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REVIEW

Checkpoint CD47 Function On Tumor Metastasis And Immune Therapy

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Abstract: The success of cancer immunotherapy on recognition checkpoints for killing cancer cells has raised a great interest of scientists in understanding new and old methods of immunotherapeutic. CD47 (cluster of differentiation 47) is a cell surface glycoprotein and widely expressed on cells, which belongs to the immunoglobulin (Ig) superfamily as a cell membrane receptor which serves in immune therapy. CD47 is an inhibitory receptor expressed on tumor cell surface and interacts with signal receptor protein-alpha (SIPR-α, also named CD172a or SHPS-1) which may escape from immune cells such as macrophage and T cells. Meanwhile, tumor cells express high CD47 protein which may secrete exosomes with high CD47 expression. The high CD47 expression-exosomes could serve the tumor metastasis process and provide transfer convenience for tumors on the microenvironment. CD47 on cancer cells can also affect the migration and invasion of cells. The high CD47 expression on tumor or CTC (circulating tumor cell) surface means the stronger migration and invasion and makes them escape from immune cells for phagocytosis such as T cells, NK (natural killer) cells and macrophage, which could be used for diagnosis and prognosis on cancer patients. Meanwhile, targeting CD47 combined with other biomarkers such as EpCAM (epithelial cell adhesion molecule), CD44, etc on cancer surface could be used to isolate CTCs from patients' blood. In terms of treatment, anti-CD47 antibody combined with another antibody such as anti-PD-L1 (programmed death-ligand 1) antibody or drugs such as rituximab, DOX or oxaliplatin also has better therapeutic effects and antitumor function to tumors. Using nanomaterials as an intermediary for CD47-related immune therapy could greatly increase the therapeutic effect and overcome multiple biological barriers for anti-CD47 antibody in vivo. In this review, we discuss the important role and the function of CD47 in tumor metastasis and also provide a reference for related research.

Keywords: CD47, immune therapy, biological target, immune checkpoint

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Introduction

Cancer has caused a huge panic which is acknowledged to be a worldwide serious public and personal health problem. In 2018, there were about 18.1 million new cancer cases and 9.6 million new cancer deaths.[1] Metastasis is the chief cause for morbidity and the highest fatality in most cancers. Cancer metastasis needs several steps after it finishes the metastasis and gets a secondary colony.[2] In microenvironment, high-expression checkpoints on cancer cell surface could stop metastasis during each metastasis step from primacy location to another organ. And the checkpoints are inhibitory receptors expressed on cancer cell surface and used to escape immune cell recognition during the process of metastasis. So the checkpoints and immune system on microenvironment play important roles in preventing tumor initiation and controlling tumor growth.[3]

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CD47 was discovered as a plasma membrane molecule, which is a kind of IAP (integrin-associated protein). CD47's structure is showing in Figure 1 with a five-membrane-spanning segments in the membrane which has a molecular weight at 45–55 kDa. And it is the receptor for thrombospondin family member and is an unusual part of the membrane protein immunoglobulin (Ig) superfamily with possessing an Ig-V-like extracellular region.[4]

CD47 is an integrin-associated protein and ligands to the transmembrane signaling protein SIRP-α (Figure 1), which is also expressed on normal cells such as red blood cells, but widely overexpressed on tumor cells, such as lung cancer,[5] colon cancer,[6] melanoma cells[7] and CTCs. SIRP-α is the ligand for protein CD47, which is a prototypic member. And the structure of SIRP-α is also shown in Figure 1. The interaction between SIRP-α and CD47 decides the function between cells and cells, which may affect tumor cell proliferation, migration, invasion and immune cell activation and apoptosis in the blood microenvironment and a series of immune environment.[8,9] The good news is that in most normal cells, due to the lack of prophagocytic signals, it will not be affected by CD47 blockade except for aging RBCs.[10] Since tumor cells frequently express pro-phagocytic signals, which make them inhibition CD47 signaling by CD47-associated molecular and cause them more easier to be exposed to phagocytosis and other immune cells.[10,11] Similarly, thrombospondins-1 ligand CD47 is an anti-inflammatory extracellular matrix protein, which could regulate regulatory T cells.[4,12] Thus, blockade CD47 may be a good choice to regulate the relation of both immune cells and tumor cells, but it may not affect melanoma cells.[7]

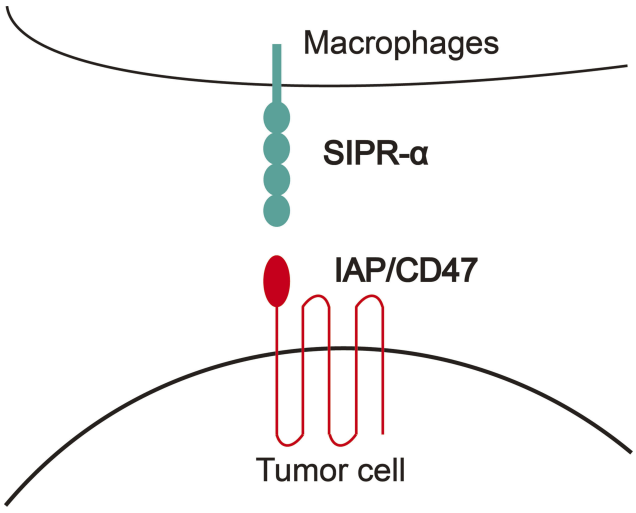

Figure 1 The structure of CD47/IAP.

In this review, we discuss the newest papers on the protein of CD47, focusing on the molecular function and mechanisms, especially, in the field of immune treatment part. Meanwhile, the potential biological effects of exosomes with high-CD47 expression which can modulate metastasis functions on cancer also have been discussed. The synergistic function between anti-CD47 and other antibody/drugs/nanoparticles on biological also been mentioned.

CD47 And Immune Evasion

CD47 is a transmembrane protein expressed on most cells but often overexpressed on tumor cells, which provides "don't eat me" signal in innate immune and adaptive signaling through binding SIRP-α to inhibit immune elimination. Whereas, SIRP-α is a regulatory membrane glycoprotein, which is particularly abundant in myeloid cells such as macrophages[13] and also in other immune cells such as dendritic cells.[14] It has been proved that blocking CD47 signaling on cancer cells could promote the stimulation of macrophages, NK cells[15,16] and activation of tumor-specific cytotoxic $CD8^+$ T cells by macrophages[17] or dendritic cells.[18] Meanwhile, the SIRP-α-inhibited macrophage injected into mice to block recognition of its ligand CD47 with the same tumor-suppressive function as injected anti-CD47 antibody could be also used to promote macrophage activity and eliminate tumor cells.[19]

It has been reviewed that blockade CD47-SIRP-α signaling on cancer cells could provoke an innate immune response and adaptive immune response to inhibit the tumor growth in vivo. First, blocking CD47 by anti-CD47 antibody could inhibit non-Hodgkin lymphoma (NHL) cell dissemination.[20] When the NHL starts to transform into circulating tumor cells and spreads to other major organs, including the central nervous system. Blocking CD47 could inhibit the dissemination from primary NHL cells by macrophage-mediated phagocytosis.[20] Meanwhile, anti-Hu5F9-G4 antibody is a novel blocking CD47 antibody which is a macrophage immune checkpoint inhibitor to induce tumor cell phagocytosis which was tested in 62 patients with advanced solid malignancy or lymphoma cancers in Phase I trial.[21] Anti-Hu5F9-G4 antibody with blocking CD47 cooperates with the antibody rituximab, which could promote cellular phagocytosis elimination of B-cell non-Hodgkin's lymphoma cells (BNL) by macrophage-mediated antibody-dependent manner.[22] Another human antibody AMMS4 got from ZF1 (an anticancer effector as

anti-Hu5F9-G4 antibody), which has significantly inhibition solid tumor growth by enhancing macrophage infiltration and reducing angiogenic ability.[23] In cynomolgus monkeys, Sun's group did not observe RBC hemagglutination even when anti-AMMS4 antibody doses were up to the 30 and 60 mg/kg.[23] Besides, when targeting cells express SIRP-α, the ligand for CD47, blockade SIRP-α makes tumor cells more susceptible to $CD8^+$ T cell-killing which was delivery tumor-associated antigen information by macrophage[17] or dendritic cells[18] in vivo. Anti-CD47 antibody synergy with anti-Blinatumomab (MT103) antibody could enable the effective control of non-Hodgkin lymphoma in a mouse model by getting the CD19/CD3-bispecific T-cell engager.[24] Various blockade CD47 and SIRP-α signaling antibodies and corresponding immune cells are listed in Table1.

CD47 has not only a related role in immune cells, but also acts as a marker for diagnosis and prognosis. In T-lymphoblastic lymphoma/leukemia (T-LBL/ALL) patients, cancer cells were detected half rate high expression for CD47 protein ($26_{high}/56_{all}$), and the level of CD47 protein and mRNA was much higher compared to the control group, and it is correlated with prognosis (P_{all} <0.05). This means CD47 has the potential diagnosis and prognosis of T-LBL/ALL.[29] Regarding T-cell acute lymphoblastic leukemia (T-ALL), CD47 agonist peptide pkhb1 could induce immunogenic cell death (ICD), as it induces calreticulin (CRT) exposure and damage-associated molecular patterns (DAMPs) release in vitro. CD47 agonist peptides have the potential as therapeutic tools to treat leukemia.[30]

Anti-CD47 antibody could also regulate various cytokines during treatment. Anti-CD47 treatment enhanced pro-inflammatory responses (including IL-2, IL-12 IFN-γ and TNF-β, and in the animal model, the CD47 therapy could increase immune cell infiltration capacity such as $CD8^+$ T-cell to tumor tissue. In the anti-CD47-treated group, T cells maintained high PD-1 and CTLA-4 levels, suggesting that anti-CD47 could be used to combine with anti-PD-1 and CLTA-4 in esophageal squamous cell carcinoma cells. It can enhance the therapeutic effect of T cell activation.[25] An antibody RRx-001 could downregulate CD47 on cancer cells and SIRP-α on monocytes/macrophages simultaneously to upregulate the innate immune in Phase III clinical trials.[31] Overexpression of CD47 could prevent stem cell-induced NK cells from IFN-γ release in vivo and vitro. In mouse and human when major histocompatibility complex (MHC) class I and II genes are inactivated[32] and CD47 is overexpressed, autologous-induced pluripotent stem cells (iPSCs) could lose their immunogenicity. Both CD47 overexpression and MHC I and II inactivation could be engineered for universal transplantation.[33] A new finding, compound CpG oligodeoxynucleotide, could enhance the antitumor ability and engulf the $CD47^+$ cancer cells by evoking the changes in the central carbon metabolism of macrophages.[34] From the above discussion and increasing evidences, blocking SIRP-α-CD47 immunological checkpoints and exploring inhibition of the SIRP-a-CD47 pathway is a fairly promising approach to the treatment of various types of human cancer cells. Glutaminyl cyclase modifies the CD47- SIRP-α axis to rise immune therapy.[35]

Table 1 CD47 Relation Antibody And Immune Cells

Antibody	Immune Cells
Anti-CD47	Macrophages,[20] T cells,[25–27] NK cells,[12,21] $CD8^+$ T cells[28]
Anti-Hu5F9-G4	B cells[22]
Anti-AMMS4	Macrophages[23]
Anti-Blinatumomab (MT103)	CD19/CD3-bispecific T cell[24]
Anti-SIRP-α antibody	$CD8^+$ T cell-killing[17]

CD47 And Exosome

Exosomes, nanoextracellular vesicles, released by almost all types of cells, exosomes have been proved to carry cargoes including proteins, lipids and RNA, and vary depending on the type of parental cell. The exosomes have a phospholipid dual structure encompassing internal proteins and an outer four transmembrane protein. It was reported that by proteomic analysis protein CD47 could be tested on mesenchymal stem cell exosomes.[36,37] Including CD47 protein about 21 kind surface N-glycoproteins on the surface of exosomes released from myeloid-derived suppressor cells (mouse mammary carcinoma-induced MDSCs) which totally contains 93 proteins. The protein expression of CD47 on exosome is consistent with the surface of parental cells expression.[38]

Besides, studies have shown that in clinics CD47 can be used as a biomarker for early diagnosis and prognosis in breast cancer patients; in 921 breast cancer clinic patients, exosome biomarkers including CD47, HER2, Del-1, miR-1246 and miR-21 were significantly higher than the healthy control group.[39] In human blood single circulating exosomes surface protein could be rapid and automated analysis, a differential CD47 expression is correlated with breast cancer status.[40]

CD47 high expression on exosome could be used as an immune checkpoint inhibitor that reduces the interaction between CD47 and SIRP-α.[41] Thrombospondin-1 (a ligand for CD47) is expressed on exosomes/ectosomes derived from T cells, and these EVs specific binding to CD47 modulation signaling between T cells and EVs. As a self-secretion, exosomes could avoid phagocytosis by monocyte macrophages, compared to non-self liposomes, especially when the CD47-mediated exosomes circulate in the mice blood.[42] Thus, the high expression of CD47 on exosomes could help circulating tumor cells (CTCs) to arrive at a new place to complete the transfer (Figure 2). At the same dose, exosomes with high expression of CD47 can enhance the phagocytosis of macrophages to tumors and inhibit tumor growth in vivo.[43] CD47 is highly expressed in cancer cells, which can secrete exosomes with high expression of CD47. Self-secretion exosomes can avoid phagocytosis by macrophages and monocytes. Therefore, self-extracted exosomes carrying CD47 expression could be a good drug-loading and have broad application prospects.

CD47 And Migration

One of the cell surface molecules involved in the transmigration process is CD47, which has a well-characterized role in transendothelial migration.[44] It has been reported that cancer cells with high invasion and metastasis ability correlate with CD47 positively level. CD47 is expressed at a higher level in NSCLC (non-small cell lung cancer cells),[45] which these cancers could easy escape immune cells to arrive lung to transfer.

CD47 regulates the actin cytoskeleton in recombinant epithelial cells to participate in cell adhesion and cell migration. This CD47 pathway is mediated by the Src family[46] and MEK/MAPK (mitogen-activated protein kinase/mitogen-activated protein kinase).[47] MEK/MAPK pathway is a chain of proteins which could affect cell cycle and cell proliferation. CD47 is expressed both on the basolateral surface of epithelia and on neutrophils,[48] and its expression in the epithelium is upregulated experimentally in response to inflammatory stimuli[49] and in inflammatory bowel disease (IBD).[50] The COX-2 protein has been shown to modulate the adhesion and migration of human intestinal epithelial cells. Blocking or knockdown CD47 by anti-CD47 antibody or specific siRNA significantly reduced collagen I-induced cyclooxygenase-2 (COX-2) expression compared to the negative group.[51] MicroRNA-133a could target inhibition CD47 protein

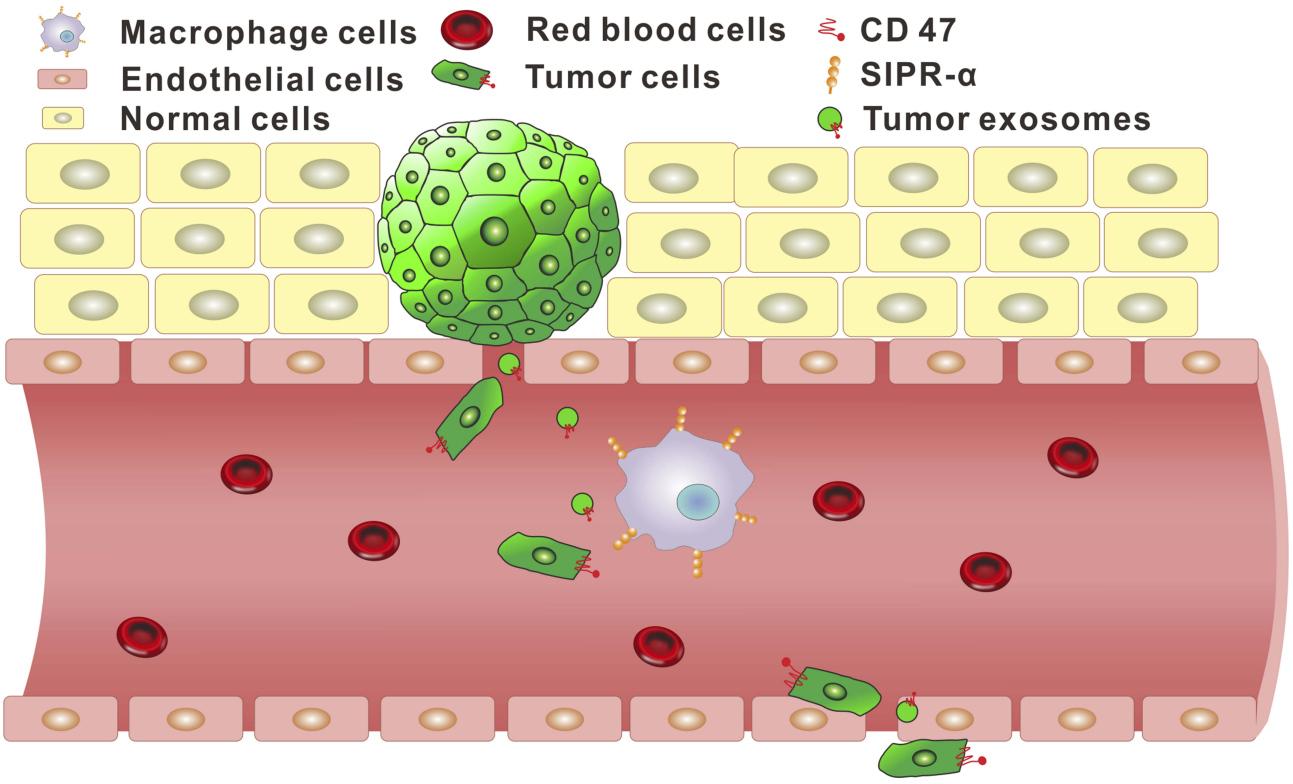

Figure 2 The function of CD47 in tumors and exosome.

to suppress the proliferation, migration and invasion of laryngeal carcinoma cells.[52]

CD47 could eliminate the phagocytosis by macrophage in vivo, so anti-CD47 antibody could promote phagocytosis. Meanwhile, using anti-SIRP-α antibody on macrophage could induce phagocytic activity and enhance antitumor effects in many cancer cells such as gastroenterological malignancies.[53] It was also reported that higher CD47 expression in high-grade serous ovarian carcinoma patient cells was significantly correlated with poor prognosis of patients which may escape from macrophage and then tend to metastasis. Relevant functional studies in ovarian cancer cells also showed that overexpression of CD47 significantly promoted migration and invasion. In addition, epithelial–mesenchymal transition (EMT) is a cells from epithelial cells to become mesenchymal stem cells process. EMT could be induced by CD47 through modulating E-cadherin and N-cadherin on high-grade serous ovarian cancer cells (HGSOC).[54] Besides, protein cdc42 is a downstream mediator of protein CD47 which regulates the cell cycle and promotes NSCLC migration/invasion.[45] Interestingly, surface expression of CD47 on cancer cells increased significantly after the adhesion to the endothelium, which helped them to stay elevated in the lung interstitium,[55] and which was effective to form metastases. CD47 also promotes cell invasion via activation PI3k/Akt pathway on human glioblastomas.[56]

Myocardial infarction-associated transcript (MIAT) knockdown on atherosclerotic cardio-cerebrovascular could upregulate CD47 expression via MIAT-knockdown and promoted phagocytosis function to macrophages in vitro and in vivo. Macrophage-relevant MIAT/miR-149-5p/CD47 pathway was a key factor in the treatment of necrotic atherosclerotic plaques.[57] Lewis Y antigen also plays an important role in embryogenesis and the immune process of intercellular recognition. CD47 and Lewis Y showed significant correlations with the clinical-pathological parameters on early ovarian cancer patients which had poor prognoses.[58] The pathway is shown in Figure 3 and Table 2.

CD47 And CTC Isolation

As we mentioned above, CD47 is an antiphagocytotic "don't eat me" signal expressed in many cancer cells, and it also proved that CD47 was expressed on CTCs to prevent macrophages or dendritic cells from attacking these tumor cells.[59] Interestingly, CD47 binds to SIRP-α,[60] SIRP-α is the specific receptor on macrophages and T cell surface and CD47 is the only upregulated protein and gene in CTCs compared with primary tumors, indicating that CD47 has

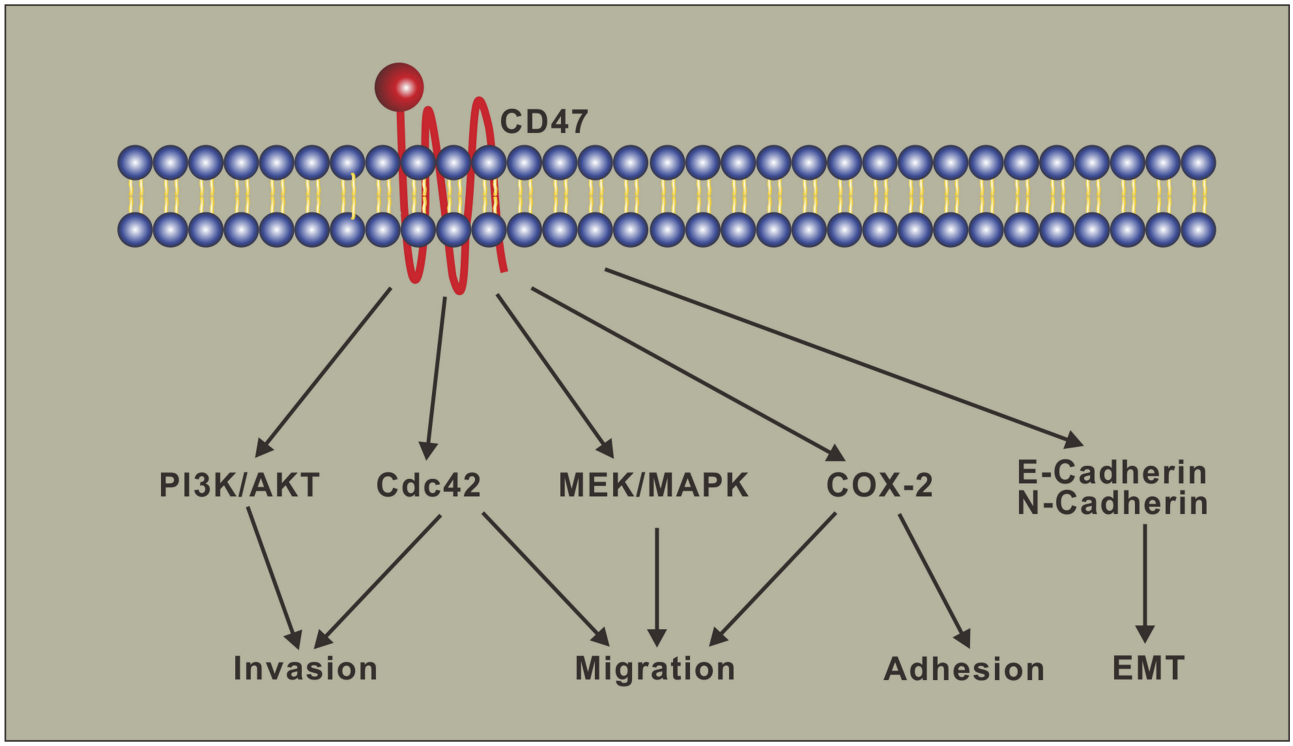

Figure 3 The relative pathway of CD47.

Table 2 CD47 Relation Pathway And Molecular

Molecular/Pathway	Cell Type Expressing The Receptor	Therapeutic Schedule	Toxicity/Defect
MEK/MAPK[47]	Kidney cells and epithelial cells	Inhibition of Src and mitogen-activated protein kinase kinase	Other pathway and protein not discussed
COX-2 protein[51]	Human embryonic intestinal epithelial cells	siRNA knockdown CD47	Other pathway and protein not discussed
E-cadherin and N-cadherin[54]	Mesenchymal stem cells (high-grade serous ovarian cancer cells)	The correlation among CD47 and poor prognosis of HGSOC patients	CD47 did not influence proliferation and cell cycle progression
Cdc42 molecular[45]	NSCLC	siRNA-mediated downregulation of CD47 and overexpression of CD47 by plasmid transfection	No significant difference in E-cadherin, N-cadherin, Snail-1 and Snail-2 proteins
PI3k/Akt pathway[56]	Glioblastomas	Knockdown CD47 by siRNA	Short-time knockdown
MIAT/miR-149-5p/CD47 pathway[57]	Atherosclerotic cardio-cerebrovascular	Knockdown MIAK	Not completely to be biomarker
CD4 and Lewis Y[58]	Ovarian cancer cells	Used as prognosis of new target for ovarian cancer patients metastasis	CD47 is not a specific marker of ovarian cancer

the survival advantage in peripheral blood CTCs.[61] Meanwhile, for patients with breast cancer, it is indicated that CD47-positive CTCs are present in the bone marrow, and this may cause cancer recurrence.[62] Chao's group study suggests that upregulation of constitutive CD47 is a key requirement for immune tolerance and transmission in non-Hodgkin lymphoma.[20,63] Consistent with the upregulation of CD47 on CTCs is a significant calreticulin down-regulation, and calreticulin is a chaperone protein that acts as a homing beacon inducing strong cytotoxicity and NK cell phagocytosis and other immune effector cells to tumor cells.

Jia' s group proved that CD47 is highly expressed on human colon patient blood CTCs, which can be used as biomarker for CTC isolation.[6] Overexpression biomarkers including CD47, EpCAM, CD44 and MET in CTCs have a relation with survival times in metastatic breast cancer.[64] In more than 200 immunohistochemical studies of breast tumors, there was a 10.3-year overall survival significant difference between MET-CD47 double-positive and double-negative patients ($p < 0.001$). EpCAM and CD44 are cancer-initiating cell markers which have the function of cell-cell adhesion, cell signaling, inflammation, and cell migration. Thus, CD47 and the tyrosine-kinase MET could be the prognostic factors for survival of patients with luminal breast cancer.[65] Primary CTC with high $MET^{+}CD47^{+}$ expression showed an excellent ability to initiate cell viability and enhanced cell metastasis in a mouse model.[65] Drug Gramicidin A (Gra A) could downregulate CD47 and CD44 to inhibit cancer stem cell (CSC) growth.[66] Meanwhile, drug Metformin could target CD47 protein to mediate miR-708 and inhibit cell self-renewal on breast CSCs.[67]

Synergism Of CD47 And Antibodies/Drugs

Synergistic drugs and antibodies are widely used in research, and synergistic drugs can improve drug efficacy compared to the individual use. A bispecific antibody derivative, called RTX (rituximab)-CD47, contains two antibody fragments in tandem that specifically bind to CD47. Rituximab is a human-mouse chimeric monoclonal antibody that can be used in human CD20. CD20 is a protein which is expressed on the surface of all B-cells. So RTX-CD47 is more effective than anti-CD47 antibody in the regulation of innate immune cells by inhibiting CD47-SIRPα on CD47-positive cancer cells with better macrophage-mediated phagocytosis assay.[68] Anti-CD47 antibody therapy combined with chemotherapy DOX could regulate autophagy and prevent cardiac toxicity to inhibit invasive breast cancer growth. Systematic protection of heart tissue activity and function in mice following synergistic treatment with CD47 and doxorubicin.[69] Another star synergist,

oxaliplatin prodrug-induced immunogenic cell death (ICD) combined with antibody anti-CD47-mediated CD47 blockade promote antigen presentation to DCs (dendritic cell) and the DC maturation. Finally, the antitumor immune antigen of ICD is transmitted. CD47 blockade and ICD induction efficiently inhibit the growth of both primary and distant tumors, inhibit tumor metastasis and prevent tumor recurrence.[70]

CD47 delivers an antiphagocytic signal to the innate immune system that signals the "don't eat me" signal. Similarly, PD-L1 (programmed death-ligand 1) is a signal to the adaptive immune system about "don't find me". The "Knobs-into-holes" technology is used to target the dual-targeting fusion protein of CD47 and PD-L1, called IAB and designed by Liu et, which was effectively induces phagocytosis of tumor cells, stimulates T-cell activation and mediates antibodies-dependent cell-mediated cytotoxicity in vivo and in vitro.[71,72] Dual inhibition CD47 and PD-L1 by siRNA could increase innate and adaptive immune responses including T cells, NK cells and IFN-γ cytokine release.[5,73]

CD47 And Nanoparticle

Nanoparticles are defined as particles between 1 and 100 nm. Nanomaterial-encapsulated drugs have the advantages of targeted delivery, sustained release of drugs, prolonged administration time and reduced toxicity and side effects. Anti-CD47 antibody-labeled SERS (surface-enhanced Raman scattering) nanoparticles bind to seven different breast cancer cell lines, and their fluorescence intensity is significantly enhanced compared to the anti-CD47 antibody alone ($p < 0.01$), concluding that CD47-targeted nanoparticles are more efficiently linked to tumor cells with high expression of CD47.[74] After packaging with nanoparticles, anti-CD47 antibody has a better function. Encapsulation anti-CD47 antibody in calcium carbonate nanoparticles in the fibrin gel could remove H^+ in the surgical wound and polarizes tumor-associated macrophages into the M1-like phenotype. When an anti-CD47 antibody is released, it can block the "don't eat me" signal in cancer cells and increase phagocytosis of cancer cells by macrophages[75] and T cells.[76] Overcoming the size of the biological barrier, the cryptic doxorubicin-loaded nanodrug is coated with CD47 peptides. This intelligent drug can efficiently reduce the phagocytosis of macrophages for the particles, which make them arrive their destinations.[77] CD47-targeted near-infrared photoimmunotherapy could significantly increase in a light-dose dependent manner to slow the tumor growth in vivo compared to antibody alone ($p=0.0002$).[78] Some papers have demonstrated that the use of anti-CD47 antibody and the chemotherapeutic drug gemcitabine in a single formulation making multifunctionalized iron oxide magnetic nanoparticles (MNPs) could keep strong efficacy against CD47-positive pancreatic cancer cells in vitro.[79] An anti-CD47 single-chain variable fragment (scFv) was chosen and conjugated to magnetic nanoparticles (MNPs) which was also utilized the $CD47^+$ on particles to bladder cells for macrophage.[80] Interesting, polyethylene glycol functionalized gold nanoparticles (polyethylene glycol AuNP) can accelerate the loss of CD47 on the membrane of aging red blood cells, enhance the aging process, and remove it in the blood through white blood cells expressed in SIPR-α.[81] Stimulated macrophages have higher phagocytic activities. Macrophages (M0) can be split into different types M1 and M2, while classically activated (M1) macrophages are more potent than alternatively activated (M2) macrophages. Carboxylated polystyrene nanoparticles with CD47 preferentially reduce phagocytic activity by the M1 phenotype. Fe_3O_4 nanoparticles (Fe_3O_4@RBC NPs) coated with biomimetic RBC membranes can evade immune cell clearance by virtue of the interactions between CD47 and SIRP-α receptor. Administration of Fe_3O_4@RBC NPs in vivo does not elicit immune responses on neither the cellular level (myeloid-derived suppressor cells (MDSCs)) nor the humoral level (immunoglobulin M and G (IgM and IgG)),[82] which means better application prospects.

Glutamine-functionalized branched polyethyleneimine (GPI) containing CD47 siRNA increases the ability of glutamine-dependent cancer cells to take up material and blocks and inhibits CD47 signals on cancer cells.[83] CD47 siRNA is effective in downregulating tumor cell CD47 protein and mRNA expression levels in vivo and in vitro. CD47 plays the role of "don't eat me" and combines with the immune receptor SIRPα to prevent phagocytosis and clearance.

Concluding Remarks

CD47 can be used as an index for the diagnosis of precancerous tumors. The CD47 interacts with its ligand signal-regulatory protein alpha (SIRP-α) (Figure 1), which influences a range of cellular activities and plays an important role in immune system and homeostasis. First, blocking CD47/SIRP-α pathway reagent provides maximum antitumor efficacy with minimal toxicity. Tumor cells express

high CD47, which could secrete high CD47 exosomes and affect the migration of tumor cells. The relative between CD47 and exosomes was first mentioned in reviews. Second, circulating tumor cells can be isolated by checkpoint CD47, a target spot for both diagnosis and prognosis in patients' cancer. So faced with such a good target, the corresponding antibody was developed such as Hu5F9-G4 and AMMS4, and its imitation organism Y, which plays an important role in blocking CD47 and treating tumors. Third, synergistic anti-CD47 antibodies with other drugs such as rituximab, DOX, oxaliplatin or anti-PD-L1 antibody could give better therapeutic effects in vivo and in vitro. Similarly, the corresponding anti-CD47 antibody and CD47 siRNA are loaded into the tumor cells through the carrier nanomaterial to achieve immunotherapy and targeted therapy for tumor cells. Therefore, given the broad potential of CD47 checkpoint function, it is certain that CD47 plays important roles in the treatment of tumors, tumor metastasis and prediagnosis.

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Author Contributions

All authors contributed to data collecting, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no competing interests in this work.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424. doi:10.3322/caac.21492
2. Kim MY, Oskarsson T, Acharyya S, et al. Tumor self-seeding by circulating cancer cells. *Cell*. 2009;139(7):1315–1326. doi:10.1016/j.cell.2009.11.025
3. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nature Rev Cancer*. 2006;6(1):24–37. doi:10.1038/nrc1782
4. Brown EJ, Frazier WA. Integrin-associated protein (CD47) and its ligands. *Trends Cell Biol*. 2001;11(3):130–135.
5. Lian S, Xie R, Ye Y, et al. Simultaneous blocking of CD47 and PD-L1 increases innate and adaptive cancer immune responses and cytokine release. *EBioMedicine*. 2019;42:281–295. doi:10.1016/j.ebiom.2019.03.018
6. Lu Y, Liang H, Yu T, et al. Isolation and characterization of living circulating tumor cells in patients by immunomagnetic negative enrichment coupled with flow cytometry. *Cancer*. 2015;121(17):3036–3045. doi:10.1002/cncr.29444
7. Anderson KL, Snyder KM, Ito D, et al. Evolutionarily conserved resistance to phagocytosis observed in melanoma cells is insensitive to upregulation of pro-phagocytic signals and to CD47 blockade. *Melanoma Res*. 2019. doi:10.1097/CMR.0000000000000629
8. Barclay AN, Brown MH. The SIRP family of receptors and immune regulation. *Nature Rev Immunol*. 2006;6(6):457–464. doi:10.1038/nri1859
9. Matozaki T, Murata Y, Okazawa H, Ohnishi H. Functions and molecular mechanisms of the CD47-SIRPalpha signalling pathway. *Trends Cell Biol*. 2009;19(2):72–80. doi:10.1016/j.tcb.2008.12.001
10. Chao MP, Weissman IL, Majeti R. The CD47-SIRPalpha pathway in cancer immune evasion and potential therapeutic implications. *Curr Opin Immunol*. 2012;24(2):225–232. doi:10.1016/j.coi.2012.01.010
11. Chao MP, Jaiswal S, Weissman-Tsukamoto R, et al. Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. *Sci Transl Med*. 2010;2(63):63ra94. doi:10.1126/scitranslmed.3001375
12. Grimbert P, Bouguermouh S, Baba N, et al. Thrombospondin/CD47 interaction: a pathway to generate regulatory T cells from human CD4+ CD25- T cells in response to inflammation. *J Immunol*. 2006;177(6):3534–3541. doi:10.4049/jimmunol.177.6.3534
13. Murata Y, Kotani T, Ohnishi H, Matozaki T. The CD47-SIRPalpha signalling system: its physiological roles and therapeutic application. *J Biochem*. 2014;155(6):335–344. doi:10.1093/jb/mvu017
14. Latour S, Tanaka H, Demeure C, et al. Bidirectional negative regulation of human T and dendritic cells by CD47 and its cognate receptor signal-regulator protein-alpha: down-regulation of IL-12 responsiveness and inhibition of dendritic cell activation. *J Immunol*. 2001;167(5):2547–2554. doi:10.4049/jimmunol.167.5.2547
15. Nath PR, Gangaplara A, Pal-Nath D, et al. CD47 expression in natural killer cells regulates homeostasis and modulates immune response to lymphocytic choriomeningitis virus. *Front Immunol*. 2018;9:2985. doi:10.3389/fimmu.2018.02985
16. Nath PR, Pal-Nath D, Mandal A, Cam MC, Schwartz AL, Roberts DD. Natural killer cell recruitment and activation are regulated by CD47 expression in the tumor microenvironment. *Cancer Immunol Res*. 2019;7:1547–1561. doi:10.1158/2326-6066.CIR-18-0367
17. Myers LM, Tal MC, Torrez Dulgeroff LB, et al. A functional subset of CD8(+) T cells during chronic exhaustion is defined by SIRPalpha expression. *Nat Commun*. 2019;10(1):794. doi:10.1038/s41467-019-08637-9
18. McCracken MN, Cha AC, Weissman IL. Molecular pathways: activating T cells after cancer cell phagocytosis from blockade of CD47 "don't eat me" signals. *Clin Cancer Res*. 2015;21(16):3597–3601. doi:10.1158/1078-0432.CCR-14-2520
19. Alvey CM, Spinler KR, Irianto J, et al. SIRPA-inhibited, marrow-derived macrophages engorge, accumulate, and differentiate in antibody-targeted regression of solid tumors. *Current Biol*. 2017;27(14):2065–2077 e2066. doi:10.1016/j.cub.2017.06.005
20. Chao MP, Tang C, Pachynski RK, Chin R, Majeti R, Weissman IL. Extranodal dissemination of non-Hodgkin lymphoma requires CD47 and is inhibited by anti-CD47 antibody therapy. *Blood*. 2011;118(18):4890–4901. doi:10.1182/blood-2011-02-338020
21. Sikic BI, Lakhani N, Patnaik A, et al. First-in-human, first-in-class phase I trial of the anti-CD47 antibody Hu5F9-G4 in patients with advanced cancers. *J Clin Oncol*. 2019;37(12):946–953. doi:10.1200/JCO.18.02018

22. Advani R, Flinn I, Popplewell L, et al. CD47 blockade by Hu5F9-G4 and rituximab in non-Hodgkin's lymphoma. *N Engl J Med*. 2018;379(18):1711–1721. doi:10.1056/NEJMoa1807315
23. Yu XY, Qiu WY, Long F, et al. A novel fully human anti-CD47 antibody as a potential therapy for human neoplasms with good safety. *Biochimie*. 2018;151:54–66. doi:10.1016/j.biochi.2018.05.019
24. Xu L, Wang S, Li J, Li B. CD47/SIRPalpha blocking enhances CD19/CD3-bispecific T cell engager antibody-mediated lysis of B cell malignancies. *Biochem Biophys Res Commun*. 2019;509(3):739–745. doi:10.1016/j.bbrc.2018.12.175
25. Tao H, Qian P, Wang F, Yu H, Guo Y. Targeting CD47 enhances the Efficacy of Anti-PD-1 and CTLA-4 in an esophageal squamous cell cancer preclinical model. *Oncol Res*. 2017;25(9):1579–1587. doi:10.3727/096504017X14900505020895
26. Jain S, Van Scoyk A, Morgan EA, et al. Targeted inhibition of CD47-SIRPalpha requires Fc-FcgammaR interactions to maximize activity in T-cell lymphomas. *Blood*. 2019. doi:10.1182/blood.2019001744
27. Johnson LDS, Banerjee S, Kruglov O, et al. Targeting CD47 in sezary syndrome with SIRPalphaFc. *Blood Adv*. 2019;3(7):1145–1153. doi:10.1182/bloodadvances.2018030577
28. Petralia MC, Mazzon E, Fagone P, et al. Characterization of the pathophysiological role of CD47 in uveal melanoma. *Molecules*. 2019;24:13. doi:10.3390/molecules24132450
29. Yang K, Xu J, Liu Q, Li J, Xi Y. Expression and significance of CD47, PD1 and PDL1 in T-cell acute lymphoblastic lymphoma/leukemia. *Pathol Res Pract*. 2018.
30. Uscanga-Palomeque AC, Calvillo-Rodriguez KM, Gomez-Morales L, et al. CD47 agonist peptide pkhb1 induces immunogenic cell death in T-cell acute lymphoblastic leukemia cells. *Cancer Sci*. 2018. doi:10.1111/cas.13885
31. Cabrales P. RRx-001 acts as a dual small molecule checkpoint inhibitor by downregulating CD47 on cancer cells and SIRP-alpha on monocytes/macrophages. *Transl Oncol*. 2019;12(4):626–632. doi:10.1016/j.tranon.2018.12.001
32. Wang D, Quan Y, Yan Q, Morales JE, Wetsel RA. Targeted disruption of the beta2-microglobulin gene minimizes the immunogenicity of human embryonic stem cells. *Stem Cells Transl Med*. 2015;4(10):1234–1245. doi:10.5966/sctm.2015-0049
33. Deuse T, Hu X, Gravina A, et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat Biotechnol*. 2019. doi:10.1038/s41587-019-0016-3
34. Liu M, O'Connor RS, Trefely S, Graham K, Snyder NW, Beatty GL. Metabolic rewiring of macrophages by CpG potentiates clearance of cancer cells and overcomes tumor-expressed CD47-mediated 'don't-eat-me' signal. *Nat Immunol*. 2019;20(3):265–275. doi:10.1038/s41590-018-0292-y
35. Logtenberg MEW, Jansen JHM, Raaben M, et al. Glutaminyl cyclase is an enzymatic modifier of the CD47- SIRPalpha axis and a target for cancer immunotherapy. *Nat Med*. 2019;25(4):612–619. doi:10.1038/s41591-019-0356-z
36. Kim HS, Choi DY, Yun SJ, et al. Proteomic analysis of microvesicles derived from human mesenchymal stem cells. *J Proteome Res*. 2012;11(2):839–849. doi:10.1021/pr200682z
37. Tong M, Kleffmann T, Pradhan S, et al. Proteomic characterization of macro-, micro- and nano-extracellular vesicles derived from the same first trimester placenta: relevance for feto-maternal communication. *Human Reprod*. 2016;31(4):687–699. doi:10.1093/humrep/dew004
38. Chauhan S, Danielson S, Clements V, Edwards N, Ostrand-Rosenberg S, Fenselau C. Surface glycoproteins of exosomes shed by myeloid-derived suppressor cells contribute to function. *J Proteome Res*. 2017;16(1):238–246. doi:10.1021/acs.jproteome.6b00811
39. Wang M, Ji S, Shao G, et al. Effect of exosome biomarkers for diagnosis and prognosis of breast cancer patients. *Clin Transl Oncol*. 2018;20(7):906–911. doi:10.1007/s12094-017-1805-0
40. Kibria G, Ramos EK, Lee KE, et al. A rapid, automated surface protein profiling of single circulating exosomes in human blood. *Sci Rep*. 2016;6:36502. doi:10.1038/srep36502
41. Koh E, Lee EJ, Nam GH, et al. Exosome-SIRPalpha, a CD47 blockade increases cancer cell phagocytosis. *Biomaterials*. 2017;121:121–129. doi:10.1016/j.biomaterials.2017.01.004
42. Kamerkar S, LeBleu VS, Sugimoto H, et al. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature*. 2017;546(7659):498–503. doi:10.1038/nature22341
43. Cho E, Nam GH, Hong Y, et al. Comparison of exosomes and ferritin protein nanocages for the delivery of membrane protein therapeutics. *J Controlled Release*. 2018;279:326–335. doi:10.1016/j.jconrel.2018.04.037
44. Cooper D, Lindberg FP, Gamble JR, Brown EJ, Vadas MA. Transendothelial migration of neutrophils involves integrin-associated protein (CD47). *Proc Natl Acad Sci U S A*. 1995;92(9):3978–3982. doi:10.1073/pnas.92.9.3978
45. Zhao H, Wang J, Kong X, et al. CD47 promotes tumor invasion and metastasis in non-small cell lung cancer. *Sci Rep*. 2016;6:29719. doi:10.1038/srep29719
46. Hanke JH, Gardner JP, Dow RL, et al. Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor. Study of Lck- and FynT-dependent T cell activation. *J Biol Chem*. 1996;271(2):695–701. doi:10.1074/jbc.271.2.695
47. Shinohara M, Ohyama N, Murata Y, et al. CD47 regulation of epithelial cell spreading and migration, and its signal transduction. *Cancer Sci*. 2006;97(9):889–895. doi:10.1111/j.1349-7006.2006.00245.x
48. Parkos CA, Colgan SP, Liang TW, et al. CD47 mediates post-adhesive events required for neutrophil migration across polarized intestinal epithelia. *J Cell Biol*. 1996;132(3):437–450. doi:10.1083/jcb.132.3.437
49. Liu Y, Merlin D, Burst SL, Pochet M, Madara JL, Parkos CA. The role of CD47 in neutrophil transmigration. Increased rate of migration correlates with increased cell surface expression of CD47. *J Biol Chem*. 2001;276(43):40156–40166. doi:10.1074/jbc.M104138200
50. Chin AC, Parkos CA. Neutrophil transepithelial migration and epithelial barrier function in IBD: potential targets for inhibiting neutrophil trafficking. *Ann N Y Acad Sci*. 2006;1072:276–287. doi:10.1196/annals.1326.018
51. Broom OJ, Zhang Y, Oldenborg PA, Massoumi R, Sjolander A. CD47 regulates collagen I-induced cyclooxygenase-2 expression and intestinal epithelial cell migration. *PLoS One*. 2009;4(7):e6371. doi:10.1371/journal.pone.0006371
52. Li H, Wang Y, Li YZ. MicroRNA-133a suppresses the proliferation, migration, and invasion of laryngeal carcinoma cells by targeting CD47. *Tumour Biol*. 2016. doi:10.1007/s13277-016-5451-x
53. Abe T, Tanaka Y, Piao J, et al. Signal regulatory protein alpha blockade potentiates tumoricidal effects of macrophages on gastroenterological neoplastic cells in syngeneic immunocompetent mice. *Ann Gastroenterological Surg*. 2018;2(6):451–462. doi:10.1002/ags3.12205
54. Li Y, Lu S, Xu Y, et al. Overexpression of CD47 predicts poor prognosis and promotes cancer cell invasion in high-grade serous ovarian carcinoma. *Am J Transl Res*. 2017;9(6):2901–2910.
55. Konrad FM, Wohlert J, Gamper-Tsigaras J, Ngamsri KC, Reutershan J. How adhesion molecule patterns change while neutrophils traffic through the lung during inflammation. *Mediators Inflamm*. 2019;2019:1208086. doi:10.1155/2019/1208086
56. Liu X, Wu X, Wang Y, et al. CD47 promotes human glioblastoma invasion through activation of the PI3K/Akt pathway. *Oncol Res*. 2019;27(4):415–422. doi:10.3727/096504018X15155538502359
57. Ye ZM, Yang S, Xia YP, et al. LncRNA MIAT sponges miR-149-5p to inhibit efferocytosis in advanced atherosclerosis through CD47 upregulation. *Cell Death Dis*. 2019;10(2):138. doi:10.1038/s41419-019-1409-4

58. Tan M, Zhu L, Zhuang H, et al. Lewis Y antigen modified CD47 is an independent risk factor for poor prognosis and promotes early ovarian cancer metastasis. *Am J Cancer Res*. 2015;5(9):2777–2787.
59. Jaiswal S, Jamieson CH, Pang WW, et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell*. 2009;138(2):271–285. doi:10.1016/j.cell.2009.05.046
60. Jiang P, Lagenaur CF, Narayanan V. Integrin-associated protein is a ligand for the P84 neural adhesion molecule. *J Biol Chem*. 1999;274(2):559–562. doi:10.1074/jbc.274.2.559
61. Steinert G, Scholch S, Niemietz T, et al. Immune escape and survival mechanisms in circulating tumor cells of colorectal cancer. *Cancer Res*. 2014;74(6):1694–1704. doi:10.1158/0008-5472.CAN-13-1885
62. Nagahara M, Mimori K, Kataoka A, et al. Correlated expression of CD47 and SIRPA in bone marrow and in peripheral blood predicts recurrence in breast cancer patients. *Clin Cancer Res*. 2010;16(18):4625–4635. doi:10.1158/1078-0432.CCR-10-0349
63. Chao MP, Alizadeh AA, Tang C, et al. Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma. *Cell*. 2010;142(5):699–713. doi:10.1016/j.cell.2010.07.044
64. Baccelli I, Schneeweiss A, Riethdorf S, et al. Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. *Nat Biotechnol*. 2013;31(6):539–544. doi:10.1038/nbt.2576
65. Baccelli I, Stenzinger A, Vogel V, et al. Co-expression of MET and CD47 is a novel prognosticator for survival of luminal breast cancer patients. *Oncotarget*. 2014;5(18):8147–8160. doi:10.18632/oncotarget.2385
66. Wang RQ, Geng J, Sheng WJ, Liu XJ, Jiang M, Zhen YS. The ionophore antibiotic gramicidin A inhibits pancreatic cancer stem cells associated with CD47 down-regulation. *Cancer Cell Int*. 2019;19:145. doi:10.1186/s12935-019-0862-6
67. Tan W, Tang H, Jiang X, et al. Metformin mediates induction of miR-708 to inhibit self-renewal and chemoresistance of breast cancer stem cells through targeting CD47. *J Cell Mol Med*. 2019. doi:10.1111/jcmm.14462
68. He Y, Helfrich W, Bremer E. Development of bispecific antibody derivatives for cancer immunotherapy. *Methods Mol Biol*. 2019;1884:335–347. doi:10.1007/978-1-4939-8885-3_23
69. Feliz-Mosquea YR, Christensen AA, Wilson AS, et al. Combination of anthracyclines and anti-CD47 therapy inhibit invasive breast cancer growth while preventing cardiac toxicity by regulation of autophagy. *Breast Cancer Res Treat*. 2018;172(1):69–82. doi:10.1007/s10549-018-4884-x
70. Zhou F, Feng B, Yu H. et al. Tumor microenvironment-activatable prodrug vesicles for nanoenabled cancer chemoimmunotherapy combining immunogenic cell death induction and CD47 blockade. *Adv Mater*;2019. e1805888. doi:10.1002/adma.201805888
71. Liu B, Guo H, Xu J, et al. Elimination of tumor by CD47/PD-L1 dual-targeting fusion protein that engages innate and adaptive immune responses. *mAbs*. 2018;10(2):315–324. doi:10.1080/19420862.2017.1409319
72. Liu X, Liu L, Ren Z, et al. Dual targeting of innate and adaptive checkpoints on tumor cells limits immune evasion. *Cell Rep*. 2018;24(8):2101–2111. doi:10.1016/j.celrep.2018.07.062
73. Lian S, Xie R, Ye Y, et al. Dual blockage of both PD-L1 and CD47 enhances immunotherapy against circulating tumor cells. *Sci Rep*. 2019;9(1):4532. doi:10.1038/s41598-019-40241-1
74. Davis RM, Campbell JL, Burkitt S, et al. A raman imaging approach using CD47 antibody-labeled SERS nanoparticles for identifying breast cancer and its potential to guide surgical resection. *Nanomaterials*. 2018;8:11. doi:10.3390/nano8110953
75. Chen Q, Wang C, Zhang X, et al. In situ sprayed bioresponsive immunotherapeutic gel for post-surgical cancer treatment. *Nat Nanotechnol*. 2018.
76. Song S, Jin X, Zhang L, et al. PEGylated and CD47-conjugated nanoellipsoidal artificial antigen-presenting cells minimize phagocytosis and augment anti-tumor T-cell responses. *Int J Nanomedicine*. 2019;14:2465–2483. doi:10.2147/IJN.S195828
77. Mo L, Zhao Z, Hu X, et al. Smart nanodrug with nuclear localization sequences in the presence of MMP-2 to overcome biobarriers and drug resistance. *Chemistry*. 2019. doi:10.1002/chem.201805107
78. Kiss B, van Den Berg NS, Ertsey R, et al. CD47-targeted near-infrared photoimmunotherapy for human bladder cancer. *Clin Cancer Res*. 2019. doi:10.1158/1078-0432.CCR-18-3267
79. Trabulo S, Aires A, Aicher A, Heeschen C, Cortajarena AL. Multifunctionalized iron oxide nanoparticles for selective targeting of pancreatic cancer cells. *Biochimica Et Biophysica Acta General Subj*. 2017;1861(6):1597–1605. doi:10.1016/j.bbagen.2017.01.035
80. Rezaei G, Habibi-Anbouhi M, Mahmoudi M, et al. Development of anti-CD47 single-chain variable fragment targeted magnetic nanoparticles for treatment of human bladder cancer. *Nanomedicine*. 2017;12(6):597–613. doi:10.2217/nnm-2016-0302
81. He Z, Liu J, Du L. The unexpected effect of PEGylated gold nanoparticles on the primary function of erythrocytes. *Nanoscale*. 2014;6(15):9017–9024. doi:10.1039/c4nr01857e
82. Rao L, Bu LL, Xu JH, et al. Red blood cell membrane as a biomimetic nanocoating for prolonged circulation time and reduced accelerated blood clearance. *Small*. 2015;11(46):6225–6236. doi:10.1002/smll.201502388
83. Wu J, Li Z, Yang Z, et al. A glutamine-rich carrier efficiently delivers anti-CD47 siRNA driven by a "glutamine trap" to inhibit lung cancer cell growth. *Mol Pharm*. 2018;15(8):3032–3045. doi:10.1021/acs.molpharmaceut.8b00076