Tumor-associated macrophages: Shifting bad prognosis to improved efficacy in cancer therapies?

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Abstract

Macrophages are innate immune cells that play an important role in the response to damaged tissue and pathogenic infection. During activation, signals from the local environment induce macrophage polarization towards either the classical pro-inflammatory phenotype (M1) or towards the alternative anti-inflammatory phenotype (M2). In cancer, M2 tumor-associated macrophages (TAMs) are associated with a poor prognosis. Notably, the Tumor Microenvironment (TME) is known to promote the M2 phenotype by dampening anti-tumor immune responses and thus promoting tumoral growth. Recent studies have demonstrated that TAMs play a major role in cancer cells resistance to chemo- and radiotherapies leading to ineffective treatment strategies. This raises the importance of including macrophage targeting strategies, either to dampen their activities or to re-educate them toward pro-inflammatory phenotype, to improve the efficiency of current and future treatments. Therefore, this mini-review aims to highlight recent discoveries demonstrating how macrophages induce cancer resistance to therapies and how re-educated TAMs could be used to improve treatment outcomes.

Introduction

Macrophages are hematopoietic cells derived from myeloid precursors in bone marrow, and play a major role in resolving pathogenic infections by recognizing Pathogen-Associated Molecular Patterns (PAMPs) via Pattern Recognition Receptors (PRRs) [1,2]. Once activated, naïve macrophages (Mo) are polarized into two main subsets; M1 or M2 [3–5].

Abbreviations

5-FU: 5-Fluorouracile; C/EBP:CCAAT-Enhancer Binding Proteins; CAR: Chimeric Antigen Receptor; CCL-C-C: Chemokine Ligand; CD: Cluster of Differentiation; CSF1: Colony Stimulating Factor 1; CXCR2-C-X-C: Chemokine Receptor Type 2; DAMPs: Damage-Associated Molecular Patterns; ERα: Estrogen Receptor Alpha; ICB: Immune Checkpoint Blockade; IFNγ: Interferon-Gamma; IL: Interleukin; IRF: Interferon Regulatory Factor; LPS: Lipopolysaccharide; M0: Macrophage-naïve macrophage; M1: Macrophage–classically activated macrophage; M2: Macrophage–alternatively activated macrophage; MAPK: Mitogen-Activated Protein Kinases; MHC–II: Major Histocompatibility Complex Type 2; miRNA: microRNA; mRNAs: messenger RNAs; PAMPs: Pathogen-Associated Molecular Patterns; PD-L1: Programmed Death Ligand 1; PI3Kγ: Phosphoinositide 3-kinase Gamma; PRRs: Pattern Recognition Receptors; PTEN: Phosphatase and Tensin Homolog; ROS: Reactive Oxygen Species; SIRP: Signal Regulatory Protein Alpha; SOCS3: Suppressor of Cytokine Signaling 3 Gene; STAT3: Signal Transducer and activator of transcription 3; TAMs–Tumor Associated Macrophages; TGF–β: Tumor Growth Factor Beta; Th: T Helper Cell; TLR: Toll-Like Receptor; TME: Tumor Microenvironment; TNF–α: Tumor Necrosis Factor Alpha; VEGFs: Vascular Epithelial Growth Factors; VISTA: V-Domain Ig Suppressor of T cell Activation

M1 macrophage polarization (often referred to as classically activated macrophages) is induced by Th1 CD4+ cells producing cytokines such as Interferon-Gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), or by toll-like receptor (TLR) activation [6–9]. For example, lipopolysaccharide (LPS) stimulation induces M1 polarization through the activation of TLR4 [9]. Once polarized towards the M1 subset, macrophages acquire a pro-inflammatory phenotype often associated with a high production of interleukin-1 (IL-1) and TNF-α [10]. Moreover, these cells exhibit an increased expression of major histocompatibility complex type II (MHC-II) and the co-stimulatory molecules CD80/86 which are associated with an enhanced ability for presenting antigens [11]. Conversely, M2 macrophage polarization (often referred to as alternatively activated macrophages) occurs under Th2 cytokines via IL-4, IL-10, IL-13 or tumor growth factor-beta (TGF-β)-stimulation of polarized macrophages (often referred to as alternatively enhanced ability for presenting antigens [11]. Conversely, M2 macrophage polarization (often referred to as alternatively activated macrophages) occurs under Th2 cytokines via IL-4, IL-10, IL-13 or tumor growth factor-beta (TGF-β) cytokines [12]. M2 macrophages exhibit an anti-inflammatory phenotype and are associated with a lower expression of MHC-II and CD80/86 but a higher expression of CD206 (a mannose receptor). This subset produces anti-inflammatory cytokines such as IL-10 and TGF-β known to dampen the immune cell response [12]. The M2 macrophage subset is mainly implicated in tissue repair processes (e.g., by boosting angiogenesis via the production of Vascular Epithelial Growth Factors (VEGFs)) and immune tolerance thus promoting a decline in local inflammation [13]. Regarding their phagocytic capacity, both M1 and M2 are more efficient than M0 macrophages [8], but M1 exhibits higher phagocytosis activity compared to M2 [8,14]. This duality and complex balance of M1 and M2 macrophage activation is crucial in several pathological conditions, including cancer.

In this mini-review, we provide an update of recent advances regarding the role of macrophages in cancer progression and the acquisition of resistance to therapeutic strategies focusing mainly on chemotherapeutic strategies. Here, we will briefly discuss the role of tumor-associated macrophages (TAMs), the main mechanisms for their polarization within tumors, and how this polarization impacts tumoral growth or regression.

Macrophages represent the most important leukocyte population to infiltrate the tumor tissue. Once in the tumor, macrophages have a dual role depending on their polarization. In general, it is accepted that the pro-inflammatory M1-polarized phenotype promotes an anti-tumor immune response whilst the anti-inflammatory properties of M2-polarized macrophages are associated with pro-tumor functions by dampening immune system responses and promoting metastasis in solid tumors [17–19]. M2 macrophages induce tumor cell proliferation and angiogenesis by producing growth factors that drive metastatic dissemination and tumoral growth [17]. Moreover, M2 macrophages produce TGF-β and IL-10 cytokines known to dampen immune cells activation, consequently inhibiting anti-tumoral immune responses [20]. When recruited to the Tumor Microenvironment (TME), the mature macrophages are converted into tumor associated macrophages referred to as TAM. The TME is known to predominantly polarize TAMs towards the M2 phenotype with a small fraction of M1 [21,22]. In addition to enhancing the polarization towards pro-tumoral M2 macrophages, cancer cells also develop mechanisms to escape the immune system. As an example, cancer cells can overexpress the marker CD47 on their cell surface, and the interaction between CD47 and the receptor Signal-Regulatory Protein Alpha (SIRPα) dampens phagocytosis activities, leading to cancer cell ignorance by macrophages [23].

Macrophages, cancer cells, and their surrounding stroma interact in a reciprocal manner. Cancer cells have been described to release vesicles and exosomes containing proteins and nucleic acids, such as microRNAs (miRNA) able to impact macrophage polarization [24,25]. Several studies have demonstrated the role of miRNAs, released by cancer cells, in inducing macrophe polarization [26–28]. For example, ovarian epithelial cancer cells express and release the miR-222–3p which, once transferred to macrophages, induces M2 polarization by inhibiting the Suppressor of Cytokine Signaling 3 gene (SOCS3) [29]. A decrease of SOCS3 expression in macrophages leads to a sustained activation of signal transducer and activator of transcription 3 (STAT3) which drives macrophage polarization toward the M2 phenotype [30]. Likewise, in colorectal cancer cells, exosomes-derived miR-1246 promotes the expression of markers such as CD206 and CD163 thus inducing M2 polarization [31].

TAMs with an M1 pro-inflammatory phenotype tend to correlate with a favorable prognosis and longer survival for patients, whilst an increased accumulation of TAMs with an M2 anti-inflammatory phenotype in tumor tissue is now commonly associated with worse patient outcomes for several tumor types, including; glioma, head and neck, lung, pancreatic, breast, ovarian, colorectal, liver, melanoma, and bladder cancer [32–42]. It has thus become increasingly apparent that the role of TAMs in current treatment modalities, such as chemotherapeutic and radiotherapeutic strategies, must be considered to have therapeutic implications and could be deemed a potential target for treatment strategies.

Involvement of TAMs in cancer therapy resistance

The M2 phenotype TAM association with bad prognoses is not only restricted to their ability to dampen the anti-tumor immune response and promote cancer cell proliferation and metastasis, but also to induce resistance to therapies by decreasing the efficacy of current treatment strategies. Here, we describe several recent studies describing how TAMs have hinders therapeutic strategies in different types of cancers by providing resistance to chemotherapeutic and radiotherapeutic strategies.

Role of TAMs in chemotherapeutic and hormonotherapy resistance

Acquired resistance of cancer cells has been related to the ability of TAMs to secrete different factors such as cytokines, nucleotides or miRNAs. Recent studies have demonstrated...
that macrophages produce and release miRNAs that modulate the TME and, consequently, cancer cell sensitivity towards treatment. For example, a study by Zhu et al. showcased that TAMs produced exosomes enriched with miR-223 which could be transferred to ovarian epithelial cancer cells [43]. By transferring miR-223, macrophages are able to downregulate the phosphatase and tensin homolog (PTEN) protein expression in ovarian cancer cells, thus promoting PI3K/Akt signaling pathway known to play a role in cancer cell survival and provide cisplatin resistance [43].

TAMs in colorectal tumors have been demonstrated to exhibit low levels of cellular miR-155, which is known to decrease Janus kinase (JAK)2/STAT3 phosphorylation, leading to increased IL-6 production by macrophages [44]. Macrophage-derived IL-6 activates the IL-6 receptor (IL-6R)/STAT3 pathway in cancer cells which downregulates miR-204 expression [45,46]. MiR-204 is known to dampen Bcl-2 and RAB22A gene expression leading to a decrease in the proliferation rate of cancer cells [47,48]. Therefore, by downregulating miR-204 in cancer cells, IL-6 produced by macrophages promotes cancer cell proliferation and provides resistance to 5-fluorouracile (5-FU) therapy [44,46]. IL-6 production by TAMs induces STAT3 activation in ovarian cancers and decreased miR-204 levels leading to cisplatin resistance [49]. Moreover, macrophages-derived IL-6 also promotes the activation of the Hedgehog pathway in breast cancer cells dampening chemotherapy efficiency [50].

TAMs, polarized to the M2 phenotype, are known to produce TGF-β and IL-10 [12]. Recent studies have demonstrated that the production of IL-10 by TAMs provides resistance to paclitaxel and carboplatin in breast cancer [51]. A study by Wei, et al. revealed that, in colorectal cancer, TAMs express a high level of C-C Chemokine Ligand 22 (CCL22) which activates the PI3K/Akt pathway in cancer cells and consequently decreases the pro-apoptotic effect of 5-FU [52]. Similarly, the secretion of CCL2 by TAM has also been linked to PI3K/Akt activation in tamoxifen resistant breast cancer cells [53]. Moreover, TAMs have been reported to contribute to tamoxifen and paclitaxel resistance in breast cancer either by interfering with the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway or chemotherapy-induced DNA damages. TAMs induce tamoxifen resistance by activating the NF-κB pathway leading to a hyperphosphorylation of Estrogen Receptor Alpha (ERα) independently from the presence of the ligand which promotes proliferation, migration and invasiveness of ER+ breast cancer cell lines [54]. Olson, et al. have demonstrated that TAMs are able to dampen the effects of paclitaxel on breast cancer cells by decreasing DNA damage and caspase activation thus dampening apoptosis induction [55]. Although the exact mechanism is not well understood, the resistance is hypothesized to be cell-contact independent and instead seems to be related to the secretome of TAMs which affects multiple signaling pathways. Another recent study demonstrated TAMs secreting nucleosides as the induction of pancreatic cancer cell resistance to gemcitabine [56]. Gemcitabine is a deoxycytidine analog which, upon incorporated into the DNA during replication, leads to cell death [57]. In this work, the authors observed that deoxycytidine release from TAMs can lead to gemcitabine resistance of pancreatic cancer cells [56].

Role of TAMs in radiotherapy resistance

Radiotherapy has been shown to induce Immunogenic Cell Death (ICD) wherein the release of tumor antigens, at the radiation site, induces immune responses. This leads to the accumulation of myeloid cells, the release of inflammatory cytokines (e.g. IL-1), monocyte/macrophage recruitment factors (e.g. IL-34, and colony-stimulating factor 1, CSF1), and pro-fibrotic mediators (e.g. TGF-β) [58]. ICD induction has been shown to contribute to anti-tumor immunity and has been seen as a promising exploitable process for cancer treatments. Unfortunately, downstream components of the immune system, such as TAMs, have been shown to either promote or suppress ICD.

Fractionated radiation therapy is considered to be immunosuppressive, stimulating the innate immune system towards a tissue repair response which promotes tumor recurrence and progression [59–62]. Macrophages have historically been noted to be relatively radioresistant, and are considered to be activated and recruited to play central roles as both the tumor-resident population of phagocytes and the central cells directing wound healing and tissue repair in tumors following radiation [63–65]. The macrophages which survive the radiation, and the recruited macrophages after, display a pro-tumoral M2 macrophage phenotype with enhanced pro-survival and pro-angiogenic activities often leading to tumor recurrence and treatment failure [66].

Interestingly, this immunomodulatory effect has been detected in distant tumors outside the field of the radiation treatment and is referred to as the abscopal effect, and highlights the importance of considering the immunological effects of ionizing radiation [67]. Notably, irradiation alone does not directly affect the production of effector molecules or cytokines in M1 or M2-activated macrophages but rather acts as an enhancer or inhibitor of inflammatory mediators [68,69]. Another important aspect regarding irradiation efficacy is hypoxia which can be compounded by TAMs as they can help modulate tumoral metabolism involved in aerobic glycolysis thus hindering the efficacy of radiotherapy [70].

The cascade of events regarding immunogenic cell death is orchestrated by several factors, of which Reactive Oxygen Species (ROS) have been implicated to play a role in the recruitment of monocytes and macrophage polarization. Depending on the environment, ROS can serve as a secondary messenger molecule that influences Mitogen–Activated Protein Kinases (MAPK) and NF-κB activity leading to the expression of pro-inflammatory genes thus promoting M1 polarization [71,72]. However, ROS have also been implicated in the early stages of M2 polarization and TAM differentiation indicating a complex relationship between ROS and macrophage polarization [73,74]. Another complication involves the dose-dependent relationship between irradiation and macrophage polarization. Local radiotherapy can affect the balance between immunosuppression and immune anti-tumor effects.
depending on the dose fractionation, the total dose, and the cancer type.

Higher dose irradiation (> 10 Gy) causes the release of damage-associated molecular patterns (DAMPs) which induce the expression of pro-inflammatory cytokines, chemokines, and effector molecules activating the ceramide pathway which triggers apoptosis via acid sphingomyelinase [75,76]. However, an increase in the number of M2-like TAMs has been observed for in vitro and model murine prostate, oral, and pancreatic cancer when exposed to > 10 Gy doses of radiotherapy [77–79]. Following the radiation of an in vitro murine oral cancer model, M2 macrophages were polarized from CD11b+ myeloid cells and were associated with tumoral recurrence and accelerated tumoral growth [79]. Lower dose irradiation (< 1 Gy), which serves as a lower toxicity regimen in comparison to higher dose irradiation, delivered during a hypofractionation treatment regime has been demonstrated to induce apoptosis of tumoral cells and stromal cells which are efficiently engulfed by macrophages, leading to the clearance of tumoral antigens and the production of anti-inflammatory mediators including TGF-β and IL-10. Specifically, lower dose irradiation has been demonstrated to reprogram M1 macrophages towards M2 TAM in both human and murine macrophages in vitro [80]. On the other hand, moderate doses of irradiation (between 1 and 10 Gy) shows a mixed M1/M2 phenotype activation. An interesting study by Prakash et al. demonstrated that irradiation of late-stage insulinoma-bearing mice induced an inflammatory response which was characterized by a decrease in M2 macrophage-associated cytokines and the induction of M1 macrophages. The groups’ results were similarly reflected in vitro, where it was noted that the expression of iNOS, NO, NFκB pp65, pSTAT3 and pro-inflammatory cytokines were down-regulated [81]. In a glioblastoma murine model treated with moderate doses of irradiation, Leblond, et al. observed a decrease in M0 macrophages concurrent with an increase in M2 TAMs. The group demonstrated that the radiation did not modify the macrophage phenotype but rather that M1 macrophages were more sensitive to ionizing radiation than M2 macrophages in both normoxic and hypoxic environments [82].

Overall, both in vitro and ex vivo studies have demonstrated that single low-dose radiation treatments are associated with anti-inflammatory M2 macrophage activation via the production of iNOS, NO, and O2, which induced the expression of pro-inflammatory cytokines (e.g. IL-1β, IL-6 and TNF-α), whilst moderate-dose irradiation enhances the pro-inflammatory properties of both M1 and M2 macrophages [62,76,81,83]. Interestingly, although local radiation cannot completely reprogram TAMs, a few studies have demonstrated that a low-to-moderate dose of whole-body irradiation in tumor-bearing mice results in a M1 cytokine expression profile [81,84–86]. This type of response has been hypothesized to be due to the mobilization of fresh reprogrammed macrophages to various lymphoid organs to infiltrate the tumor site. Of course, it should be noted, that whole-body irradiation studies have been applied mainly to mice as this radiation regime is not applicable to human patients [65].

Notably, the full molecular mechanisms of exactly how TAMs promote therapeutic resistance is beyond the scope of this mini-review and the authors highly recommend more in-depth reviews [87–89].

Role of TAMs in immunotherapy resistance

Although this mini-review is mainly focused on TAMs resistance to chemo-, hormone- and radiotherapy, the importance of TAMs in other treatment strategies, such as immunotherapies, is also relevant. Immunotherapy strategies are a highly promising approach to cancer treatment. Several immunotherapies have been developed, including immune modulators agents like cytokines (to enhance the immune response), adoptive cell transfer (which includes the chimeric antigen receptor (CAR) T-cell therapy), and monoclonal antibodies therapies (which enable either the direct targeting of cancer cells or the inhibitory molecules known to dampen immune responses) [90]. In the case of monoclonal antibody therapies, several studies have revealed that macrophages may impact treatment efficacies in certain circumstances. For example, the expression of programmed death ligand 1 (PD-L1) by TAMs, and by cancer cells, has been described to inhibit T cell activation which dampen anti-cancer immune responses and consequently promotes tumor growth [91,92]. Indeed, the interaction between PD-L1 and PD-1, expressed by T cells, induces T cell inactivation and decrease proliferation [93,94]. The immune checkpoint blockade (ICB) strategy aims to inhibit this PD-L1/PD-1 interaction with monoclonal antibody anti-PD-L1 or anti-PD-1 to avoid T cell inhibition [95,96]. Unfortunately, although this strategy seems efficient for several cancer types, recent evidence has indicated that macrophage-derived granulin dampens CD8+ T cell infiltration into metastatic pancreatic tumors and drives resistance against anti-PD-1 therapy. The inhibition of granulin, produced by macrophages, promotes CD8+ T cell infiltration and enhances ICB therapy [97]. Additionally, macrophages also express the V-Domain Ig Suppressor Of T Cell Activation (VISTA) protein [98]. VISTA shares a homology with PD-L1, and can also play a role in the dampening T cell activation [99]. The upregulation of VISTA expression by macrophages, after ICB treatment in prostate and melanoma cancer, has been hypothesized to represent a compensatory pathway implicated in ICB resistance [100].

Thus, collectively, these studies indicate the major impact of TAMs on cancer cells and chemo-hormone-, radio- and immunotherapy resistance.

Macrophages targeting therapeutic strategies to improve current treatment

The TME is known to polarize TAMs toward the M2 phenotype and the phenomenon is now commonly associated with a poor prognosis in various cancers. The participation of TAMs in chemo-, immune- and radiotherapy resistance is considered an important aspect of treatment strategies thus the following section will highlight the potential of targeting TAMs. Specifically, strategies to reduce tumor progression and therapy resistance via the re-education of M2 TAMs towards deeper reviews [87–89].
the M1 phenotype or the reduction of M2 TAMs to skew the M1/M2 population towards a pro-inflammatory ratio will be discussed.

**Specific depletion of pro-tumorigenic TAMs**

Melittin, a major compound of bee venom, has been observed to target pro-tumorigenic M2 TAMs in a Lewis lung carcinoma mouse model, and to reduce the M2 population without affecting pro-inflammatory M1 TAMs [101]. The mechanisms associated with this decrease in M2 TAMs are not currently well known but a decrease of angiogenesis was observed in the tumor stroma of mice injected with melittin. Melittin has also been coupled with other peptides, such as the pro-apoptotic peptide d–(KLAKLAK)₉, to target M2 TAMs and induce cell death by mitochondrial-dependent apoptosis thus leading to decreased angiogenesis, tumor growth rates, and tumor weight [102]. The same melittin–d(KLAKLAK)₉ compound has also been associated with enhanced anti-tumor effects of immunotherapy in breast cancer models [103].

Another study demonstrated the specific depletion of CD163⁺ TAMs using CD163 antibodies conjugated with cytotoxic lipid nanoparticles loaded with doxorubicin. The CD163⁺ TAMs depletion induced an infiltration of activated T cells in tumors thus leading to tumor regression in melanoma mice models [104].

**Re-education of TAMs toward anti-tumorigenic macrophages**

*In vitro* transcribed messenger RNAs (mRNAs) are a promising new approach to re-educating M2 TAMs toward anti-tumorigenic M1 macrophages. These synthetic mRNAs enable researchers to transiently translate the proteins of interest and target specific cells. *In vitro* transcribed mRNA of the Interferon Regulatory Factor (IRF) protein family, specifically IRF5 and IRF5 activating kinase IKKβ which are highly expressed in M1, have been used to successfully reprogram TAMs in pro-inflammatory macrophages. The studies demonstrated a lower tumor size *in vivo* for glioblastoma and melanoma lung metastases in mice [105,106].

Direct injections of IL-21, a type I cytokine mostly produced by T cells and natural killer T cells [107], in tumors also succeeded in reprogramming TAMs in anti-tumorigenic M1 phenotype when combined with immunotherapeutic Anti-Her2/neu treatment in breast cancer cells [108]. M2 repolarization toward M1 can also be achieved by combining siRNA targeting IKKβ, a NF-κB regulating kinase, as its silencing could lead to M1 repolarization and STAT6 inhibition to impair IL-4/α mediated M2 activation pathway. IKKβ siRNA and STAT6 inhibitor molecules were combined in a pH-activated micellar nanodrug targeting M2 peptides, designed to only activate in the acidic environment of the TME and not have negative side effects in healthy tissues with a more neutral pH [109]. The system was able to successfully repolarize M2 to M1 TAMs and suppress tumor growth and metastasis both *in vitro* and *in vivo* in murine models whilst minimizing the inflammatory and toxic effects in the liver and lungs. Interestingly, paclitaxel has recently been discovered to induce M2 to M1 reprogramming through TLR4 activation in mice models of breast and melanoma tumors. Paclitaxel exposure blocked IL-4/STAT6-mediated M2 activation and led to increased NF-κB activation, repolarizing TAMs to M1 phenotype expressing markers such as TNF-α and IL-12 [110].

Di Mitri, et al. have demonstrated that prostate tumors with no expression of tumor suppressor gene PTEN are highly infiltrated with TAMs expressing C–X–C Chemokine Receptor Type 2 (CXCR2), which leads to M2 pro-tumoral phenotype when activated with its ligand CXCL2. They established that these tumors were sensitive to treatment by a CXCR2 antagonist which induced TAMs re-education towards M1 anti-tumoral phenotype and therefore tumor inhibition. The authors also suggested combining treatment with a CXCR2 antagonist with infusions of CXCR2-KO activated monocytes, which showed similar results as CXCR2 treatment in their prostate cancer mice models, to further increase the efficacy on tumor inhibition [111]. M1 to M2 re-education could also be achieved by incubating isolated peritoneal macrophages with various polysaccharides, for example from common buckwheat or guava seeds. Incubated macrophages showed elevated expressions of M1 cytokines such as IL-6 and TNF-α in a dose dependent way. Treatment of MCF7 breast cancer cells with the supernatant of polysaccharides polarized macrophages culture medium induced a decreased MCF7 cell growth [112].

Radiotherapy also exhibited the ability to induce M2 to M1 repolarization. Irradiation of *ex vivo* colorectal cancer tissue samples revealed an increased expression of M1 markers in flow cytometry indicating increased M1/M2 ratio, which was also observed after irradiation of cancer cells in 3D co-cultures with macrophages. This M2 to M1 shift could be at least partly achieved with the help of cancer cells exosomes. Indeed, co-cultures of macrophages and exosomes harvested from irradiated cancer cell lines led to M1 polarization compared to co-cultures of macrophages and exosomes from non-irradiated cancer cell lines [113].

**Other therapeutic strategies with macrophages targeting**

A few other therapeutic strategies have also been developed for macrophage targeting, although interestingly their aim is not to impact M1/M2 ratio unlike the previous strategies, but rather to prevent tumors from escaping the immune system. As an example, the expression of CD47 cell surface markers by cancer cells is a “don’t eat me” signal for macrophages to ignore cancer cells. Immunotherapy using blocking antibodies against CD47 has been shown to reinduce cancer cells phagocytosis by macrophages *in vitro* and total cancer eradication in acute myeloid leukemia patient–derived xenograft mice models. Preclinical studies are currently ongoing with CD47 antibodies, such as magrolimab, in combination with other cancer treatments like azacitidine [114,115].

Macrophages targeted for cancer treatment can enable the recruitment of other cells to the tumor such as T cells. TAMs

secretes anti-inflammatory proteins and cytokines to create an immunosuppressive environment thus avoiding the recruitment of T cells which could participate in tumor clearance. Kaneda, et al. showed that phosphoinositide 3-kinase gamma (PI3Kγ) can act as a switch between immune suppression and immune stimulation, as PI3Kγ expression inhibits NF-κB and activates CCAAT–Enhancer–Binding Proteins (C/EBP), leading to immune suppression and tumor growth. Molecules specifically inhibiting PI3Kγ expression in macrophages will inhibit C/EBPβ and stimulate NF-κB activation, thus allowing an immunostimulatory transcriptional program to restore CD8+ T cell activation and cytotoxic activity, leading to tumor growth inhibition and extended survival in mouse models [116].

Conclusion

Whilst M2 macrophages induce tumor cell proliferation and angiogenesis driving metastatic dissemination and tumoral growth, M1 macrophages promote an anti-tumor immune response. Thus, the accumulation of TAMs with an M2 anti-inflammatory phenotype in tumor tissue is associated with poor patient outcomes for several tumor types. Recent studies have demonstrated that TAMs produce exosomes enriched with miRNAs able to confer resistance to chemotherapeutics, including cisplatin and 5-FU. The NF-κB pathway has also been indicated as a possible mechanism of chemotherapeutic resistance to paclitaxel. Other treatment options, such as local radiotherapy, can affect the balance between immunosuppression and immune anti-tumor effects depending on dose fractionation, total dose, and the cancer type. After a high dose of radiation an increase in the number of M2-like TAMs has been observed for in vitro and model murine prostate, oral, and pancreatic cancer. In lower doses, irradiation has been demonstrated to reprogram M1 macrophages towards M2 TAM in both human and murine macrophages in vitro. Conversely, moderate doses of radiation show mixed M1/M2 activation wherein the destruction in M0 macrophages could explain the increase in the M2 macrophage population. To combat these issues of treatment resistance, research has been orientated towards the reduction and re-education of TAMs. Depletion has been achieved via melittin, pro-apoptotic peptides, and cytotoxic lipid nanoparticles, whilst re-education induction has found success in using miRNAs, siRNAs in pH-sensitive nanodrugs, and polysaccharides derived from buckwheat and guava seeds. Other strategies also include the blocking of CD47 “don’t eat me” signals of cancer cells and using PI3Kγ as an immune stimulatory switch. Overall, recent research demonstrates that M2 TAMs – and the balance between M1/M2 macrophages in tumoral tissue – have a profound effect on cancer response to treatment, and the subsequent rise in resistance, and must be addressed to provide effective anti-cancer therapies.

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References


