Cancer Progression Related with Tumor-associated Macrophages

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Abstract Tumor-associated macrophages are one of the main populations of inflammatory cells in cancers that favor tumor cell growth and survival. Tumor-derived factors such as VEGF-A and CSF-1 recruit the macrophages in tumor micro environment and alter their phenotype in M1 to M2 or tumor-associated macrophages by secretion of several cytokines including IL-4, IL-13 and VEGF-A. In return tumor-associated macrophages released growth factors and cytokine that helps in cancer cell proliferation and metastasis. Tumor-associated macrophage secreted cytokines promotes the angiogenesis and lymphangiogenesis that assist tumor cell to metastasize in distant organs. Importantly, tumor-associated macrophages promote an immunosuppressive environment with the help of other immune cells in the tumor-bearing host that helps tumor to grow unchecked and unchallenged. In addition, tumor-associated macrophages induce resistance against cancer therapy and boost tumor regrowth after therapy. In this review, we discuss the role of tumor-associated macrophages in the pathobiology of cancer. Understanding of the crucial role of tumor-associated macrophages in cancer progression may help to assess potential therapeutic strategies.

Keywords Tumor-associated Macrophages, Cancer Progression, Therapy Resistance, Immune Suppression

1. Introduction

Cancer remains the most common cause of death in several countries, following cardiovascular diseases [1]. Genetic mutations and deregulated signaling pathways contribute to the development of cancer phenotype that leads to uncontrolled cell growth [2-5]. Growing body of evidence suggest that the Tumor Microenvironment (TME) play crucial role in development and progression of cancer [6-8]. The TME in which tumor exist typically contains a wide variety of cells including malignant and non-malignant cell populations [9]. Non-malignant populations include fibroblasts, stromal cells, bone marrow-derived inflammatory cells and immune cells such as T-cells, natural killer cells and macrophages [9,10]. Macrophages are crucial

cellular component of TME, where they are commonly termed Tumor-associated Macrophages (TAMs). Macrophages are the key component of innate immunity and perform multiple functions including tissue growth, tissue repair, homeostasis, and both inhibition or promotion of cell proliferation [11,12]. Macrophages are first line of defense against invading pathogens as these cells phagocytose microbes and present antigens to T-cells. However, TME alters the phenotype of macrophages from classically activated or M1 macrophages that exhibit inflammatory functions to alternatively activated or M2 macrophages or TAM that exhibit anti-inflammatory functions. TAMs originate from circulating monocytes which infiltrated in TME and programmed by tumor-secreted factors such as vascular endothelial growth factor-A (VEGF-A) and colony-stimulating factor-1 (CSF-1) [13]. TME alters the macrophages to TAM phenotype and induce them toward Tumor-supportive M2-polarized macrophages [14-16]. Tumor supporting functions of TAM includes encouragement of tumor growth by secretion of growth factors and induction of angiogenesis and lymphangiogenesis for metastatic spread [13,17].

In this article, we review the role of TAM in providing favorable conditions for tumor progression. In addition, we have discussed how TAM induced immunosuppression and therapy resistance help unchecked growth and survival of tumor cell. We have listed the tumor-derived factors responsible for macrophage polarization and TAM-derived factors that induce immune suppression and therapy resistance.

2. The Role of Tumor Associated Macrophage in Cancer Progression

Recruitment of macrophages and converting them to TAM in the TME is recognized as key features of cancer progression. TAMs have integral role in cancer progression by supporting tumor cell proliferation, survival, angiogenesis, lymphangiogenesis as well as migration and invasion (Fig.1). Furthermore, TAMs guide tumor cells for intravasation of at the primary tumor site and extravasation for distant metastatic [13, 18-20]. Role of TAM in cancer progression is discussed below.

Figure 1. Tumor associated macrophages and cancer progression. Cross-talk between cancer cells and macrophages in TME promotes the polarization of macrophages to TAM or M2 phenotype. In return the TAM secreted factors in TME induce the tumor growth, aggressiveness, immunosuppression and therapy resistance.

2.1. Tumor Growth

Unchecked tumor growth is the result of several factors such as altered signaling, continuous supply of growth factors, immune evasion, therapy resistance, and overall change in TME. The presence of TAM is positively correlated with endometrial cancer, breast cancer, and renal cell cancer proliferation [21,22]. TAMs inhibit the cytotoxicity of T-cells and NK cells in TME by secreting large amounts of IL-10 that helps in tumor growth [23]. TAM-derived adrenomedullin is found to induce angiogenesis and tumor growth in melanomas by interacting endothelial cells eNOS signaling pathway [24]. In addition, TAM-derived adrenomedullin activate macrophage also in autocrine manner and inhibition of adrenomedullin receptors on TAMs reduces the angiogenesis and tumor growth [25,26]. Co-cultured of macrophages with tumor cells shown to facilitate tumor cell proliferation by secreting several growth factors and cytokine [23]. The cytokine IL-23 that induce inflammatory responses is believed promote tumor growth by upregulating MMP9, that are crucial for tumor growth [27,28]. Further, TAMs depletion proven to prevent tumor growth, suggesting its contribution in growth of disease [29]. Activated macrophages are also found to induce chromosomal instability in the bystander cells. Bystander cells are the normal cells that are surrounding the tumor or infected cells. Bystander effects have been studied extensively to both radiation and chemotherapeutic treatment [30-33]. In present scenario, human intestine infection with *Enterococcus faecalis* activates macrophages that secretes increased TNF- α , that acts upon the bystander epithelial cells and induces a pro-proliferation and anti-apoptotic pathways [34]. This result shows the deleterious effects of activated macrophages in the cancer

progression.

2.2. Invasion and Metastasis

Cancer metastasis is one of the hallmarks of cancer, which leads to the formation of new tumors at distant organ is the major cause of cancer-related death [35,36]. Tumor associated macrophages are known to influence the process of metastasis by altering the TME in which tumor exist [22,37]. The intravasation of tumor cell is shown to be associated with TAMs in mammary tumors, suggesting its crucial role in cancer metastasis [38]. Macrophages and tumor cells works in coordination to help each other, for example CSF-1 produced by tumor cells promotes macrophage migration and macrophage-derived epidermal growth factor (EGF) promotes tumor cell invasion. Inhibition of CSF-1 or EGF attenuates the migration of macrophages and tumor cells [20]. TAM, derived from lung cancer tissue found to express high level of Cathepsin K, PDGF-A, VEGF-A, COX-2, MMP-9, HGF and UPA. Interestingly, conditioned medium obtained from TAM significantly promoted cell migration and invasion in SPC-A1, A549, and H460 lung cancer cells. Further, anti-MMP-9 and anti-UPA monoclonal antibodies inhibit TAM-induced invasion in lung cancer cells [39]. Further, TME induced signals significantly contribute in the metastasis of tumor cells. Extracellular matrix components in TME have been suggested to alter the functions of TAM that promote the growth and aggressiveness of the tumor cells. Sulfate proteoglycan such as versican shown to be expressed in tumor cells activate macrophages in Lewis lung carcinoma through TLR-2/TLR-6 signaling axis and that leads to growth and aggressiveness of tumor [40].

Macrophage produced proteolytic enzymes such as serine proteases, cathepsins and MMPs have been implicated in degradation of the ECM and thereby promoting the cancer cells invasion. Moreover, depletion of TAM produced cathepsin B and S found to inhibit the tumor invasion [41], suggesting the role of TAM in tumor invasion.

2.3. Angiogenesis and Lymphangiogenesis

Cancer growth and aggressiveness depend on angiogenesis and lymphangiogenesis triggered by signaling molecules in TME. Continuous supply of nutrients and oxygen by newly formed blood vessels helps in the growth of tumor cells and provide an opportunity to escape into circulation and metastasize to distant organs. TME induced changes in macrophage promotes the secretion of pro-angiogenic factors [17,27,42]. Role of TAM in angiogenesis has been demonstrated in several animal models of prostate cancer, breast cancer, ovarian cancer, melanoma and cervical cancer [27,43,44]. Although, tumor cell-derived factor are capable to initiate tumor angiogenesis, presence of TAMs expedite the process of angiogenesis [18]. Further, depleting TAMs by clodronate encapsulated reduce blood vessel density in the tumor tissue [45]. Further a positive correlation between the recruitment of TAM and angiogenesis was found in several human cancers including gastric cancer, melanoma, breast cancer, pulmonary adenocarcinoma, B-cell non-Hodgkin's lymphoma and glioma [46-53]. Overexpression of CSF-1 enhances the infiltration of TAMs in TME that induce malignant progression in the mammary epithelium of MMTV-PyMT mice [54]. Further, inhibition of CSF-1 or its receptor with the help of short-interfering RNA (siRNA) in mice model decreased the macrophage infiltration [17,55]. TAM-derived VEGF-A is believed to be a significant contributor in tumor angiogenesis and its level correlates with TAM density [51,56]. A subset of TAM that propagate in hypoxic conditions was shown to be associated with VEGF-A and pro-angiogenic gene expression [57]. Further, tumor-released CSF-1 (M-CSF), also induces the VEGF-A expression through NF- κ B activation that triggers the pro-angiogenic functions of macrophages [20,58]. In ovarian cancer mouse model, TAM were shown to be a key source of MMP9 and positively correlated with tumor growth and angiogenesis [27]. In addition, enhanced expression of MMP9 in pancreatic cancer TME facilitated the release of VEGF-A [59]. In addition, re-polarization of the TAM to M1 inhibits the pro-angiogenic function and induces the expression of anti-angiogenic factors such as IFN- β and CXC-chemokine ligand 9 (CXCL9) [60]. Increased expression of oncogene GLI1 is shown to regulate the macrophages induced angiogenesis in oral cancer [61]. While the inhibition of GLI1 shows a promising anti-cancer therapeutic strategy [62,63], the detailed mechanism by which it regulates the macrophages has to be studied in detail.

It has been demonstrated recently that TAM not only

participate in angiogenesis but also in lymphangiogenesis in TME by producing VEGF-A, VEGF-C, VEGF-D and MMP-9 [64,65]. TAM expressing VEGF-C, VEGF-D, and VEGFR-3 found to elevate tumor lymphatic microvessel density (LVD) in cervical cancer [65]. VEGF-C released by TAMs plays a significant role in peritumoral lymphangiogenesis and lymphatic metastases in cervical cancer [65]. Further, VEGF-C expression was positively associated with lymphangiogenesis and angiogenesis in bladder cancer (Miyata Y 2006) [66]. Furthermore, TAM expressed lymphatic endothelial growth factors are known to promote lymphangiogenesis [65,67]. CD11b⁺ macrophages express lymphatic endothelial markers, including LYVE-1 and Prox-1 and shown to contribute in lymphangiogenesis under pathological conditions [68]. Further, TAM produced factors such as VEGF-C has been shown to enhance the intratumoral lymphangiogenesis and regional lymphovascular invasion in breast cancer and mantle cell lymphoma [69,70]. Further, TAM demonstrated to induce VEGF-C expression in Lewis lung carcinoma cells [71]. Interestingly inhibition of VEGFR-3 (receptor of VEGF) suppresses tumor lymphangiogenesis and metastasis of tumor to distant organs [72]. In addition, depletion of VEGFR-3⁺ TAM significantly reduces the secretion of VEGF-C, VEGF-D and LVD in TME [73]. VEGF-A not only induce lymphangiogenesis by recruiting macrophages it promotes proliferation and migration of VEGFR-2 expressing lymphatic endothelial cells in a skin cancer model [74,75]. Further in fibrosarcoma model, VEGF-A induces peritumoral lymphangiogenesis and participates in lymphatic metastasis [76]. TAM regulates lymphangiogenesis, by producing enzymes such as MMPs that control matrix remodeling [77]. MMP-9 and MMP-2 regulates the formation of lymphatic vessels and their downregulation or inhibition reduces lymphangiogenesis, the tube-forming properties and invasive ability of LEC [78,79].

3. Tumor Associated Macrophage Induce Immunosuppression

Coordinated action of both innate and adaptive arms of the immune system is essential to induce phagocytosis or apoptosis in tumor cell. Macrophages possess the unique ability of phagocytosis and present tumor antigens to induce adaptive immunity to orchestrate the antitumor immune response. However, an immunosuppressive factor present in the TME alters the antitumor phenotype of macrophage and induces their pro-tumorigenic characteristics that allow tumor immune evasion. Tumor cells escape from immune system is one of critical events that regulate tumor survival, growth, and metastasis. TAM adopts a poor antigen-presenting capability and produce immunosuppressive cytokine such as IL-10 and TGF- β that suppress the functions of T cells [80]. TAM-derived cytokines and proteases, such as IL-10, TGF- β , and arginase

1, have reported to contribute in immunosuppression (Fig. 1) [81-83]. TGF- β is known to promote tumor-associated macrophage polarization phenotype, which further induce TGF- β secretion and deepens immunosuppression [84]. TGF- β also inhibits the cytolytic activity of natural killer (NK) cells and decreases dendritic cells (DCs) migration that leads to the poor antitumor response [85-88]. Further, macrophage produced TNF- α and IL-10 induce the expression of programmed cell death 1 (PD-L1) that reduce the T cell proliferation induce T cell dysfunction [89]. Moreover, PD-L1 blocking found to enhance the capacity of T cells to eliminate tumor cells. Regulatory T cells (Tregs) are commonly found in various tumors and known to counteract T cell-mediated immune responses [90-93].

Moreover, infiltration of Tregs in tumors is found to be correlated with a lack of responsiveness to therapy [94]. Compelling data demonstrated that Tregs inhibit host T-cell activity against tumor associated antigens and impair efficacy of anticancer immunotherapeutic strategies [95]. Further removal of these cells has been shown to increase the natural anti-tumor T-cell responses [96]. Moreover, depletion of Tregs found to enhance the antitumor immunity in cancer patients [96]. CCL22/CCR4 axis is found to mediate the intratumoral recruitment of Treg [97]. Importantly, TAMs produce the chemokine CCL22 and CCL20, and mediate the trafficking of Tregs in ovarian and colorectal cancer [92,98,99]. Further, TGF- β promotes CD4⁺ T cells differentiation into Th2 cells and reduces the efficacy of efficient antitumor immune response [100]. Moreover, TGF- β also reduces the antitumor activity of CD8⁺ T cells by suppressing the expression of several cytolytic genes, such as IFN- γ , granzyme A, granzyme B, and FAS ligand [85,101]. Furthermore, TGF- β also stimulates tumor growth by the maintenance of Treg cell differentiation [85]. In inflammatory TME, TAMs recruit monocytes and other immunosuppressive cells such as Tregs and Myeloid-derived suppressor cells (MDSCs) by secreting several signaling molecules [99,102]. MDSCs are diverse population of immature granulocytes, monocytes, and dendritic cells and are known for its ability to inhibit cytotoxic T-cell responses. The secreted factors from TAMs recruit each leukocyte population with the MDSC phenotype. TAM-derived chemokine CCL17 and CCL22 attract CCR4 expressing monocytes, immature dendritic cells, and other lymphocytes in TME and subsequently contribute in the immunosuppression [103].

4. The Role of Tumor Associated Macrophage in Therapy Resistance

Growing body of evidence suggest that TAMs modulate the efficacy of several anticancer therapy and facilitate tumor regrowth, and spread after the treatment. Macrophage toxins were shown to reduce the antitumor efficacy of doxorubicin mice bearing immunogenic leukemia or

lymphoma [104]. Further, antitumor activity of the taxane docetaxel was found to be enhanced by depletion of TAMs and subsequently activation or expansion of M1-like macrophages in 4T1-Neu mammary tumor implants. In addition, in vitro experiments demonstrated that docetaxel-treated monocytes are able to enhance tumor-specific, cytotoxic T cell responses [105]. Further, Trabectedin; a DNA-damaging agent was shown to inhibit the growth of mouse fibrosarcomas by depleting TAMs, suggesting that the antitumor activity of some cytotoxic agents may depend on their ability to reprogram or deplete protumoral immune cells [105,106]. In addition, inhibition of CSF1R in mouse models of glioblastoma reduces the tumor volume by re-polarizing them to a state regulated by GM-CSF that has been suggested to be anti-tumoral [107]. Similarly CSF1R inhibitors found to deplete TAMs and enhance chemotherapy responses in cervical and breast cancer models [108]. Likewise, TAM depletion improved the efficacy of paclitaxel (PTX, a taxane) in MMTV-PyMT mouse mammary tumors [19]. PTX was found to increase the recruitment of macrophages to the tumors by upregulating the expression of CSF1 that increased TAM numbers in PTX-treated tumors which limits the infiltration of CD8⁺ cytotoxic T cells and possibly decrease their tumoricidal activity. These findings indicate that TAMs can limit the therapeutic efficacy of PTX in breast cancer, by suppressing the antitumor immune responses [19]. Moreover, released lysosomal enzymes, cathepsins B and S, shown to protect tumor cells from PTX-induced cell death and reduce the efficacy of PTX [109]. In addition, gemcitabine and 5-fluorouracil induce monocytes/MDSCs to release cathepsin B [110]. Further, co-culture of macrophage with cancer cells demonstrated that cathepsins protect cancer cells from cytotoxic effects doxorubicin and etoposide [109]. Interestingly, downregulation of TAM-secreted VEGF or placental growth factor (PIGF) decreased vessel leakiness, normalized the vasculature, and enhanced chemotherapy delivery to tumors [60,111]. The cytotoxic drugs affect multiple cell types in TME as well as TAM that may influence the function and ability of TAMs to respond to a given chemotherapeutic drug [112]. Furthermore, application of several extracellular bacteria including anaerobe *Clostridium* and *Shigellae*, which produce several lethal toxins, were explored for their anti-tumor activities [113-120]. Bacterium *Shigellae* induced apoptosis shown to deplete TAMs that leads to complete tumor regression in breast cancer model [115]. Few studies correlated the greater numbers of TAM in mouse tumors with poor tumor responses to irradiation [121]. Local irradiation of orthotopic human glioblastoma is shown to enhance the infiltration of CD11b⁺ myeloid cells [122]. In addition, CXCL12 was found to attract the F4/80⁺TIE2⁺ macrophages in the lung and mammary tumors following irradiation [123]. Further TAM-induced immunosuppression is shown to be correlated with the activation of several transcription factors such as NF- κ B, STAT3 and STAT6, however the specific mechanism still unexplored [124].

5. Macrophage Polarization from M1 to M2

M1 or Classical and M2 or alternative polarization of macrophages, mimic the Th1–Th2 activation of T cells, denote two opposite functional state of macrophage. Growing body of evidence suggest that M1 phenotype of macrophage is stimulated by pro-inflammatory cytokines such as IFN- γ , TNF, or microbial products such as TLR ligands that promotes elevated antigen presentation, high secretion of IL-23 and IL-12 and higher level of reactive oxygen intermediates (ROI) and nitric oxide (NO) [125]. Whereas, M2 macrophages are known as resting phenotype which promotes healing-type situations without infections which can be further induced by IL-10, IL-4 or IL-13. Several inflammatory mediators, signaling pathways, and transcription factors participates in the regulation of macrophage polarization. Further, immune cells such as, polarized T cells also participate in macrophage polarization [126]. IFNs and TLR activates STAT1 that promotes macrophage polarization toward the M1 phenotype via STAT/IRF signaling, while activation STAT6 by IL-4 and IL-13 promotes macrophage function toward the M2 phenotype [16]. TLR4 stimulated by lipopolysaccharide (LPS) and other microbial ligands; induce macrophages polarization towards M1 by MyD88 and TRIF signaling pathways which subsequently activates nuclear factor kappa B (NF- κ B). NF- κ B pathway is known to regulate the expression several inflammatory genes such as IL-6, IL1B, TNF α and cyclooxygenase 2 (COX2). Further, NF- κ B signaling activates the transcription factor interferon-responsive factor 3 (IRF3), which promotes the expression and secretion of type I interferon, including IFN α and IFN β that subsequently activates the transcription factor STAT1. IRF3 and IRF5 are shown to be involved in regulating M1 polarization and M1-associated gene induction [127,128]. Further, IFN induced chemokine such as CXCL9 and CXCL10 are typical characteristic of M1 macrophage [129]. Macrophage polarization is extremely dynamic process that can be reversed under pathological and physiological conditions [130,131]. Macrophages polarization towards M2 phenotype can be induced by IL-4, IL-13, and IL-10 via activation of STAT6 or STAT3 [132,133]. In various pathophysiological settings, the similar signaling pathway can switch macrophages to M1 or M2 phenotype. Accumulating data suggest that microRNAs (miRNAs), such as miR-155 and miR-146 also regulate macrophages polarization through TLR4/IL-1R signaling pathways [134-138]. Furthermore, let-7c was found to express at a greater level in M2 than M1-type macrophages. In addition, when M2 macrophages were re-polarized to M1-type macrophages, let-7c expression level was decreased, implicating the role of let-7c in macrophage polarization [139].

6. Conclusions

Macrophages are extremely versatile and plastic immune cells that can be altered and perform wide range of functions in TME. Another important pathway to study will be the macrophages associated DNA damages in tumor cells. Macrophages induced production of ROS and NO induces all types of damages including the lethal DNA double strand breaks, which is evident by the increased chromosomal aberrations and γ H2AX [140]. Even though DNA repair proteins like FANCD2, RAD51 [141] try to resolve the damages, it is important to note that the improper repair of macrophages assisted DNA damages will also lead to carcinogenesis and cancer progression. Tumor cell-derived factors hijack the regular functions of macrophages and induce M2 macrophage phenotype that are known as TAM. Macrophage polarization is determined by the inducer that can be cytokine, growth factors or bacterial products. The M2 macrophages are divided into M2a, M2b, M2c and M2d subtypes that functions in tumor promotion, and commonly known as TAMs. Growing body of evidences suggest that TAMs significantly influence the tumor progression, immune suppression and therapy resistance. In the light of this evidence, attempts are being made to target the cytokines and growth factors that polarize M1 macrophages to TAM or M2 in TME. Importantly, repolarization of macrophages from M2 to cytotoxic M1 phenotype hold potential for cancer therapy. The re-education of TAM from M2 to M1 by histidine-rich glycoprotein (HRG), which downregulate macrophage PIGF, promoted normalization of blood vessels, and enhances the efficacy of chemotherapy. Re-programming of macrophages from M2 to M1 can be crucial for effective treatment and to develop novel therapeutics interventions.

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