Mechanism of exosomes in the tumor microenvironment in the abscopal effect (Review)

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Received September 15, 2022; Accepted November 1, 2022

DOI: 10.3892/ijo.2022.5450

Abstract. Previously, the abscopal effect, which is an antitumor therapeutic effect on untreated tumor locations elsewhere in the body as a result of treatment of the targeted region, was rarely reported, and its mechanism remains unknown. Increasing evidence has shown that the immune system is implicated in the abscopal effect, and that combining immunotherapy and radiation can assist to improve its frequency. Understanding how different types of cells and cell-derived exosomes cause the abscopal effect in the tumor microenvironment (TME) is crucial to increasing the clinical occurrence of the abscopal effect in the TME.

Contents

1. Introduction

Multiple novel radiotherapies have emerged in recent years, including brachytherapy, carbon ion radiotherapy and proton therapy, which can be further subdivided into photothermal therapy and photodynamic therapy (1-3). The abscopal effect describes the shrinkage of unirradiated tumors that occurs concurrently with irradiated tumors in patients with multiple tumors. Since Mole's first proposal of this effect (4), there had only been a few cases reported, until the association with the immune system was demonstrated by previous research that found the abscopal effect was not observed in mice with immunodeficiency (5). With immunotherapy becoming a more effective treatment for tumors (6,7), clinical studies have revealed that a combination of radiotherapy and immunotherapy, such as the immune checkpoint inhibitor anti-cytotoxic T lymphocyte-associated antigen-4 (anti-CTLA-4), anti-programmed death-1 (anti-PD-1) and anti-programmed cell death 1 ligand 1 (anti-PD-L1), produces the abscopal effect in the treatment of lung cancer (8), prostate cancer (9), melanoma (10), breast cancer (11), liver cancer (12), type B3 thymoma (13) and glioblastoma (GBM). As a result, the mechanism of the abscopal effect and how to produce it by combining immunotherapy and radiotherapy has become a hotspot in tumor research, which is also the focus of the present review.

2. Immune mechanism of the abscopal effect

The immunological mechanism of action behind the abscopal effect remains unknown, although various studies have shown that it is dependent on T cells (5,14,15) and macrophages (7). Thus far, it is known that tumor cells are triggered to produce tumor-associated antigens (TAAs) when their DNA is damaged. TAAs are then phagocytosed by antigen-presenting cells (APCs) before being activated by major histocompatibility complex (MHC) molecules on $CD8^+$ T cells. Since $CD8^+$ T cells not only have a direct impact on primary tumors but also reach untreated tumors via the blood and lymph circulation, they attach to tumor cells and destroy them, thus exerting an antitumor effect (16).

Exosomes are secreted by cells for intercellular signal transduction and information exchange. They carry nucleic acid, proteins and lipids to the target cell by acting on its surface or fusing with it (17). Exosomes derived from various cells serve an immunosuppressive or immunoenhancing role (18), and participate in carcinogenesis, proliferation and metastasis (19), thus playing distinct roles in the abscopal effect. Furthermore, there are alterations in exosome secretion following radiation (20,21). The subsequent sections discuss the mechanisms by which distinct cells produce exosomes after radiation to modify the abscopal effect.

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Key words: abscopal effect, exosomes, radiotherapy, immunotherapy

3. Role of tumor cells

Tumor cells become more immunogenic after being irradiated because their DNA is damaged, thus causing the production of TAAs. The endoplasmic reticulum, which contains calreticulin and the disulfide isomerase ERp57, migrates to the plasma membrane and delivers an 'eat-me' signal to APCs, enhancing their phagocytosis and abscopal action. On the other hand, due to the formation of cytoplasmic double stranded DNA (dsDNA) induced by radiation, GMP-AMP synthase (cGAS) and dsDNA initiate the formation of cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) (22). The increased cGAMP level combines with Stimulator of interferon genes (SINGs) to help regulate the activity of downstream immune stimulating genes, and ultimately promote the maturation and migration of dendritic cells and the activity of $CD8^+$ T cells, thus playing an antitumor role (23,24). Certain exosomes produced by tumor cells can enhance the antitumor effect. They are highly rich in proteins, such as CD40L, which activates the CD40 signaling pathway in dendritic cells (DCs), and induces DCs to mature and produce IL-12, thus promoting anti-tumor immunity (25).

Previous research indicated that tumors with neoepitopes, such as epidermal growth factor receptor (EGFR) vIII in GBM, are more vulnerable to the abscopal effect (7), albeit the mechanism is unknown and requires additional exploration. This tumor type also produces exosomes with high EGFRvIII levels (26).

The radiation-induced damage to DNA, on the other hand, can be repaired and therefore blocked by the DNA exonuclease 3 repair exonuclease 1 inside the tumor, and the degree to which the calreticulin is exposed will be lowered by tumor cell autophagy. Furthermore, CD47 on the plasma membrane can inhibit phagocytosis of tumor cells by the immune system and emit a 'do-not-eat-me' signal (27) to offset the impact of 'eat-me' signals (28). Furthermore, the expression of PD-L1 on the surface of tumor cells increases, and the combination of PD-L1 and PD-1 on the surface of $CD8^+$ T cells acts as an immunological brake, weakening the action of effector T cells (29-31), and therefore limiting the abscopal effect (Fig. 1).

There are immunosuppressive exosomes as well as immunoenhancing exosomes. Some of them produce cell membrane proteins, including PD-L1 and MHC. According to a previous study, PD-L1 immunosuppression on tumor cell-derived exosomes is considerably greater than that on the membrane (32). These PD-L1-rich exosomes perform the same function as PD-1 on the tumor surface, and their immunosuppressive impact is amplified when they express both PD-L1 and MHC molecules (33). Furthermore, certain cancer types produce exosomes that increase PD-1 expression on $CD8^+$ T cells (34) and increase PD-L1 expression on the surface of macrophages (35), shielding tumor cells from $CD8^+$ T cells. Exosomes rich in TGF-β and IL-10 may increase tumor migration and invasion (36), and tumor-derived exosomes can cause tumor immune evasion via the T-cell immunoglobulin and mucin domain 1 (TIM-1) signaling pathway (37). Furthermore, tumor-derived exosomes are high in Fas-L (38), which acts on the surface of T cells to trigger their death, thus markedly reducing antitumor immunity. Meanwhile, these exosomes' surfaces may be rich in integrin β 3 (ITGB3), which can activate focal adhesion kinase and influence intracellular signaling cascades, promoting tumor spread (39). The distinction is that tumor cells from the original location secrete exosomes rich in integrin β-like 1 (ITGBL1) to act on the distant site, thus activating the EVs-ITGBL1-CAFs-TNFAIP3-NF-κB signaling axis (40) to modify the tumor microenvironment (TME) and accelerate tumor metastasis, severely reducing the abscopal effect. A previous study has found that GBM-induced exosomes contain CD274, DNA and other chemicals that influence the transcriptional activator 3 STAT3 signaling pathway and promote macrophage polarization to M2 cells (41). Finally, exosomes from lymphoma cells include the inhibitor of apoptosis protein survivin, which inhibits natural killer (NK) cell surveillance and cytotoxicity (42).

Aside from proteins, nucleic acids in exosomes play an important role in the abscopal action. DNA, for example, can stimulate the STING signaling system, boost antitumor immunity and promote the abscopal effect (43). Furthermore, colorectal cancer-induced long non-coding RNA (lncRNA)-rich exosomes operate on the TME to stimulate the proliferation and differentiation of T helper (Th)17 cells and enhance the antitumor action (44). Meanwhile, microRNAs (miRNAs or miRs) in exosomes can influence IL-10 activation, resulting in an immunosuppressive TME that inhibits the development of the abscopal effect. Among these miRNAs, miR-212-3p has been shown to diminish MHC-2 expression and induce immunological tolerance in DCs, allowing tumors to escape immune surveillance (45). It has been reported that miR-934 (46), miR-301a-3p (47), miR-21-3p, miR-125b-5p and miR-181d-5p (48) can cause macrophages to polarize toward M2 cells, hence promoting tumor spread. These tumor-derived exosomes can act not only on the local TME, but also on the distant tumor site via the circulatory system.

There are numerous strategies for reducing the release of tumor-induced exosomes, which limit the abscopal effect. The first is to target and block exosomes from being released via routes such as endosomal sorting complex required for transport (ESCRT), tumor susceptibility gene 101 protein and other ESCRT proteins or ESCRT auxiliary molecules (49). The second is to influence the exosome acceptance pathway. The third option is to disrupt the functional pathway, such as by utilizing anti-PD-L1 or anti-PD-1 antibodies to prevent exosomes from acting on cells. In addition, radiotherapy can reduce the secretion of exosomes that promote tumor proliferation and metastasis by 25.8% (50). Furthermore, brachytherapy can boost the release of particular exosomes rich in high mobility group to induce macrophage polarization to M1 cells (51), hence considerably enhancing the abscopal effect. A combination of radiotherapy and immunotherapy can restrict the production and activity of immunosuppressive exosomes, and it remains to be explored which combined option should be utilized to do so while also increasing the release of immunoenhancing exosomes.

Upon radiotherapy, tumor cells will release damage-associated molecular patterns (DAMPs) such as ATP, high mobility group box 1 protein (HMGB1), nucleic acid, prostaglandin E2 (PGE2), sphingosine 1-phosphate, IL-6 and granulocyte macrophage colony stimulating factor (GM-CSF) (29,52,53) to exert their different biochemical effects.

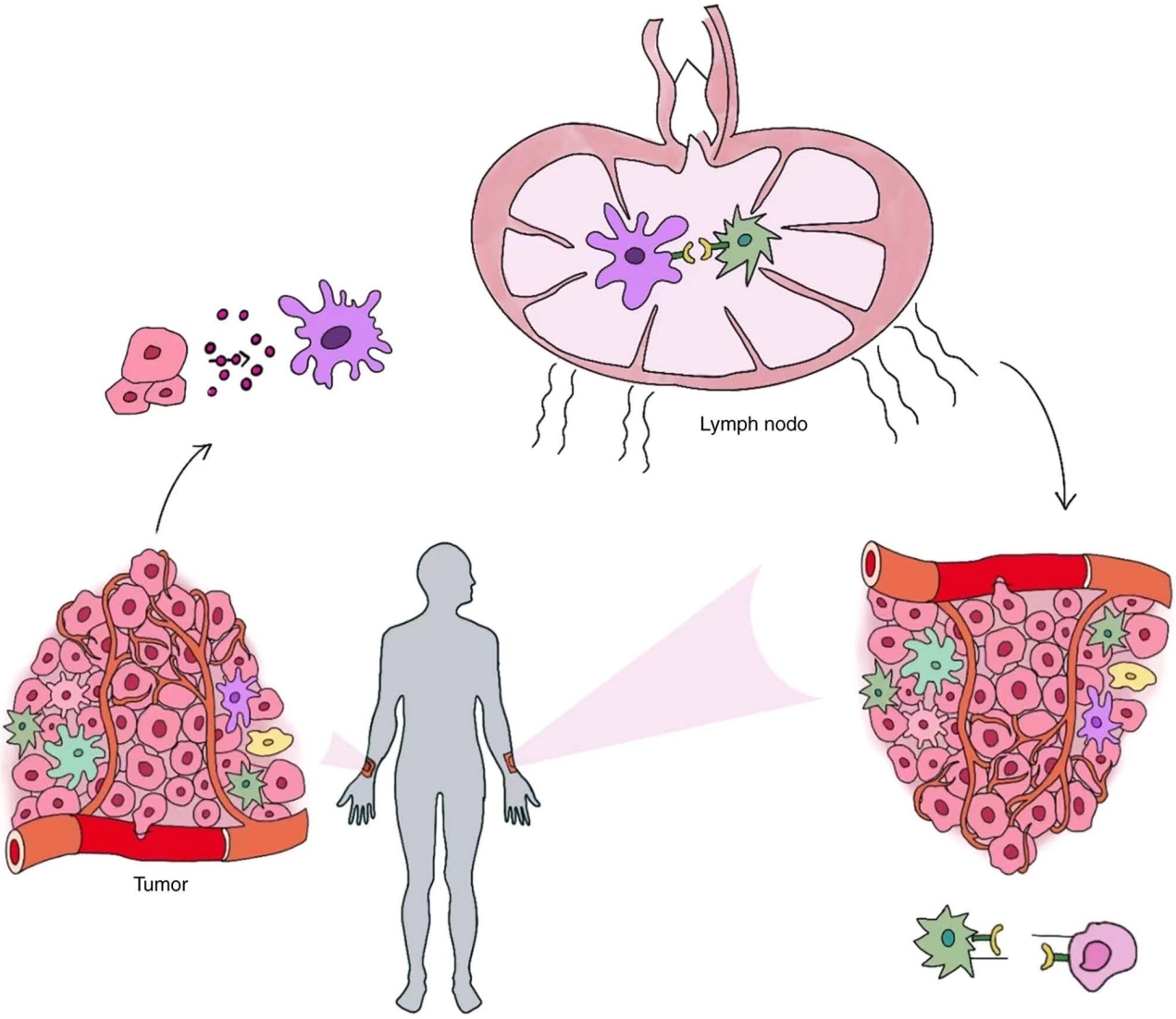

Figure 1. Mechanism of the abscopal effect. For patients with multiple tumors, when one of the tumors at a particular site is irradiated, this tumor releases the TAA to activate APCs. APCs deliver TAAs in the lymph nodes through the MHC1 molecular mechanism and activate CD8+ T cells. Then $CD8^+$ T cells reach the distant and unirradiated tumor site through the circulatory system, and kill the target cells through the Fas or perforin pathway. TAA, tumor-associated antigen; APCs, antigen-presenting cells.

4. Roles of tumor-associated molecular patterns

HMGB1 has a two-fold effect. On one hand, HMGB1 binds to receptor for advanced glycation end product (RAGE), a self-receptor, to promote tumor development and immunological tolerance while also inhibiting the abscopal effect. When its oxidation sites are blocked, however, HMGB1 increases immunity and enhances the abscopal effect (54,55).

ATP's role. The production of ATP at the start of radiation can stimulate the activation of DCs and effector T cells, boosting the abscopal effect (12,56-58). However, a previous study found that high ATP release could cause PD-1 overexpression in tumor cells as well as regulatory T cell aggregation. It could also cause the decrease of the entire immune process via APCs that promote immunological tolerance (59), thus substantially reducing the abscopal effect. Meanwhile, radiotherapy-induced dying tumor cells predominantly release ATP, which is converted to adenosine monophosphate (AMP) and adenosine by ecto-5'-nucleotidase expressed on macrophages, thus activating the A2a adenosine receptor on macrophages and suppressing macrophage-mediated antitumor immunity (60). In addition, ATP and adenosine diphosphate (ADP) are cleaved into AMP by CD39, and then CD73 converts AMP to adenosine, thereby inhibiting the stimulation of $CD8^+$ T cells, activating regulatory T cells (Tregs) and promoting the differentiation of M2 macrophages to inhibit antitumor immune responses (61). CD39 and CD73 together play an important role in transforming an ATP-mediated proinflammatory TME into an adenosine-mediated immunosuppressive microenvironment (62). Furthermore, ATP and its metabolites ADP and AMP all have immunosuppressive roles in inhibiting the abscopal effect; thus, sedatives of several target adenosines are being studied in the clinical practice to restore the abscopal effect.

TGF-β. TGF-β affects the generation and activity of a range of immune cells. It regulates acquired immunity by directly

stimulating Treg cell proliferation while suppressing the production and function of $CD8^+$ T cells and antigen-presenting DCs. Similarly, TGF-β regulates the innate immune system by decreasing NK cells and controlling the complex activities of macrophages and neutrophils, as well as counteracting the anti-CTLA-4 and anti-PD-1 effects of immunosuppressive medications. As a result, research recently being conducted aimed to diminish TGF-β's impact on immunological drugs and prevent the reduction in acquired immunity by limiting its function, thus improving the abscopal effect (63,64).

To summarize, tumor cells perform a dual role in the abscopal effect. In general, tumors tend to block the abscopal effect for self-protection as the disease advances. As a result, efforts should be made to increase tumor cell immunogenicity and immunostimulation while decreasing their immunosuppressive effect.

Effect of APCs. DCs are the most effective APCs. Their Toll-like receptor (TLR) receives radiotherapy-induced TAAs via a TLR-signaling network (65), thus allowing DCs to identify, phagocytose and process TAAs. After swallowing TAAs, APCs can produce antigen peptides and costimulatory signals that activate T cells and amplify the abscopal effect. This boosting effect is smaller than that obtained directly from APCs (66), but it is sufficient to promote the abscopal effect. Thus, the tumor is driven to create 'danger signals', which activate DCs and promote the T cell response via MHC molecules. As a result, MHC-1 and MHC-2-rich exosomes generated by mature DCs have been used in clinical immunotherapy (67). Furthermore, exosomes generated by DCs during irradiation can strongly activate NK cells to destroy tumor cells (68). In comparison, despite their large number, immunological DC-induced exosomes had a significantly lesser effect than mature DC-induced microvesicles, which was analogous to their role in the abscopal effect (69). Activated DCs significantly enhanced the $CD8^+$ T/Treg ratio in both primary (irradiated) and secondary (unirradiated) tumors, thus amplifying the abscopal effect (70). Furthermore, the insensitivity of DCs to irradiation, in comparison to that of tumor cells, ensures their survival and activity under high-dose irradiation, thus facilitating that their relatively high antigen-presenting effects are retained in the TME when high-dose irradiation is required to ensure therapeutic outcomes (71).

Tumor cell-induced DCs, on the other hand, produce TGF-β, IL-27 and other molecules that inhibit their own activity, activate Tregs and limit antitumor immunity function, thus decreasing the immune response and the abscopal effect (72,73). Immature DCs severely impede the function of effector T cells and suppress the immune response, resulting in the body's failure to generate the abscopal effect (74). One of the most essential features of solid tumors is a hypoxic TME, which substantially inhibits APC activity and reduces the antitumor impact. To reinforce the abscopal effect (75), it is worthwhile examining methods to improve DCs' antitumor immunological action while decreasing their immunosuppressive role.

Role of macrophages. Tumor-associated macrophages (TAMs) phagocytose tumor cells by recognizing their TAAs. TAMs can cause the abscopal effect in two ways: i) By attaching to T cells to achieve the antitumor immunological response or ii) By self-activation to reach the distal tumor location and kill tumor cells. Previous research has demonstrated that anti-PD-L1 can directly activate macrophages to boost the abscopal effect (7), and that it is a promising technique to promote the combination of activated macrophages with targeted radiation to enhance tumor cell damage and, thus, the abscopal effect (76).

TAMs are categorized into several subtypes, the most common of which are M1 and M2. M1 cells primarily contribute to antitumor immunity by directly phagocytosing tumor cells and secreting cytokines such as TNF to macrophages of the M1 phenotype. M1 macrophages can improve antitumor immunity by promoting the activation of effector T cells and the maturation of DCs by producing cytokines such as TNF-β, IL-6 and IL-23 (77). Meanwhile, M1 macrophages produce exosomes that interfere with the NF-κB signaling pathway while activating the caspase 3 signaling pathway, resulting in M1 macrophage polarization (78). M2 macrophages, on the other hand, predominantly suppress the immune system through the production of angiogenesis factors, growth factors and proteases, thus hastening the development of malignant tumors. M2 macrophages release exosomal miR-590-3p, which passes through the target LATS1 and activates YAP/β-catenin to decrease the immunological response (79). They also release integrin αMβ2-rich exosomes, which activate the MMP-9 signaling pathway in receptor tumor cells, as well as apolipoprotein E-rich exosomes, which promote tumor spread and proliferation (80,81). When M2 macrophages produce miRNA-21, it inhibits cell death, increases PI3K/AKT signaling pathway activation by downregulating PTEN (82), and boosts drug resistance in gastric cancer cells as well as tumor proliferation and metastasis, thus decreasing the abscopal effect.

Furthermore, M1 and M2 cells can convert into each other type, resulting in a shift in macrophage activity between antitumor and pro-tumor effects, which has a marked impact on the development of the abscopal effect.

Hypoxia (83) and suppression of NF-κB activity within the TME (84) can both cause M2 polarization in macrophages, resulting in an increase in the M2/M1 cell ratio. M2 macrophages produce a considerable quantity of TGF-β, which suppresses APC activity, and promotes tumorigenesis and development (85). Furthermore, the production of IL-4 by $CD4^+$ T cells promotes M2 macrophage polarization (86), a process triggered by IL-10 and IL-11 (87). Meanwhile, inflammatory cytokines produced by cytotoxic T lymphocytes (CTLs), such as IFN, drive M1 macrophage polarization (88).

The polarization of M1 and M2 cells is mutually hostile and complex, and it is strongly associated with radiation. According to a previous study, when a cumulative quantity of 10 Gy is obtained based on a daily dosage of radiation of 2 Gy/fraction/day, the number of M1 cells increases while the number of M2 cells declines (89). When local low-dose irradiation is administered, M2 cells polarize back to M1 cells, promoting CTL penetration into the TME (90), improving the therapeutic effect. On the other hand, another study found that M2 cells were less sensitive to radiation than M1 cells, so that the ratio of M2/M1 cells increased when the cumulative radiation dosage reached a particular level (91). These seemingly contradicting outcomes are strongly linked to the radiation

dose and method. In any case, M2 infiltration and polarization during radiation has a significant impact on patients' prognosis, and diminish their survival rate (92). Therefore, it is worth investigating how to activate M1 cells while suppressing M2 cell activation, as well as how to re-convert M2 cells into M1 cells to minimize the M2/M1 cell ratio.

TAMs steadily increase the expression of PD-1 while exerting antitumor immunological actions. Since PD-1 expression is inversely correlated with macrophage phagocytic and antigen-presenting capabilities (93), tumor escape can be inhibited by reducing PD-1 expression, and anti-PD-1 treatment can boost macrophage antitumor efficacy. TAMs, on the other hand, overexpress indoleamine 2,3-dioxygenase (IDO) (94), which inhibits CTL activity. Furthermore, CD40 on the surface of APCs will be highly expressed, weakening their function; thus, currently, studies are using multi-functional radiotherapy-associated biological materials to inhibit the expression of CD40 by delivering an anti-CD40 antibody to a tumor *in situ*, which allows the antitumor effects to be maintained (95).

TAMs also cause exosomes containing miR-29a-3p and miR-21-5p to inhibit STAT3, resulting in significant Treg proliferation (96), whereas exosomes containing miR-155 can control the TME, prompting macrophages to polarize into M1 cells (97,98). When TAMs produce lysyl oxidase like 4-rich exosomes, they promote the production of their own PD-L1, resulting in an immunosuppressive phenotype that inhibits the activity of $CD8^+$ T cells (99), significantly decreasing antitumor immunity and the development of the abscopal effect. Furthermore, macrophages can produce GM-CSF-rich exosomes to enhance tumor angiogenesis (100), supply oxygen and nutrients to tumors, and hence provide possibilities for tumor migration and invasion.

Despite the several conditions that can restrict APCs' function, it has been observed that when antigens are available, boosting APCs function alone can induce a sufficiently significant abscopal effect. As a result, targeted activation of APCs contributes to the intensification of the abscopal effect.

Role of T cells. There are various T cell types, and all play crucial roles in the generation of the abscopal effect, with some of them enhancing each other, while others antagonize each other.

$CD8^+$ T cells. $CD8^+$ T cells have a potent antitumor function (101). The release of exosomes by $CD8^+$ T cells is important, as they ingest exosomes originating from tumor cells and immune cells (102) to regulate their own functions and antitumor actions, which enhances the abscopal effect. CTLs recognize tumor cells via their TCR on the cell surface and eliminate them via two traditional mechanisms, namely i) The Fas-based molecular and ii) The perforin-granzyme-based processes (103). In the first mechanism, Fas ligands (FasL) exist on the T cell surface, and the surface of these exosomes is also rich in FasL, which acts on the Fas receptor on the surface of tumor, thus mediating tumor cell apoptosis (104,105). FasL bind to their receptors on tumor cells, activate the caspase protein, and promote the apoptosis of tumor cells through the Fas/FasL pathway. The second mechanism involves the release of perforin through CTLs. In terms of the first mechanism, both $CD8^+$ T and NK cells release exosomes rich in CD56 and perforin (106). Perforin mediates the lysis and destruction of receptor tumor cells, thereby 'punching a hole' in the membrane of the tumor cell, allowing the granzyme to enter the tumor cells, and eventually leading to the rupture and death of tumor cells. Therefore, the degree of infiltration of $CD8^+$ T cells is positively correlated with their antitumor effects.

The killing effect of CTLs can be activated when they are targeted by the auto-specific antigen of certain tumors, such as melanoma-associated antigen 1 (107) in melanoma and EGFRvIII (7) in GBM; therefore, these tumors are more susceptible to the abscopal effect. Furthermore, $CD8^+$ T cells stimulate DCs by releasing exosomes rich in cytoplasmic DNA, and DCs are activated via the cGAS/STING signaling pathway (108). By creating immunological synapses and suppressing apoptosis, DCs stimulate $CD8^+$ T cells, forming a powerful immune impact that can boost the abscopal effect.

During the antitumor process, $CD8^+$ T cells in the TME are gradually fatigued, and the depleted $CD8^+$ T cells produce a high number of lncRNA-rich exosomes. These exosomes impact the production of INF-γ and IL-2 in normal $CD8^+$ T cells (109), and the ratio of $CD8^+$ T/Tregs decreases, thus blocking the abscopal effect. Furthermore, CTLA-4 expression in $CD8^+$ T cells would gradually increase, thus reducing the lethal impact of CTL on malignancies. Furthermore, PD-1 expression on CTLs increases during the antitumor process, thus blocking TCR signals by inactivating CD28 (110). CTLs unite with tumor cells that are highly expressing PD-L1 to further block their tumor-killing impact. Notably, IFN-γ secreted by effector T cells, which reduces the abscopal effect, increases PD-L1 expression on tumor cells. Aside from CTLA-4 and PD-1, it has recently been shown that Lag-3, Tim-3 and TIGIT likewise suppress CTL function while conducting their own (111).

Role of Tregs. Tregs have a high immunosuppressive impact (112), and their primary role is to induce immunological tolerance re-establishment. PD-1 expression on Tregs can enhance Treg self-proliferation and $CD8^+$ T cell death (113), as well as strongly block CTL activity, and this immunosuppressive impact is regulated by IL-2. The level of IL-2 in the body is positively correlated with the immunosuppressive ability of Tregs; that is, when the number of IL-2 declines, the number and activity of Tregs decrease (114), and when the number of IL-2 increases, the number and activity of Tregs increase (114). As a result of their dependence on IL-2, Tregs' function can be impeded. Moreover, Tregs counteract the tumor immunity in a variety of ways (115), including the release of immunosuppressive factors such as IL-10 and TGF-β. The tumor sends a 'recruitment' signal to Tregs, such as C-X-C motif chemokine ligand 12, C-C motif chemokine ligand (CCL)17, CCL22 and CCL28, to increase the number of Tregs (116), resulting in a further decrease in the $CD8^+$ T/Tregs ratio in the TME, thus inhibiting the abscopal effect and enhancing tumor immune tolerance.

The presence of CD73 on the surface of Treg-derived exosomes is required for Tregs to mediate immunosuppressive effects, and its mechanism is similar to that of Th17 cells, which facilitates the conversion of ATP to ADP and AMP (117,118). When the TCR on the surface of Tregs is activated, it increases exosome secretion, inhibits the tumor-killing activity of CTLs and inhibits the proliferation of effector T cells (119). The

activated TCR promotes the release of IL-4 and IL-10, which is linked to miR-150-rich exosomes generated by Tregs (120). Treg-secreted miR-146a-5p-rich exosomes can play a role via suppressing STAT1 and interleukin 1 receptor associated kinase 2 (121).

Tregs serve an important function in preventing DC maturation. They produce exosomes and deliver them to DCs for intercellular communication with miRNAs in DCs (122), which significantly reduces antitumor immunity. Let-7d miRNA is encased in exosomes and preferentially acts on Th1 cells, reducing their proliferation and immunity by inhibiting cyclooxygenase 2 (123). Certain Treg-derived exosomes have a 25-100-fold higher IL-35 concentration in the cell surface, and IL-35 acts on target cells to promote the expression of PD-1, Tim3 and Lag3 (124), suppressing antitumor immunity. In summary, Tregs can suppress antitumor immunity and the abscopal effect by secreting various exosomes.

After radiotherapy, CXCR4 antagonist can promote the depletion of Tregs and enhance the antitumor and anti-metastasis therapeutic effect (125,126). Depletion also encourages T cells to mature into effector memory T cells (127), as well as inhibiting Treg proliferation, resulting in a higher $CD8^+$ T cell/Treg ratio. Tregs are less vulnerable to radiation than CTLs, and their increased activity can be maintained even at larger doses (128); therefore, the radiation dose can be utilized to modify the $CD8^+$ T cell/Treg ratio. According to a previous study, hypo-fractionated stereotactic radiation therapy can increase the number of $CD8^+$ T cells while decreasing the number of Tregs (129).

Dual effect of Th17 cells. Th17 cells, a novel assistant cell distinct from Th1 and Th2 cells (130), have a dual effect on tumors, resulting in their 'double-edged sword' function in the occurrence and progression of the abscopal effect. Th17 infiltration was also observed in tumors that showed the abscopal effect, such as prostate, lung and breast cancer, and melanoma (131,132). Th17 cells, on the one hand, can release CCL20, recruit DCs to the tumor, activate $CD8^+$ T cells and release IFN-γ to increase the antitumor response (133,134), thus contributing to the abscopal effect. Th17 cells, on the other hand, have an immunosuppressive effect after entering tumors. They produce IL-17, attract myeloid-derived suppressor cells (MDSCs), and promote their own proliferation and development (135,136). The concentration of VEGF is positively correlated with the concentration of IL-17 (137), and IL-17 can increase tumor angiogenesis, thus enhancing tumor proliferation and metastasis. Meanwhile, the anti-abscopal extracellular nucleotidase CD39 and CD73 on the surface of Th17 cells decompose ATP into AMP to achieve an immunosuppressive effect (117).

The role of Th17 cells in malignancies, on the other hand, is complex, and their processes are unknown. Meanwhile, their effects differ depending on the tumor type. Thus, further research is required. To summarize, different types of T cells play diverse roles in the abscopal effect. Only by further studying and specifying the role and mechanism of distinct T cells, as well as the connections between T cells and other cells, the human body's antitumor immunity will be better understood and maintained.

Role of MDSCs. MDSCs, which are immature myeloid cells produced and secreted by the bone marrow, are recruited to the TME to control the immune response and build an immunological-tolerant TME. Meanwhile, additional MDSCs will be created by the bone marrow, which is triggered by chronic inflammatory signals sent out by the TME (138), thus creating a feedback loop that interferes with tumor therapy. According to a previous study, tumor cells in a model lacking MDSCs were quickly removed by activated antitumor immune cells (139), indicating the critical role of MDSCs in the immunosuppressive process. Exosomes secreted by MDSCs play an important role in suppressing the abscopal effect and promoting tumor immune escape, proliferation and migration. Thanks to their contents (>4,000 types of protein), MDSCs-derived exosomes and MDSCs support each other, strengthen the immunosuppressive effect, and promote tumor proliferation and survival (140).

It has been demonstrated that, even during radiation, MDSCs suppress the abscopal effect, since they arrive at the tumor site 10 days after radiotherapy (139), and decrease antitumor immunity by releasing cytokines such as TGF-β and strongly expressing PD-L1 (141). Proliferation of MDSCs can be aided in a variety of ways. VEGF has been shown to play a significant role in promoting the proliferation of MDSCs (142), and an increase in MDSCs and a decrease in effector T cells have been observed in tumor models with high VEGF expression (143), implying that VEGF inhibits the abscopal effect by promoting MDSCs.

IL-6 is linked to aggressive tumor development and recruitment of MDSCs (144), and this process reduces IL-6-silencing small hairpin RNA, upregulates miR-155 and miR-21, and activates STAT3, thus initiating and enhancing proliferation of MDSCs (145). Furthermore, miR-9, miR-494 and miR-21 in these exosomes govern the cell cycle, boost MDSCs proliferation and expansion, increase MDSCs accumulation in the TME, and enhance MDSCs immunosuppressive capacity, all of which promote tumor progression (146,147). The involvement of nucleic acid in MDSCs exosomes is clearer, since MDSCs-derived exosomes contain a high concentration of nucleic acid. Their miR-146a content, which is 18-fold higher than that of cells, acts on TNF receptor-associated factor 6 and other NF-κB pathway-related receptors, thereby regulating the genesis and apoptosis of cervical cancer cells (148,149).

Moreover, the proliferation of MDSCs is promoted by PGE2, which activates the p38MAPK/ERK signaling pathway to enhance the release of TGF-β (150,151). HMGB1 is a high-content protein in MDSCs-derived exosomes, which can induce the production and accumulation of MDSCs. Other MDSCs-derived exosomes rich in TGF-β1, IL-10 and IL-6 may be ingested by macrophages and T cells, causing a significant increase in Tregs to play a stronger immunosuppressive effect, a reduction in the proliferation of Th cells, weakened CTL cytotoxic activity and a slight increase in the lymphocyte apoptosis rate. Specifically, the content of TGF-β1 within these exosomes is 4.3-fold higher than that within the cell, thus promoting tumor angiogenesis and metastasis (148,152,153). Moreover, it has been shown that MDSC-derived exosomes are associated with the resistance of chemotherapeutic drugs (152).

In summary, MDSCs can inhibit the generation of the abscopal effect via a variety of mechanisms. Furthermore, the greater the accumulation of MDSCs in the TME, the stronger its immunosuppressive ability, the more conducive to tumor

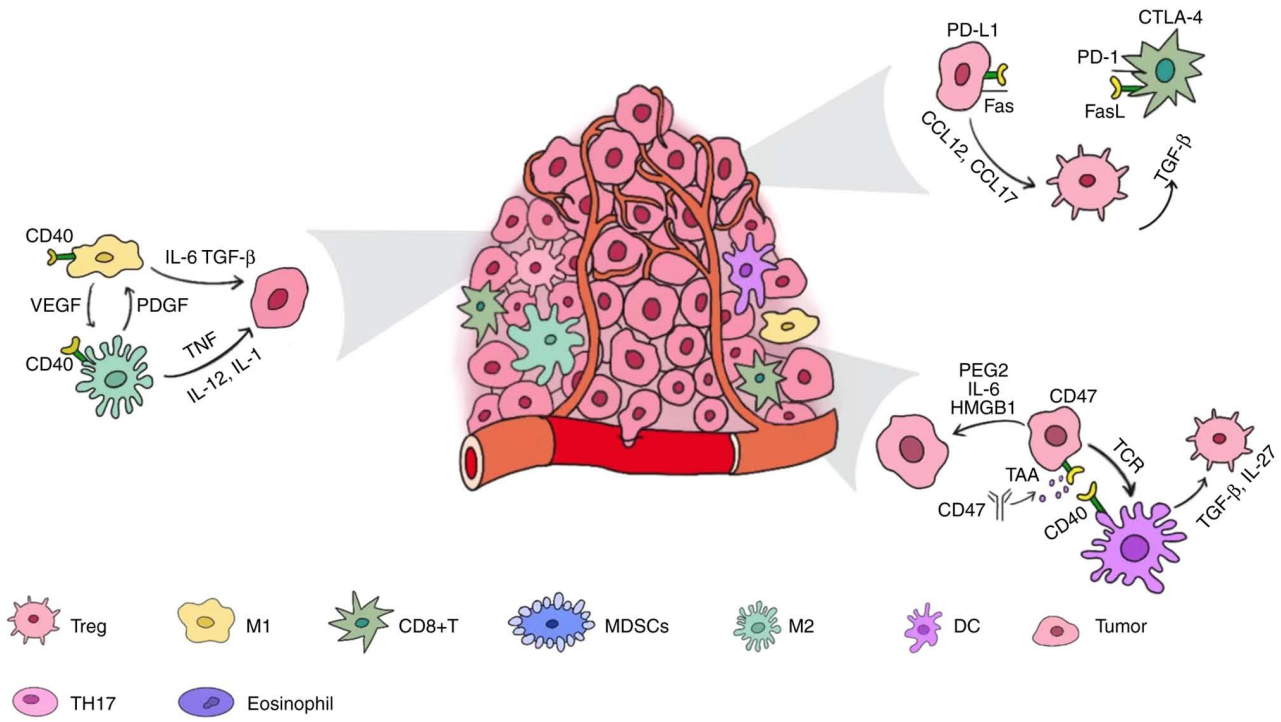

Figure 2. Role of some cells in TME in the abscopal effect. Tumor cells act on tumor cells through direct interaction between cells or the release of various substances. DCs, $CD8^+$ T, and regulatory T cells regulate the intensity of the abscopal effect. CD47, PD1 and other inhibitory factors can be inhibited by corresponding antibodies to activate anti-tumor immunity. DCs can also act on T cells and activate regulatory T cells by releasing TGF-β and IL-27. Anti-CD40 can prevent the decline of the anti-tumor immunity of DC cells. M1 type macrophages and M2 type macrophages can convert to each other and express CD40 in the same way as DCs. M1 and M2 type macrophages play a diametrically opposite role in tumors. TME, tumor microenvironment; DCs, dendritic cells; PD-1, programmed cell death-1.

growth, ultimately having a negative effect on the overall treatment of the tumor. As a result, they may be utilized as a prognostic marker in patients, and targeting MDSCs can help restore antitumor immunity and boost the abscopal effect.

Role of eosinophils. Eosinophils have been observed to infiltrate numerous tumors, directly interact with tumor cells, govern tumor formation by modifying the TME and contribute to antitumor immunity via a number of pathways. Eosinophils have high levels of IL-1 receptor 1, TLR4 and RAGE expression. They also recruit to the tumor site following radiotherapy-induced DAMPs such as HMGB1 and IL-33 signals (154).

Eosinophils have a two-pronged effect on malignancies. On one hand, they cause $CD8^+$ T cells to penetrate tumors, normalize blood vessels and drive macrophage polarization to M1 cells, resulting in a marked antitumor action (155). Although the underlying mechanism is unknown, eosinophils can significantly boost the abscopal effect. On the other hand, eosinophils play an inhibitory function in immunity (156). When triggered by thymic stromal lymphopoietin, they restrict DC maturation and block tumor apoptosis by secreting a substantial quantity of IL-10, IL-4, IL-5 and IL-13 (157), thus increasing tumor growth. TGF-β (158) is also released by eosinophils to impact the abscopal effect. Furthermore, they produce IDO (159) to enhance TME's immunosuppressive function, boost M2 polarization of macrophages in the TME by releasing IL-13 and IL-4 (160), and decrease the antitumor effect. As a result, inhibiting the tumor-promoting function of eosinophils, so that they improve the antitumor action of macrophages to promote the abscopal effect, is worth investigating.

There are vigorous battles between antitumor cells, such as DCs and NK, $CD8^+$ T, M1 cells and CTLs, and immunosuppressive cells, such as M2 cells, MDSCs and Tregs, during the generation of the abscopal effect after radiotherapy (Fig. 2). Such battles are regulated by cells with dual functions, including Th17 cells and eosinophils (Fig. 3), resulting in complex and mutual influences on these antitumor and immunosuppressive cells: $CD8^+$ T cells and Tregs interact to produce adenosine (72); $CD8^+$ T cells bind to eosinophils; the TME enhances the immunosuppressive effect of MDSCs; and MDSCs and Tregs mutually promote the expansion of each other.

Immunosuppressive cells gradually win the fight to suppress the abscopal effect later in the process. Despite the fact that the current immunotherapy has overcome the disadvantages of antitumor cells, the lack of knowledge of the underlying mechanism, combined with the complexity of the aforementioned battles, hampers the formation of the abscopal effect over time (Fig. 4).

5. Clinical application

Radiotherapy combined with immunotherapy. Radiotherapy is an important cancer treatment that can boost the abscopal effect. Radiotherapy was previously only utilized to treat local malignancies due to technological limitations and its harmful effects on normal tissues. In addition to its cell-killing impact,

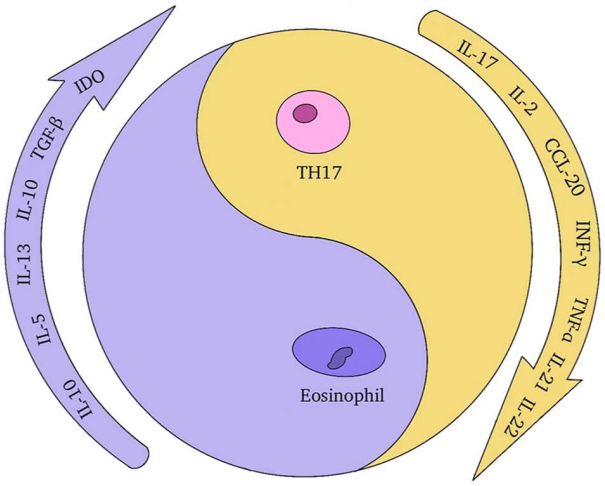

Figure 3. Dual role of eosinophils and Th17 cells in the abscopal effect. The role of eosinophils and Th17 cells in the abscopal effect is similar to the 'Yin' and 'Yang' in Tai Chi, and they play a diametrically opposite yet complementary role. Both eosinophils and Th17 have a dual role, and they can not only promote tumorigenesis but also play an anti-tumor effect. Specifically, eosinophils secrete IL-10, IL-5, IL-13, TGF-β, and IDO, while Th17 releases IL-17, IL-2, CCL20, IFN-y, TNF-a, IL-2, and IL-22. IDO, indoleamine 2,3-dioxygenase.

radiation modulates immunomodulation. Radiotherapy, for example, renders tumor cells more vulnerable to T cell attack. After each 10-25 Gy low-fraction radiation session, the expression of MHC-1 molecules on the surface of human melanoma cells was increased (161), which enhances the presentation of antigens, making it easier for these tumor cells to be destroyed and removed by T lymphocytes. Furthermore, various immune cells respond differently to radiation; for example, a radiation dosage of 0.94 Gy strongly inhibits Treg proliferation (162). Due to the lack of studies on different tumor radiotherapy doses and the susceptibility of different cells to radiotherapy, it is difficult to utilize radiotherapy alone to overcome the inhibitory impact of the TME, which is why the abscopal effect was uncommon in the past.

However, immunotherapy compensates for this rarity. Multiple clinical and pre-clinical studies (Table I) have shown that, compared with the effect of radiotherapy or immunotherapy alone, the combination of radiotherapy and immunotherapy can significantly increase the incidence and intensity of the abscopal effect (7,13,163-166). Commonly used immune checkpoint inhibitors are anti-CTLA-4, anti-PD-1 and anti-PD-L1 antibodies, all of which strengthen the effect of T cells on tumor cells and can be combined with radiotherapy to enhance the incidence rate of the abscopal effect. A recent study showed that the combination of 8 Gy x3F radiotherapy and anti-PD-L1 monoclonal antibody can enhance the abscopal effect, significantly reduce MDSCs and promote $CD8^+$ T cell infiltration (167). Another clinical study indicated that radiotherapy combined with immune adjuvant GM-CSF treatment can trigger the abscopal effect in 30% of patients with cancer (168). Similarly, anti-CD40 antibody could maintain the antitumor effect of APCs (95), and FMS-like tyrosine kinase 3 ligand could recruit and stimulate APCs (169), so both of them can enhance the effect of APCs, and increase the incidence of the abscopal effect when combined with radiotherapy. In addition, when combined with tumor cells, both anti-CD47 and anti-CD73 antibodies can promote APCs to phagocytose tumor cells (170-172), and further exert their antitumor role.

However, immunotherapy also has defects such as cross-reactions. Specifically, while anti-CD47 eliminates tumor cells, it may accidentally injure the red blood cells that also carry CD47 on their surface (173), leading to anemia. Besides, the overlap and systemic toxicity of a combination of radiotherapy and immunotherapy are still difficult to deal with, although a reasonable combination can overcome immunosuppression and promote the generation of the abscopal effect (16). Moreover, immune-related adverse reactions are worrying. Even if radiotherapy can overcome the drug resistance of anti-PD-1 antibody, it does not have a long-lasting therapeutic effect on ~80% of patients with non-small cell lung cancer (NSCLC), due to tumor oxidative metabolism obstacles (174). The dosage and sequence of the combination of radiotherapy and immunotherapy for different tumors, or different combination options from different tumor radiotherapy and immunotherapy methods are currently unclear; therefore, it is necessary to further explore immunotherapy options and timings for different tumor types to find the best timing, dosage and sequence of the combination of radiotherapy and immunotherapy. Emerging methods and technologies may help to understand how to generate the abscopal effect and promote its incidence rate, which is beneficial to the treatment and prognosis of patients. These benefits are subsequently described in the present review.

New radiotherapy technologies are conducive to the abscopal effect. The development of radiotherapy technology has made a great progress. Compared with traditional radiotherapy methods, new technologies may be more conducive to the generation of the abscopal effect. Stereotactic body radiation therapy combined with immunotherapy is well tolerated and relatively safe, and there were cases of lung cancer as well as head and neck squamous cell carcinoma where the abscopal effect was generated by such a combination (163,166). In addition, technologies such as intensity-modulated radiation therapy, stereotactic ablative radiotherapy or proton therapy can change the range of radiotherapy according to the tumor size and greatly reduce radiation toxicity (3), thus overcoming the toxicity caused by combined therapy. Besides, high-dose radiation (HDR) brachytherapy can protect adjacent healthy tissues by bringing the emission source into the tumor tissue, thereby reducing radiotherapy-induced toxicity (175). Research has shown that a combination of HDR brachytherapy with anti-PD-1 or anti-CD137 antibodies can produce the abscopal effect (176). When these new radiotherapy approaches are combined with different immunotherapy methods, the best combination option and timing may be found, which may overcome the limitations caused by the toxicity of radiotherapy in the past and help to improve the current type of combination of radiotherapy and immunotherapy.

Triple therapy including radiotherapy and immunotherapy. As aforementioned, the combination of radiotherapy and immunotherapy can achieve in an improved way the antitumor effect and reduce the drug resistance to immunotherapy; however, such combination also has a limited effect on the generation of an abscopal effect sufficiently strong in

Table I. Compared with radiotherapy or immunotherapy alone, the combination of radiotherapy and immunotherapy can significantly increase the incidence and intensity of the abscopal effect.

Tumor type	Radiation dose	Types of immunotherapy	Combined treatment sequence
Melanoma	30x10	CTLA4	Immunotherapy before radiotherapy
Colon adenocarcinoma	3x8 Gy	Anti-PD1/anti-CD137	Immunotherapy after radiotherapy
Glioblastoma	10 Gy	Anti-PD-L1	Immunotherapy after radiotherapy
NSCLC	9 Gy x 3/6 Gy x 5	Anti-CTLA-4	Immunotherapy after radiotherapy
Adenocarcinoma	30 Gy (10 fractions)	Atezolizumab	Immunotherapy after radiotherapy
Head and neck squamous cell carcinoma	4,500 cGy	Atezolizumab	Immunotherapy before radiotherapy
NSCLC	3 fractions of 12 Gy	Anti-PD-L1	Immunotherapy before radiotherapy
NSCLC	8 Gy x 3/12 Gy x 3	Anti-PD-1	Immunotherapy before radiotherapy

NSCLC, non-small cell lung cancer; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; PD-1, programmed cell death-1; PD-L1, programmed cell death 1 ligand 1.

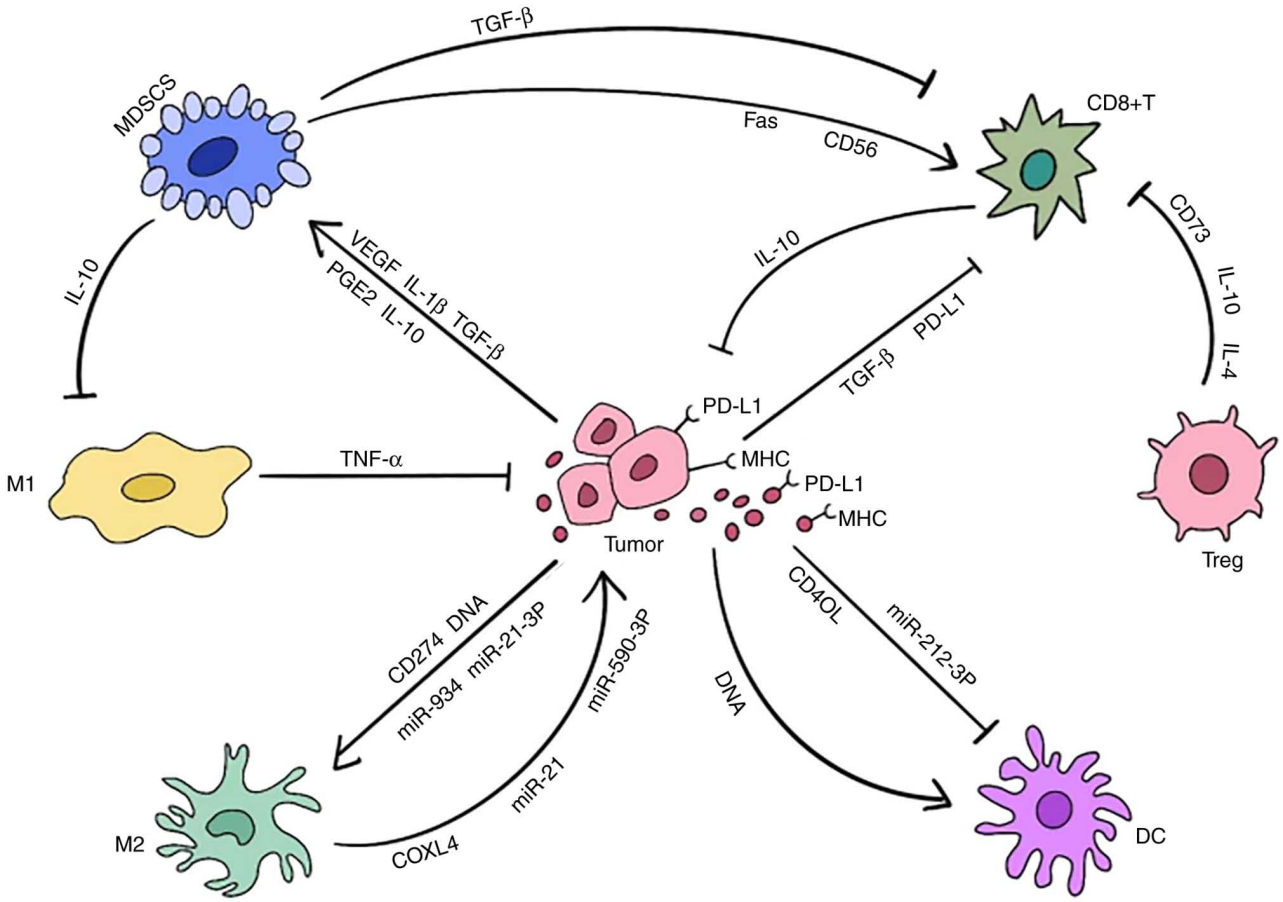

Figure 4. Various cells in the TME form an interactive network through the release of exosomes to affect anti-tumor immunity. TME, tumor microenvironment.

certain tumors such as NSCLC. The underlying mechanism of such limitation may be the regulation of the ERK signaling pathway to act on Src homology region 2-containing protein tyrosine phosphatase 2 (SHP2), which regulates tumor cell proliferation (177) and is the main effector mediating the downstream signal transduction of PD-1 in T cells (178). Previous research has shown that the triple therapy of SHP2 inhibitor, anti-PD-L1 antibody and radiotherapy can increase the ratio of M1/M2 cells and CTL/Treg lymphocytes to stimulate antitumor immunity (174). In addition, the oxidative phosphorylation (OXPHOS) of tumor mitochondria may be another cause of the aforementioned poor effect of the combination of radiotherapy and immunotherapy (179). The triple therapy of IACS-010759, an OXPHOS inhibitor, combined with anti-PD-1 antibody and radiotherapy can promote the abscopal effect (180) and resolve the problem of anti-PD-1 resistance in NSCLC. Other triple or quadruple therapies could be used to overcome the disadvantages of the

traditional combination of radiotherapy and immunotherapy. For example, genetic ablation of the TGF-β signaling pathway components added to the conventional radioimmunotherapy could trigger a powerful antitumor response (63), as well as a combination of anti-PD-1 treatment after radiotherapy (181) and targeted suppression of antitumor immunity. Similarly, exosomes within the TME can also enhance the therapeutic effect, and ultimately promote the abscopal effect to prolong the survival time of the patient.

Oncolytic virus. In oncolytic immunotherapy, an oncolytic virus is often injected locally into tumors, which has a tropism for malignant tumor cells and can replicate in tumor cells to eventually promote their lysis (182). Oncolytic virus replication can induce the death of tumor immunogenic cells, send out immunological danger signals, promote tumors to produce TNF-α, and induce the body to produce strong immune effects (183), thereby enhancing the occurrence of the abscopal effect. The shrinkage of distal tumors after the local injection of an oncolytic virus has been reported, and the mechanism is similar to that of the abscopal effect (184). When oncolytic viruses are used in combination with immune checkpoint inhibitors and radiotherapy their effects can be enhanced. Specifically, the oncolytic adenovirus is currently one of the most promising oncolytic viruses (185). Recently, a patient with Hodgkin's lymphoma infected with the new coronavirus experienced systemic tumor regression. The reason may be that the coronavirus triggered antitumor immunity in his body (186); therefore, this novel coronavirus may also have the potential to be developed as an oncolytic virus to promote the abscopal effect.

Smart material technology. Smart material technology is divided into nanoparticles and intelligent radiotherapy biomaterials (187). Within nanoparticles, nanoparticle-delivered drugs have great potential for improving the antitumor immune effect. Nano-immunotherapy, which is the combination of nanoparticle-delivered drugs and immunotherapy, can be achieved in three different ways, and these nano-drugs are used to target cancer cells and the TME (58). When targeting cancer cells, nanoparticle-delivered drugs cause the immunogenic death of tumor cells and can be combined with immunotherapy to greatly promote antitumor immunity (188). Moreover, when combined with photodynamic radiotherapy to treat primary tumors, nanodrugs can promote the occurrence of abscopal effects (189). Calcium carbonate nanoparticles with anti-CD47 activity have been developed (190). When targeting the TME, nanodrugs such as antigen capture nanoparticles can capture TAAs to activate DCs, and thus promote the abscopal effect (191). Certain nanodrugs can also act on immunosuppressive molecules, such as IDO, TGF-β and IL-2 (192) to reshape the TME, which is beneficial for antitumor immunity.

The second type of smart material technology, smart radiotherapy biomaterials, also promotes the abscopal effect. For example, a hydrogel formed by alginate can capture the drug formed by the combination of ^{131}I-labeled catalase and the immune adjuvant CpG, and the immune checkpoint inhibitor of the combination of the hydrogel and the drug can produce powerful antitumor immunity and the abscopal effect, which has been observed in experimental mice (193). Compared with traditional technologies, these new technologies have improved treatment methods to reduce toxicity towards normal tissues and/or lymphocytes, have improved targeting ability, are beneficial to patients, and can reduce the cost of treatment for patients.

6. Conclusions

Tumor metastasis has caused the suffering and mortality of >90% of patients with cancer. The abscopal effect can be used to combat tumor metastasis. The biggest advantage of this abscopal effect is the inhibition and elimination of distant and metastatic tumors. Therefore, by further studying the underlying mechanism and improved using of new technologies and methods to enhance the abscopal effect, an improved treatment plan for patients with cancer could be developed. Furthermore, new radiotherapy and immunotherapy approaches based on cells and exosomes that play a role in the abscopal effect are beneficial to increase the incidence of abscopal effects in clinical practice. Besides, its needs to be taken into consideration how to reduce the toxicity caused by treatment, relieve the suffering of patients, and reduce the cost of treatment. It is worth noting that microwave ablation has been found to induce the abscopal effect in clinical practice (194), and the effect of oncolytic viruses is similar to that of the abscopal effect. Therefore, investigating the mechanism of oncolytic viruses may help to find another way to promote the abscopal effect.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Students' Innovation and Entrepreneurship Training Program (grant nos. S202210632275, S202210632129 and 202110632052).

Availability of data and materials

Not applicable.

Authors' contributions

JW and GK wrote the manuscript. ZW and CL created the figures. JW and JL revised the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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