Review

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2018, Vol. 46(11) 4418–4426

DOI: 10.1177/0300060518799612
journals.sagepub.com/home/imr
SAGE

Targeting the CD47-SIRPα signaling axis: current studies on B-cell lymphoma immunotherapy

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Abstract

The function of the immune system in cancer initiation and progression has been widely examined. Notably, immunotherapy has become a promising approach for cancer treatment. CD47, a member of the immunoglobulin superfamily, plays an important role in the immune regulation of cancer by binding to SIRPα Multiple studies have detected high CD47 expression on the surface of tumor cells, which indicates poor prognosis. Treatments that block the interaction of CD47 and SIRPα significantly suppress tumor growth and metastasis through diverse mechanisms, such as phagocytosis, antibody-dependent cellular cytotoxicity, and apoptosis. Recently, several studies have reported increased CD47 expression on different types of lymphoma cells, indicating that the CD47-SIRPα pathway can be used as a therapeutic target in lymphoma. This review focuses on the role of CD47-SIRPα in B-cell lymphoma and discusses promising therapeutic strategies targeting the CD47-SIRPα axis, which yield insights into the immunotherapy of B-cell lymphoma.

Keywords

CD47, SIRPα, immunotherapy, combination strategy, immunoglobulin, B-cell lymphoma

Date received: 7 May 2018; accepted: 15 August 2018

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Introduction

Non-Hodgkin lymphoma (NHL) is a common lymphoid malignancy. According to the American Cancer Society, the morbidity of NHL currently ranks seventh among all cancers.[1] Although chemoimmunotherapy improves the survival of CD20-positive B-cell lymphoma, many patients experience disease relapse, and some show drug resistance to both conventional chemotherapy and rituximab, suggesting that additional approaches are needed to more effectively treat lymphoma patients.[2] Immunotherapy is a novel approach for treatment of B-cell lymphoma that is relapsed or refractory after chemotherapy. Chimeric antigen receptor T cell (CAR-T) therapy uses genetic engineering to alter T cells to produce transmembrane proteins on the cell surface with an extracellular antibody fragment domain that recognizes a tumor antigen; such therapy shows high response rates in refractory B-cell lymphoma.[3] As a fusion protein containing both an antigen recognition domain and T cell signaling domain, the CAR specifically activates T cell-mediated antitumor immune responses.[4] In contrast, immune checkpoint inhibitors, such as programmed cell death-1 or programmed cell death ligand-1 (PD-1/PD-L1) monoclonal antibody (mAb), can activate T cells to attack cancer cells; thus, they offer new options for patients with lymphoma.[5] PD-1 is an immune checkpoint receptor expressed on the surface of T cells; by binding to PD-L1, predominantly expressed on tumor cells, PD-1 attenuates the T cell-mediated anti-tumor immune response.[6]

The innate immune system also plays an important role in anti-tumor responses. Macrophages are important components of innate immunity, which can inhibit tumor growth through phagocytosis. CD47 is a 50-kDa ubiquitous cell membrane protein in the immunoglobulin superfamily. By interacting with integrin $\alpha v\beta 3$ and thrombospondin, CD47 participates in regulation of cell motility, adhesion, migration, and platelet activation.[7–10] Signal regulatory protein-α (SIRP-α) is another immunoglobulin superfamily transmembrane receptor primarily expressed on the surface of myeloid cells, including macrophages, granulocytes, monocytes, and dendritic cells.[11] The interaction of SIRP-α with CD47 phosphorylates its immunoreceptor tyrosine-based inhibition motif, then activates the inhibitory tyrosine phosphatases SHP-1 and SHP-2 to suppress phagocytosis.[12,13] CD47 was first identified on human ovarian tumors. A growing number of studies have reported high CD47 expression on diverse types of cancers, including breast cancer, hepatocellular carcinoma, and colon glioblastoma; high CD47 expression is associated with poor prognosis.[14–17] Recently, CD47 was reported to be expressed on NHL.[18] Therefore, targeting CD47 may be a novel strategy for lymphoma treatment. This review focuses on the role of the CD47-SIRPα pathway and effects of therapeutic strategies targeting this pathway in B-cell lymphoma.

Mechanisms and effects of blocking CD47 in cancer

CD47 expressed on tumor cells and tumor stem cells has been identified as a "don't eat me" signal, and binding of CD47 to SIRPα contributes to inhibition of macrophage phagocytosis, thus facilitating cancer immune evasion.[19–21] Indeed, Liu et al.[22] reported that high CD47 expression led to the progression of ovarian cancer by inhibiting macrophage phagocytosis; CD47 downregulation by shRNA, or its inhibition by mAb, promoted phagocytosis and macrophage infiltration in tumor cells.[22] Anti-CD47 antibodies also induced macrophage-mediated phagocytosis and suppressed

tumor growth in myeloma, hepatocellular carcinoma, and glioblastoma[23–26]; additionally, blockage of CD47 increased M1 macrophages, affected overall macrophage distribution, and promoted the migration of macrophages into the tumor.[25–26] These studies demonstrated that blocking the CD47 inhibitory signal promoted an innate immune response through macrophage-dependent phagocytosis.

The anti-tumor mechanisms of blocking CD47 include: 1) Activation of the antibody-dependent cellular cytotoxicity (ADCC)-mediated innate immune response. For example, Kim et al.[27] showed that high CD47 expression was associated with a reduction in natural killer (NK) cell-mediated cytotoxicity in head-and-neck squamous cell carcinoma (HNSCC); treatment with anti-CD47 antibody remarkably increased NK cell-mediated cytotoxicity against HNSCC.[27] Chao et al.[28] observed that the use of anti-CD47 antibody caused NK cell-mediated ADCC in lymphoma, in an Fc receptor-dependent manner. 2) Promotion of adaptive immunity. Tseng et al.[29] reported that antigen-specific CD8+ T cells proliferated after macrophage phagocytosis induced by the use of an anti-CD47 antibody in colon cancer, whereas the number of regulatory T cells was reduced; an increase in CD8+ T cells showed good anti-tumor effects *in vivo*, indicating that the use of an anti-CD47 antibody enabled adaptive T cell immune responses and overwhelmed the regulatory T cell-mediated immune evasion in cancer.[29] Similarly, another study showed that the anti-CD47 antibody inhibited tumor progression by enhancing the antigen-specific CD8+ T cell response, which was activated by dendritic cell-mediated tumor antigen presentation to T cells.[30] Recently, Soto-Pantoja et al.[31] reported that blocking of CD47 induced a cytotoxic T cell-dependent anti-tumor immune response and enhanced the effects of irradiation in fibrosarcoma; CD47 knockdown in CD8+ T cells also significantly enhanced their tumoricidal activity. Moreover, elevated CD47 expression was associated with reduced CD8+ T cell infiltration in melanoma.[31] 3) Direct induction of apoptosis. Several studies showed that the blockage of CD47 by the antibody MABL (specific for an extracellular domain of CD47) promoted apoptosis of leukemia and myeloma cells.[32–33] Additionally, MABL-induced apoptotic activity in myeloma cells was not affected by chemotherapy, indicating an independent anti-myeloma role for MABL-induced apoptosis.[33] 4) HIF-1-mediated inhibition of cancer stem cells (CSCs). Zhang et al.[34] reported that CD47 gene transcription was dependent on HIF-1 in breast cancer. CD47 expression also contributed to maintenance of breast CSCs. Therefore, downregulation of CD47 by blockage of HIF-1 may inhibit breast CSCs.

Therapeutic strategies targeting CD47-SIRPα in B-cell lymphoma

Increasing evidence has demonstrated that increased CD47 expression by different types of B-cell lymphoid malignancies was associated with tumor progression and dissemination. For example, Chao et al.[28,35] reported that CD47 mRNA expression was significantly increased in various B-cell lymphoma cells, and that high CD47 expression indicated poor survival and disease progression in diffuse large B-cell lymphoma (DLBCL), B-cell chronic lymphocytic leukemia (B-CLL), and mantle cell lymphoma. Subsequently, increased CD47 expression was detected in disseminated lymphoma samples; further, CD47 knockdown reduced disease involvement at secondary sites in a lymphoma xenograft model, indicating that lymphoma dissemination was CD47-dependent.[28,35] Starr et al.[36] also reported that CD47 was highly expressed in both nodal and intravascular DLBCL cells, indicating

that CD47 plays an important role in the intravascular dissemination of DLBCL. Therefore, blocking the CD47-SIRPα pathway may be an effective approach to treat B-cell lymphoma.

Strategies targeting the CD47-SIRPα pathway in B-cell lymphoma are diverse. First, CD47 can be directly blocked by monoclonal antibodies. Second, CD47 can bind to recombinant polypeptides derived from SIRPα, such as the SIRPα-Fc fusion protein. Additionally, the interaction of CD47 and SIRPα can be inhibited by an anti-SIRPα antibody.[37] Goto et al.[38] reported increased expression levels of CD47 on the surface of primary effusion lymphoma (PEL) cell lines, and showed that CD47 knockdown by siRNA or anti-CD47 antibody increased the phagocytosis of PEL cells by macrophages; they also demonstrated that the use of an anti-CD47 antibody significantly inhibited the growth and metastasis of PEL cells in a xenograft mouse model.[38] Similarly, Chao et al.[28,35] demonstrated that both anti-CD47 and anti-SIRPα antibodies promoted macrophage phagocytosis of NHL cells, in a manner dependent on the level of CD47 expression; additionally, the use of an anti-CD47 antibody inhibited tumor growth and extended the survival of both localized and disseminated lymphoma *in vivo*. Interestingly, although the anti-CD47 antibody did not fully eliminate the lymphoma, it reduced the growth rate of the tumor and prevented both extranodal and hematogenous spread in a macrophage-dependent manner, demonstrating the inhibitory effect of anti-CD47 antibody on lymphoma dissemination.[28,35] Uno et al.[39] reported that the anti-CD47 monoclonal antibody (mAb), MABL, enabled apoptosis in JOK-1 cells, but did not induce cell death in CD34+ progenitor cells; additionally, treatment with MABL prolonged the survival of xenograft mice; the median survival of mice injected with MABL was longer than that of mice treated with fludarabine, demonstrating the efficacy of MABL in eradicating B-CLL.[39] To reduce hemagglutination from MABL, Sagawa et al.[40] developed an S-S diabody, a disulfide-stabilized dimer of a single-chain antibody fragment of MABL; the S-S diabody induced apoptosis in B-CLL cells, but not in normal leukocytes. The experiment in B-CLL-transplanted mice also revealed the anti-tumor effects of the S-S diabody, which blocked tumor growth and improved survival.[40] Additionally, CD47 ligation by an anti-CD47 mAb induced apoptosis and cell death in B-CLL through the caspase-independent pathway; apoptotic cells were then eliminated by dendritic cell-mediated phagocytosis.[41–42]

However, CD47 is also widely expressed on normal cells, including red blood cells, platelets, and mesenchymal stem cells, weakening the specificity of the antibody towards tumor cells.[43] Therefore, several studies have developed bispecific antibodies (BsAbs) to specifically limit CD47 neutralization to tumor cells. Piccione et al.[44] reported that BsAbs co-targeting CD47 and CD20 increased the phagocytosis of NHL cells; BsAb also reduced the tumor burden in both localized and disseminated NHL mouse models and significantly prolonged survival, compared with either anti-CD47 antibody or rituximab monotherapy.[44] Métayer et al.[45] showed that both anti-CD47 and anti-CD19 antibodies induced phagocytosis in Burkitt's lymphoma cells. Subsequently, Dheilly et al.[46] developed a CD47/CD19 dual-targeting BsAb and demonstrated its binding selectivity and anti-lymphoma effects, which were mediated by antibody-dependent cellular phagocytosis. Although these BsAbs showed satisfactory binding selectivity, the interaction of the functional Fc fragment of these BsAbs with the Fc receptor of macrophages led to phagocytic systemic toxicity and premature off-target effects that

reduced the accumulation of BsAbs on the tumor cell surface. Recently, van Bommel et al.[47] built a novel BsAb containing single-chain fragments of variable regions (scFv) of anti-CD47 antibody and scFv (derived from the anti-CD20 antibody rituximab) to resolve this limitation. They demonstrated that this novel BsAb specifically induced antibody-dependent cellular phagocytosis in CD20+/CD47+ malignant B-cell lymphoma in an Fc-independent manner, and that it enhanced the anti-tumor effect of the mAbs daratumumab, alemtuzumab, and obinutuzumab.[47]

In addition, combination strategies based on blockage of the CD47-SIRPα pathway exerted synergistic anti-lymphoma effects. Liu et al.[48] reported that the combination of Hu5F9-G4 and rituximab resulted in phagocytic elimination of lymphoma and significantly prolonged the survival time of NHL model mice. Similarly, Chao et al.[28] reported that an anti-CD47 antibody enhanced phagocytosis induced by rituximab in different lymphoma cell lines, but not in normal peripheral blood cells; notably, combination therapy with an anti-CD47 antibody and rituximab eliminated lymphoma in some xenograft mice and induced long-term survival without disease relapse. The improved anti-tumor effect originated from anti-CD47 antibody-mediated Fc-independent and rituximab-mediated Fc-dependent macrophage phagocytosis, but not through NK cells or complement.[28] The anti-SIRPα mAb MY-1 also facilitated the phagocytosis of Burkitt's lymphoma cells induced by rituximab; MY-1 significantly enhanced the inhibitory effect of rituximab on lymphoma growth *in vivo*.[49] Furthermore, the safety and anti-lymphoma effects of several agents blocking CD47 alone or in combination with rituximab have been investigated in phase 1 and 2 clinical trials (Table 1).

In addition to their synergistic effects with tumor antigen-specific antibodies, antibodies targeting CD47-SIRPα may combine with other agents to augment treatment efficacy. For example, an anti-CD47 mAb triggered type III programmed cell death in B-CLL cells, demonstrating involvement of the caspase-independent pathway in CD47-mediated tumor destruction; anti-CD47 mAb-induced type III programmed cell death was associated with F-actin dynamics. Thus, additional F-actin regulators may enhance the anti-tumor effect of the anti-CD47 mAb. Additionally, because caspase-dependent apoptosis enables cell death in B-CLL, therapeutic strategies that combine anti-CD47 mAb with caspase modulators may constitute promising approaches.[50] TTI-621, a soluble SIRPα Fc fusion protein that blocks CD47, significantly increased the phagocytosis of lymphoma cells by macrophages *in vitro* and tumor-associated macrophages derived from xenograft DLCBL tumors. TTI-621-stimulated macrophages

Table 1. Clinical trials of combination strategies targeting CD47-SIRPα

NCT number	Agent	Strategy	Type of lymphoma	Phase
NCT02663518	TTI-621	Single agent; combination with rituximab	NHL	Phase I
NCT02367196	CC-90002	Single agent; combination with rituximab	NHL	Phase I
NCT02953509	Hu5F9-G4	Single agent; combination with rituximab	NHL; DLCBL; indolent lymphoma	Phase I+II
NCT03013218	ALX148	Combination with rituximab	NHL	Phase I

Abbreviations: DLBCL: diffuse large B-cell lymphoma; NHL: non-Hodgkin lymphoma.

Table 2. Therapeutics targeting CD47-SIRPα in lymphoma.

Drug	Type of tumor	Mechanism	Combined agents	Refs
Anti-CD47 antibody	PEL	Phagocytosis	/	38
MABL	B-CLL	Apoptosis	/	39
S-S diabody	NHL	Apoptosis	/	40
Anti-CD47 antibody	B-CLL	Apoptosis	/	41
CD47/CD20 BsAb	NHL	Phagocytosis	/	44
CD47/CD19 BsAb	Burkitt's lymphoma	ADCP	/	46
CD47/CD20 scFv	B-cell lymphoma	ADCP	/	47
Anti-CD47 antibody; Anti-SIRPα antibody	NHL	Phagocytosis	Rituximab	28
Anti-CD47 antibody	Burkitt's lymphoma	ADCP	Anti-CD10 antibody; anti-CD19 antibody	45
Hu5F9-G4	NHL	Not mentioned	Rituximab	48
MY-1	Burkitt's lymphoma	Phagocytosis	Rituximab	49
Anti-CD47 antibody	B-CLL	Type III PCD	F-actin regulators; caspase modulators	50
TTI-621	DLBCL	Phagocytosis	Macrophage agonists	51

Abbreviations: ADCP: antibody-dependent cellular phagocytosis; BsAb: bispecific antibody; B-CLL: B-chronic lymphocytic leukemia; DLBCL: diffuse large B-cell lymphoma; NHL: non-Hodgkin lymphoma; PCD: programmed cell death; PEL: primary effusion lymphoma; scFv: single-chain fragment of variable regions.

exhibited a highly phagocytic phenotype upon exposure to cytokines (interferon (IFN)-γ, IFN-α, and interleukin-10) or Toll-like receptor agonists (lipopolysaccharides, Poly (I:C), and R848), suggesting that blockage of CD47 with macrophage regulators may serve as a potential combination therapy.[51] Furthermore, Gautam et al.[52] observed that the Hsp70-peptide complex transformed M2 macrophages into tumor-inhibiting M1 macrophages in Dalton's lymphoma; additionally, SIRPα expression on macrophages was elevated after treatment with Hsp70-peptide complex. Therefore, the combination of Hsp70 with an anti-SIRPα antibody may have synergistic anti-lymphoma effects[52] (Table 2).

Conclusion

Tumor immune escape is a primary mechanism of lymphoma progression and dissemination. Therefore, immunotherapy has become a hotspot of lymphoma treatment in recent years. The CD47-SIRPα axis plays an important role in the immune regulation of lymphoma. Studies targeting the CD47-SIRPα pathway have shown significant anti-lymphoma effects, mainly through the activation of innate immunity, mediated by macrophage phagocytosis, or direct promotion of apoptosis. However, anti-CD47 antibodies have some limitations: 1) CD47 is not solely expressed on lymphoma cells; it is also expressed on normal cells, resulting in toxic effects and antibody exhaustion. Bispecific antibodies co-targeting CD47 and other tumor-specific antigens may improve the binding specificity of antibodies and tumor cells, enhancing safety and efficacy. 2) Most studies have reported that anti-CD47 antibody monotherapy does not fully eliminate lymphoma; combination strategies that activate adoptive immunity or involve the use of the anti-CD20 antibody, macrophage agonists such as IFN-γ, IFN-α, interleukin-10, and other agents (e.g., caspase modulators and F-actin regulators), may have lasting and effective anti-lymphoma activities. 3)

The efficacies of different methods of blocking CD47, such as anti-CD47 antibody or scFv derived from an antibody, remain unknown. Therefore, strategies based on blockage of the CD47-SIRPα axis require further evaluation in pre-clinical studies and clinical trials, and may provide new directions for lymphoma treatment.

Declaration of conflicting interest

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship and/or publication of this article: This research was supported by the National Natural Science Foundation of China (No. 81670178), The National Key Research and Development Program of China (No. 2016YFC090150X), the Research Project for Practice Development of National TCM Clinical Research Bases (No. JDZX2015113), and the Funds of Science Technology Department of Zhejiang Province (No. 2018C03016-1).

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