the secretion of cytokines on APCs and T cells. During primary T cell activation, the absence of CD55 on APCs and on T cells enhances T cell proliferation and amplifies induced IFN-γ-producing cells; the effect was factor D- and, at least in part, C5-dependent, indicating that local alternative pathway activation is essential [9]. CD55-deficient APCs produced significantly more C5a and IL-12 and promoted a greater number of IFN-γ-producing T cells; this process was dependent on the C5a receptor expressed on APCs and demonstrated a correlation among CD55, local complement activation, IL-12 and T cell-produced IFN-γ [48]. Furthermore, the co-stimulation of human naïve $CD4^+$ cells through CD97/CD55 interaction d drives the activation, expansion, and function of T regulatory type 1 (Tr1) cells. Moreover, Tr1s proliferate and maintain their differentiated $IL\text{-}10^{high}$ phenotype via CD55 re-stimulation, thereby demonstrating that CD55 inhibits T cell function in an IL-10 dependent manner [49]. However, Fang et al. reported that the lack of CD55 on the APCs of naïve mice did not alter their T cell stimulating activity. In contrast, APCs derived from CD55-knockout mice treated with inflammatory stimuli were more potent T cell stimulators than those derived from similarly treated wild-type mice. The acquisition of a higher T cell stimulating activity by APCs in challenged CD55-knockout mice required C3 and C5aR and was correlated with decreased surface PD-L1 and/or increased CD40 expression [50], suggesting that CD55 inhibits T cell immunity in the context of systemic complement activation and inflammation but does not play an intrinsic role in either T cells or APCs during T cell-APC interaction. Capasso et al. reported that the direct stimulation of CD55 on $CD4^+$ T cells with CD97 can modulate the T cell activation but does not interfere with CD55-mediated complement regulation [51], suggesting that the role of CD55 spans beyond complement regulation. This evidence implies that the underlying mechanisms of CD55 in the T cell response are complex and diverse and warrant further investigation. Furthermore, a clinical study based on the roles of CD55 in T cell response in patients with osteosarcoma reported the potential of tumor vaccine targeting CD55 for cancer treatment [52].

CD46 in T cell anti-tumor immune responses: Some studies reported that CD46 could restrict T cell responses through Tr1 and T helper type 1 (Th1) cells. Activation of human CD4 T cells by anti-CD46 and anti-CD3 crosslinking leads to the induction of Tr1 [53]; these cells secret a large amount of IL-10 and inhibit CD4 T-cell proliferation. Besides, the ligation of CD46 with a physiologically relevant ligand, such as C3b or a pathogen, also induces Tr1 cell generation [54]. Furthermore, the co-stimulation of human $CD4^+$ T cells with CD46 and CD3 leads to the differentiation of a "switched" Th1 population, which shuts down IFN-γ secretion and upregulates IL-10 [55]; and blocking CD46 can inhibit IL-10 production [56]. CD46 signaling in $CD4^+$ T cells leads to a strong reduction in miRNA-150 levels. Compared with IFN-γ-secreting Th1 cells, CD46-induced "switched" IL-10-secreting Th1 T cells increase the expression of miRNA-150, suggesting that CD46 controls both Th1 activation and regulation by a miRNA-150-dependent mechanism [55].

These findings indicate that CRPs play important roles in inhibiting the T cell response in tumor immunity (**Figure 1**). However, the mechanisms underlying the CRP inhibition of T cell responses remain clear and warrant further studies. Apparently, these data support the fact that developing approaches targeting CRPs is helpful in improving the anti-tumor T cell responses in tumor immunotherapy.

Role of CRPs in immunotherapy

Anti-tumor monoclonal antibodies (mAbs), such as rituximab and ipilimumab, are being increasingly used for cancer therapy [57]; these mAbs target different antigens to kill tumor cells directly or indirectly by different mechanisms (**Figure 2**) [1, 57, 58]. These mAbs are primarily

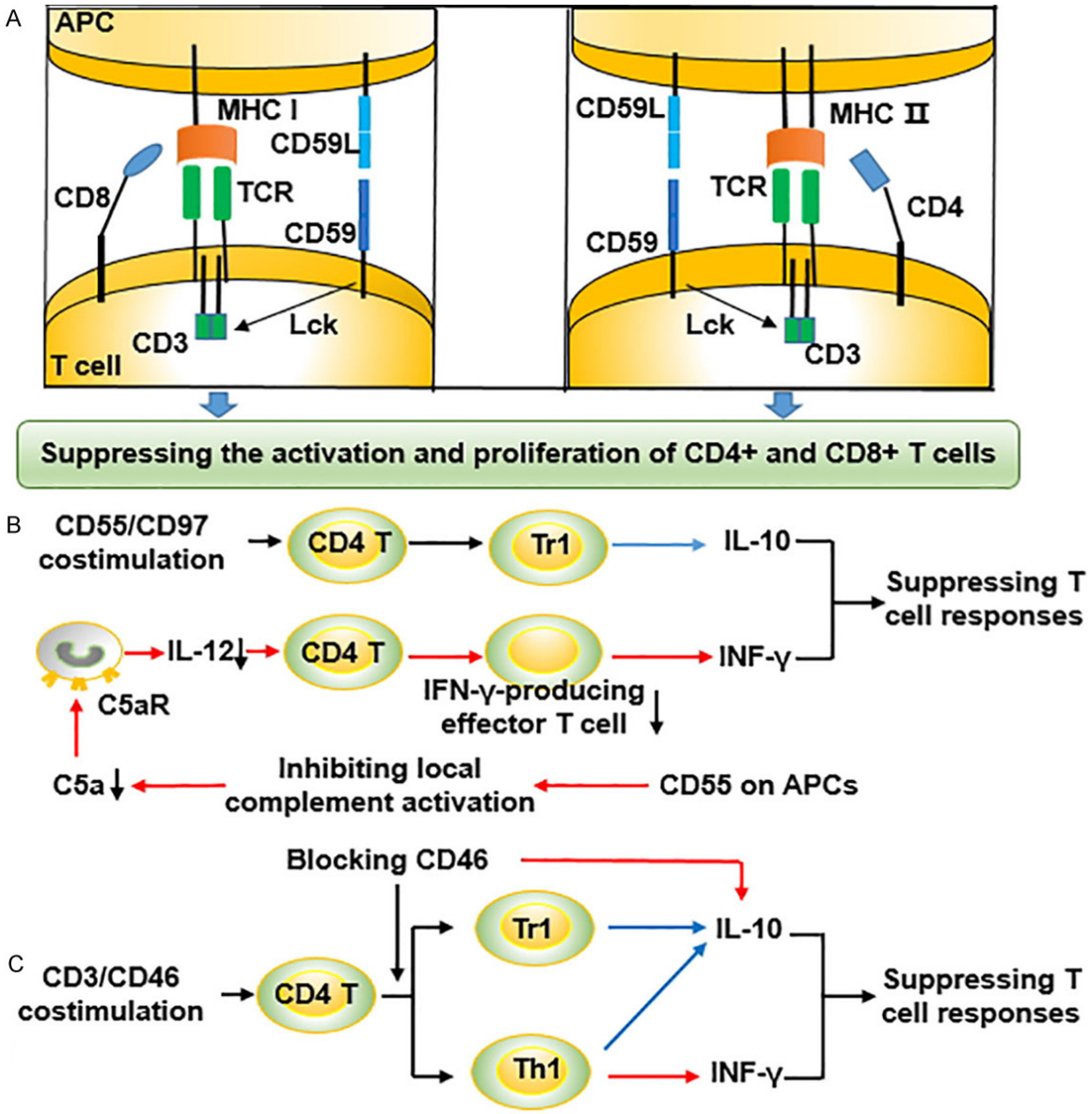

Figure 1. Mechanisms suppressing T cell responses by CRPs. A. APCs produce the MHC class II-peptide complex to present antigens to $CD4^+$ T cells and MHC class I-peptide complex to present antigens to $CD8^+$ T cells by binding TCR, producing phosphorylated ITAM of CD3 and ultimately altering gene expression for their activation. The up-regulated CD59 on T cells binds to its ligand on APCs to suppress the activation and proliferation of $CD4^+$ and $CD8^+$ T cells. The signaling from CD59 to the TCR/CD3 complex may be mediated by Lck; however, several mechanisms remain unclear to date. B. CD55 suppresses T cell responses though two pathways: (1) co-stimulation of naïve $CD4^+$ T cells with CD55/CD97 generates Tr1, leading to the release of IL-10. (2) red lines, CD55 can inhibit the production of INF-γ by inhibiting the release of IL-12. CD55 on APCs inhibits local complement activation, leading to reduced C5a. By binding to its receptor on APCs, C5a upregulates IL-12 production, leading to the differentiation of T cells into IFN-γ-producing effector T cells. CD55 may suppress T cell responses by other mechanisms; however, these mechanisms warrant further investigation. C. Co-stimulation of $CD4^+$ T cells with CD3/CD46 generates Tr1, resulting in the release of IL-10 (blue lines), which also leads to the shutdown of the release of INF-γ (red lines) and increases in the release of IL-10 on Th1 (blue lines). Besides, blocking CD46 on activated $CD4^+$ T cells suppresses the release of IL-10 (red lines).

IgG1 and IgG2, and their Fc region can activate the complement by binding C1q, leading to CDC and enhanced ADCC. However, tumor cells resist mAb-mediated killing, resulting in the poor efficacy of mAbs in tumor immunotherapy. Thus, understanding the relevant resistance mechanisms is essential to improving the therapeutic efficacy of mAbs.

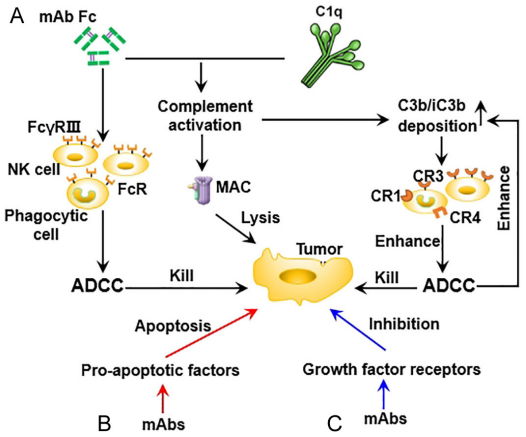

Figure 2. Effector mechanisms of anti-tumor mAbs to kill tumors in tumor immunotherapy. A. mAbs binding to the target antigen on the tumor cell surface can be recognized by Fcγ-receptors (FcRs) expressed on immune cells, such as natural killer (NK) cells, and macrophages, or some T-cell subsets, resulting in ADCC. C1q binding to the mAb Fc region can activate the classical pathway of the complement system and result in the deposition of C3b or iC3b on cell surface, which are opsonizing agents for cells having CR3. In addition, complement activation can lead to the formation of the MAC, resulting in CDC. ADCC can be further enhanced by complement receptor 3 (CR3) binding to iC3b deposited on cell surface, thereby enhancing FcR-mediated effector-cell binding and combining the above described mechanisms. B. Some mAbs can target pro-apoptotic factors such as the TNF receptor, TRAIL-R and could induce apoptosis in tumor cells via Fas or TRAIL pathways. C. Growth arrest is caused by mAbs targeting receptors for growth factors (e.g. cetuximab, rituximab).

Some studies have reported that the secretion of sCRPs and the overexpression of mCRPs by tumor cells are the primary reasons for the poor efficacy of anti-tumor mAbs in tumor immunotherapy. For instance, the inhibition of CFH binding CLL cells by the human recombinant CFH-derived short-consensus repeat 18-20 (hSCR18-20) significantly boosts rituximab-induced CDC [59]. In addition, a single nucleotide polymorphism (rs3766404) in the CFH gene is significantly related to event-free survival in rituximab-treated follicular lymphoma (FL) [60]. Mamidi et al. reported a reduced expression of mCRPs (CD59, CD55 and CD46) on tumor cells, including breast cancer cells, ovarian cancer cells, and lung cancer cells, by siRNAs, leading to increased trastuzumab- and pertuzumab-induced CDC. Besides, the antibody-induced C3 opsonization of these tumor cells was found to be markedly enhanced [61]. In B cell acute lymphoblastic leukemia cells, the simultaneous loss of expression of CD55 and CD59 significantly increased the sensitivity to the CDC induced by rituximab, ofatumumab or alemtuzumab. In addition, the retrovirally induced increment in CD55 or CD59 expression protects the cells from CDC [62]. The data obtained from clinic scrutinizing patients with breast cancer treated with adjuvant trastuzumab demonstrated that patients with an overexpression of mCRP had a considerably shorter survival than those with a low mCRP expression [29]. Overall, these findings indicate that CRPs inhibition could significantly reduce the resistance of tumor cells to the mAb-induced complement-mediated attack.

Approaches targeting CRPs to enhance tumor killing

Based on the importance of CRPs in tumor immunity, T cell responses and immunotherapy, CRPs are crucial targets and potential therapeutic strategies targeting CRPs have been developed in immunotherapy (**Figure 3**). However, these results have been obtained *in vitro* or in murine models, and there is a lack of such clinical data from humans. Hence, some problems, such as their effects and safety, warrant resolution to avoid unwanted side effects, such as hemolytic disease (wherein CD59 is highly expressed in erythrocytes).

Studies regarding approaches targeting sCRPs focus on CFH

Approaches targeting sCRPs comprise utilizing their autoantibodies, neutralizing sCRPs and blocking them from binding the tumor cell surface. In 2010, Nita et al. reported autoantibodies to CFH in the serum of patients with early NSCLC [63]. Later, the CFH autoantibody was proven to belong to the IgG3 subclass. Purified CFH autoantibodies isolated from patients with lung cancer could restrict the binding of CFH to A549 cells, increase C3b deposition, and result in complement-dependent tumor cell lysis [64], suggesting that using recombinant CFH autoantibodies which directly target CFH to treat patients with cancer may be a feasible approach in the future. However, a limitation of IgG3 antibodies is their short serum half-life [65], which needs further studies. Besides, rituximab-induced CDC in B-cell chronic lymphocytic leukemia (B-CLL) cells derived from patients significantly enhanced by neutralizing CFH with anti-CFH mAbs [66]. hSCR18-20 can block the binding of CFH to the tumor cell surface by representing the main binding domain of CFH. In

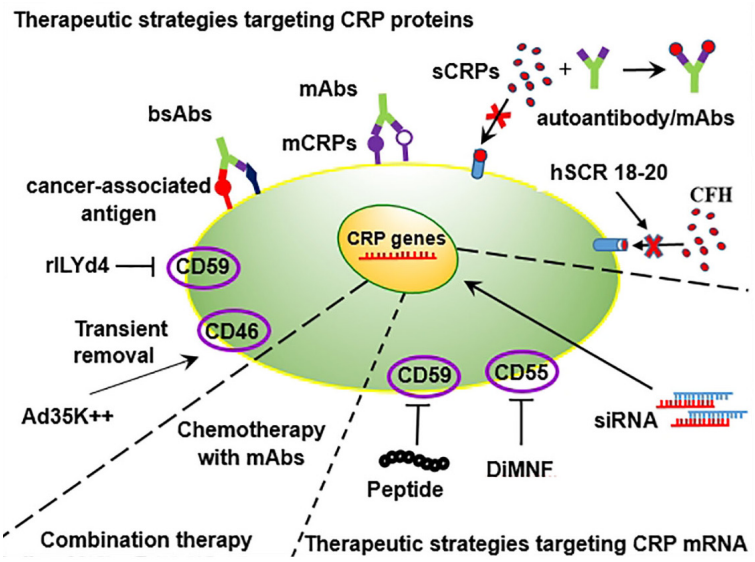

Figure 3. Approaches targeting tumor complement regulatory proteins (CRPs). As several tumors upregulate the expression of mCRPs and sCRPs to circumvent complement-mediated killing, certain strategies have been developed to block/inhibit CRPs. At the protein level, CRP function can be blocked by directly targeting those using antibodies of different formats (e.g. autoantibody, bsAbs and anti-CRP mAbs). Besides, amino acid peptides (e.g. rILYd4) and small recombinant protein (e.g. Ad35K^{++}) can block CRPs in particular. hSCR 18-20 can inhibit the binding of CFH to the tumor cell surface. At the gene level, the expression of CRP can be regulated by interfering with the expression level (e.g. siRNA, peptide, aryl hydrocarbon receptor modulator DiMNF). Otherwise, chemotherapeutic drugs can downregulate mCRPs, thereby increasing the therapeutic efficacy.

CLL cells, rituximab- or ofatumumab-induced CDC was significantly enhanced by utilizing hSCR18-20 *in vitro* [59, 67]; however, their effects *in vivo* warrant further investigation.

Approaches targeting mCRPs

SiRNA-mediated RNA interference (RNAi) is the most efficient approach for silencing specific genes encoding CRPs. For example, in uterine serous carcinoma cell lines, the downregulation of CD55 and CD59 by siRNA significantly increases trastuzumab-induced CDC and ADCC *in vitro* [68]. The critical problem with this approach is the delivery of CRP-directed siRNA to specifically target tumor cells *in vivo* and the ideal delivery of CRP-specific siRNA to tumor cells warrants the conjugation of targeting molecules, such as receptor ligands or antibodies to lipid carriers. Cinci et al. reported that anti-mCRP siRNAs were encapsulated in transferrin-coupled lipoplexes for precise delivery to transferrin receptor CD71^{high} expressing SW-480, DU145, and BT474, resulting in the efficient silencing of all three mCRPs (up to 90%) and a significant increase in CDC on CD71^{high} tumor cells [69]. AtuPLEX, a cationic lipid-based siRNA delivery system, can deliver anti-mCRP siRNAS to HER2 overexpressing SKOV3, SK-BR-3, Calu-3 and BT474 cancer cells, thereby resulting in an 85%-90% reduction in the mCRPs expression and the augmentation of CDC [61].

Neutralizing mCRPs should avoid damaging healthy tisues and cells because of their widespread expression in healthy cells. Neutralizing mCRPs using an tibodies to them could sinificantly increase mAb induced CDC and ADCC to tumor cells such as CLL cells and lung carcinoma cells [2, 70], thereby augmenting the therapeutic effects of anti-tumor mAbs. rILYd4, a CD59 inhibitor, can sensitize rituximab-resistant lymphoma cells to ofatumumab- and rituximab-induced CDC both *in vitro* and *in vivo* [71, 72]. In addition, rILYd4 did not adversely mediate the hemolysis of CD59-expressing erythrocytes *in vivo* [71]. Ad35K^{++}, a small recombinant protein, can enhance the efficacy of trastuzumab, alemtuzumab and rituximab by transiently removing CD46; further, it is safe in CD46 transgenic mice and macaques [73]. Overall, these *in vivo* and *in vitro* results suggest that the neutralization of CRPs through appropriate CRP inhibitors is feasible.

Perhaps, simultaneously targeting tumor-associated antigen and mCRPs on tumor cells to inhibit mCRPs could avoid damage to healthy tissues and cells. Hence, bispecific antibodies (bsAbs) are designed to improve efficacy of mAbs in immunotherapy. Junnikkala et al. combined biotinylated anti-CD59 antibody with anti-GD-3 gangliosides on the surface of human melanoma cells, causing a substantially increased killing effect of the complement to the tumor cells [74]. In cervical carcinoma cells, BsAbs (anti-Ep-CAM*anti-CD55) were designed to target CD55 and Ep-CAM, resulting in a two-fold increase in C3 deposition *in vitro* [30]. In

CLL cells, two bsAbs targeting CD20 and CD55 or CD59 could kill 4-25-times more cells than the anti-CD20 recombinant antibody by the CDC *in vitro*; further, the two bsAbs completely prevent the development of human/SCID lymphoma in mice [75].

Interestingly, chemotherapeutic drugs can improve the efficacy of anti-tumor mAbs in immunotherapy as adjuvant therapy. In lung carcinoma cell lines, cisplatin can downregulate the expression of CD55 and CD59, thereby enhancing trastuzumab-induced CDC [70]. In FL cell lines, fludarabine can downregulate the expression of CD55, thereby increasing rituximab-induced CDC [76]. In CLL cell lines, sorafenib can downregulate three mCRPs by inhibiting the STAT3 phosphorylation, thereby increasing ofatumumab-induced CDC [37, 77]. In diffuse large B-cell lymphoma cell lines, a recent study reported that gemcitabine (GEM) could enhance the anti-tumor efficacy of rituximab by upregulating the expression of CD20; the mechanism underlying this upregulation correlated with GEM-induced NF-κB activation [78]. These studies suggest that the appropriate combination could result in maximum therapeutic outcomes in different tumors.

Other approaches inhibiting the expression of CRPs are anticipated at the transcriptional levels. Repressor element-silencing transcription factor (REST) is reportedly expressed as a truncated protein and is involved in the overexpression of CD59 in neuroblastoma and colorectal cancer [79, 80]; this truncated isoform of REST can be targeted with peptides to sensitize tumor cells to CDC killing in neuroblastoma. In addition, selective aryl hydrocarbon receptor (AHR) modulators can regulate AHRs, thereby inhibiting the expression of pro-inflam matory genes. 3', 4'-dimethoxy-α-naphthoflavone (DiMNF) is an AHR modulator that inhibits the expression of CD55. The fluorescein reporter assay shows that DiMNF acts on the promoter of CD55 [81], suggesting that DiMNF can target CD55 to inhibit its expression. However, studies regarding the expression of CRPs at the transcriptional levels are limited at present.

Conclusions

Although CRPs can protect healthy human tissues and cells from complement attack, the upregulation of CRPs on tumor cells could render these cells safe from complement attack. While these can promote tumor growth that leads to a poor prognosis in patients, they could also inhibit mAb-induced complement-mediated tumor killing, resulting in the poor efficacy of anti-tumor mAbs in immunotherapy. Furthermore, CRPs suppress the T cell response in tumor immunity. These functions of CRPs may be complement-dependent or beyond complement regulation. To date, several mechanisms remain unclear and warrant further studies.

Based on the roles of CRPs in immunity and immunotherapy, targeting CRPs in immunotherapy is essential to treat tumors. Inhibiting CRPs not only improves complement attack on tumor cells and the therapeutic efficacy of mAbs but also enhances the killing of T cells to tumor cells. Although some approaches have been developed to target CRPs, we lack patient data to assess the effects and safety of those approaches; thus, we expect to obtain data from patients for future investigations. Further, all approaches should avoid complement-mediated immune disorders due to the widespread expression of CRPs in normal cells. In the future, the role of CRPs in immunity and immunotherapy should be further investigated to develop novel strategies to enhance tumor killing by targeting CRPs and to help elucidate the mechanisms of escape of tumors and their resistance to anti-tumor mAbs.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Muying Ying, Department of Molecular Biology and Biochemistry, Basic Medical College of Nanchang University, Bayi Road 603, Nanchang 330006, PR China. Tel: 86-0791-85788326; Fax: 86-0791-85788326; E-mail: yingmuying@ncu.edu.cn

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