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REVIEW

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Pro-tumor activities of macrophages in the progression of melanoma

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ABSTRACT

Macrophages are located in essentially all tissues due to their "janitor" function. Macrophages can exert either anti- or pro-tumor activities depending upon the specific tumor microenvironment they inhabit. Substantial evidence indicates that macrophages, owing to their plasticity, can be reeducated to adopt a protumoral phenotype within a tumor microenvironment through the help of growth factors in the microenvironment and intercellular interactions. As the lethality of malignant melanoma is due to its aggressive capacity for metastasis and resistance to therapy, considerable effort has gone toward treatment of metastatic melanoma. In the present review, we focus on the pro-tumor activities of macrophages in melanoma. Based upon the information presented in this review it is anticipated that new therapies will soon be developed that target pro-tumor activities of macrophages for use in the treatment of melanoma.

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Introduction

Malignant melanoma is considered as one of the most lethal cancers due to its aggressive metastasis and resistance to therapy. Worldwide it has been reported to affect 232,000 people, leading to 55,000 deaths in 2012.[1] In western countries, people, especially those with diminished skin pigment, are at increased risk as a result of sunbathing.[1] In mainland China, it is anticipated that melanoma will result in 20,000 new cases annually,[2] of which primary skin melanoma will account for 50–70% and mucous membrane 22.6% of the cases.[3]

Macrophages, originally identified by Elie Metchnikoff,[4] can engulf and digest malignant cells within the body.[5] Initially, macrophages were considered to be the guardians of our body against microbes as well as tumors,[6] including melanoma.[7] Macrophages are classified into 2 different extremes of a continuum ranging from M1 to M2 macrophages in terms of the Th1/Th2 paradigm. M1 is a pro-inflammatory macrophage exhibiting defense reactions against tumors while M2 is an anti-inflammatory macrophage which is beneficial to tumors.[8,9] Recently, evidence has been presented which suggests that macrophages within the context of a tumor microenvironment show a preference to adopt to a protumoral phenotype (M2)in primary and/or metastatic sites *in vivo* with the help of growth factors in the microenvironment and through intercellular interactions.[10] We have described targeting macrophage anti-tumor activity to suppress melanoma progression in another review.[11] In the current review, we focus on discussing the protumor activities of macrophages in melanoma and how such activities can be used for therapeutic purposes in the treatment of melanoma.

Macrophage recruitment to melanoma

Melanomas release molecules that can recruit macrophages to melanoma sites. Alterations in macrophage population patterns are observed during the progression of a malignant melanoma.

Monocyte chemoattractant protein-1 (MCP-1)

MCP-1, acting as a potent macrophage-recruiting molecule,[12] is expressed in human malignant melanoma.[13] A mutant of MCP-1 that lacks the amino acids 2–8 at the N-terminal was reported to be overexpressed when transfected in thigh muscle and secreted into the systemic blood circulation.[13] Such an effect in turn leads to a reduction in MCP-1 expression by melanoma cells.[13] Blocking of MCP-1 function inhibits macrophage recruitment and partially reduces the angiogenesis and growth of malignant melanomas.[13] The capacity of MCP-1to enhance tumor angiogenesis is related with inducing the secretion of TNF-α, IL-1α and vascular endothelial growth factor (VEGF) through macrophage recruitment as well as exerting potential direct autocrine/paracrine effects upon the melanoma cells.[13] In a melanoma xenograft study, the tissue growth was substantially reduced, which is due to no production of MCP-1 of human melanoma cell line IIB-MEL-J.[14] When transfected with an MCP-1-expression vector, MCP-1 was produced and *in vivo* tissue growth increased.[14] The application of MCP-1 inhibitor, as well as macrophage depletion with clodronate-laden liposomes, have been shown to reduce tumor growth and macrophage recruitment, which then induces necrotic tumor

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masses.[14] Anti-tumor effects with restraint stress could reduce macrophage trafficking by suppressing MCP-1 production.[15]

However, MCP-1 may exert a biphasic effect in melanoma, with high levels promoting tumor rejection, whereas low or intermediate levels of MCP-1 support tumor growth.[16]

VEGF-C

Vascular endothelial growth factor C (VEGF-C) is a protein that is a member of the platelet-derived growth factor / vascular endothelial growth factor (PDGF/VEGF) family.[17] A substantial number of human tumors express VEGF-C, including malignant melanomas.[17] One of the most critical steps in tumor progression is completed through the interactions of tumor cells with lymphatic vessels. VEGF-C-overexpressing human melanomas result in enhanced macrophage recruitment as well as melanoma progression.[18] Furthermore, in skin areas surrounding VEGF-C-transfected melanomas, increased levels of peritumoral macrophages have been observed. VEGF-C does not appear to exert any direct effects on tumor cells, as VEGF-C-overexpressing cells do not change the proliferation of control cells, and addition of recombinant VEGF-C to control cells did not affect their growth rate *in vitro*.[18] Like MCP-1, VEGF-C may exert biphasic effects in melanoma. An increase in the recruitment of macrophages, which can enhance host-tumor defense capabilities, may be related to the reduction in growth observed in VEGF-C-overexpressing melanomas.[18] This conclusion follows from results showing that increased densities of peritumoral macrophages were correlated with tumor growth suppression.[18]

Polarization to pro-tumor M2 type

Polarization of macrophages to M2 plays a vital role in the outcome of melanoma patients. It results from the presence of growth factors and exsomes that can be released by both melanoma cells and macrophages, or from Treg cells, as well as by intercellular interactions.

Transforming growth factor-β1

All 3 isoforms of TGF-β has been found to be expressed in cultured malignant cells[19-21] and *in situ*.[22-24] TGF-β1, that can be produced by M2 type macrophages, plays a pivotal role in macrophage polarization to the pro-tumor M2 type.[9] It has been reported that tumor cells which produce high levels of TGF-β1 can stimulate monocytes/macrophages, and, in this way, support tumor growth and immune escape.[25,26] Alternative activation (M2) was shown to be mostly associated with responses of macrophages to anti-inflammatory mediators, such as glucocorticoids.[27] This mechanism appears to represent a major component involved with increasing surface contact sites of the TGF-β1 receptor.[28] In the presence of glucocorticoids, TGF-β1 stimulates mature macrophages to polarize to the M2 type.[28] TGF-β1 can also suppress nitric oxide release resulting from M1 polarized macrophages[29] and suppress M1 polarized macrophages to increase melanoma survival through stroma remodeling.[30] However, it has also been reported that tumor cells exposed to TGF-β1 experience enhanced susceptibility to NK-mediated extermination.[31]

IL-10

Interleukins are a group of cytokines (secreted proteins and signal molecules) that were first seen to be expressed by white blood cells (leukocytes).[32] Alternative activation (M2) can be a response of macrophages to Th2 cytokines, such as IL-10. IL-10 acts on macrophages by down-regulating class-II MHC antigens[32] and expression of the co-stimulator molecule, B7 on macrophages,[33] which then inhibit cytokine production by Thl cells.[34] IL-10 production is not confined to Th2 cells, as it has been reported that IL-I0 could be produced by melanoma cells.[35] Melanoma cells, in turn, then have the potential to use IL-10 as a means to modulate immune responses such as induction of M2 polarized macrophages.[35] Moreover, it has been shown that M2 macrophages also release IL-10.[36]

Adrenomedullin (ADM)

ADM is a multifunctional molecule involved with tumor angiogenesis and widely expressed in a variety of tumor types,[37-39] such as melanoma.[40] Levels of ADM and its receptor are increased in human melanoma, suggesting a role in melanomagenesis. Tumor-associated macrophages (TAM) are identified as the major source of ADM in melanoma.[41] TAM-derived ADM can induce the phosphorylation of endothelial nitric oxide synthesis in endothelial cells via a paracrine mechanism and polarize macrophages to an M2 phenotype via autocrine mechanisms to enhance melanoma tumor angiogenesis and tumor growth.[41] Based upon data generated from a mouse melanoma study,[41] a mathematical model has been derived to assess these interactions among mouse melanoma cells, Th2/Th1 cells and M2/M1 macrophages. With this model it is possible to investigate the role of re-polarization between M1 and M2 macrophages on tumor growth, and the findings obtained indicate that melanoma growth is associated with a type-II immune response as it can result from large numbers of Th2 and M2 cells.[42]

CD73

Nucleotidase CD73 expression is upregulated in melanoma.[43] Tumor macrophage infiltration can be dramatically decreased and the microenvironment substantially altered following inhibition or knockdown of tumor CD73, due to their effects upon the polarization of M1 or M2 macrophages.[44] Yegutkin et al reported that host CD73 knockout did not affect B16F10 melanoma infiltration by macrophages in B16 melanoma.[45] However, suppression of tumor cell CD73 or chemical inhibition of CD73 decreases macrophage infiltration. One conclusion from such results is that CD73 plays a role in the regulation of macrophage infiltration. Pro-neoplastic and pro-angiogenic M2 phenotypes of tumor-associated macrophages are often observed in hypoxic regions of a tumor[46]; and, a downregulation of pro-M1 cytokines are found in response to any reductions in CD73.[44] Such changes in the microenvironment contribute to macrophage polarization resulting in a pro-neoplastic M2 phenotype that can then regulate the progression of a tumor.[46]

Arginine metabolism

Arginine metabolism plays a role in macrophage polarization. This relationship follows from findings which show that

macrophages using arginine can induce nitric oxide synthase to produce nitric oxide (NO) as M1 types and ornithine through arginase as M2 types.[47] The number of M1 type macrophages varies as a function of tumor progression and location.[48] Mostly within peritumoral locations, considerable numbers of M1-type macrophages were reported to be present *in situ* and in thin melanomas. In contrast, within tumors of advanced stages and in melanoma metastases decreased numbers of these macrophages were found in peritumoral, as well as in intratumoral locations.[48] In both peritumoral and intratumoral locations, the percent of M2 type macrophages (arginase-positive) was lower than that of M1 type macrophages in thin melanomas. Macrophages -induced NO release was shown to be dependent on tumor microenvironment, with high levels being observed as associated with IFN-γ while low levels associated with more advanced tumors.[48]

The macrophage mannose receptor (MR)

MR is upregulated in the alternative anti-inflammatory/pro-tumoral M2 macrophage and has been shown to be essential for cytokine production.[49] In the mouse melanoma model with lung metastasis, recruitment of CD68+CD11b+CD11c− monocytes was abrogated in C57BL/6 mice without MR (i.e., MR−/−) and fewer lung colonies were observed in MR−/− mice as compared with that in the wild type.[49]

Exosome

Exosomes are microvesicles of 20–100 nm diameters which can be released by tumor cells.[50] As a result of their nanoscale size, exosomes readily penetrate and interact with local tumor cells. Moreover, these microvesicles can affect other cell types distal to the advancing tumor cell front.[51] Melanoma cells release exosomes which influence the tumor immuno-microenvironment,[50] via effects upon the cytokine and chemokine profiles of the macrophages.[52] Tumor cells treated with melanoma cell-derived exosomes respond vastly different from those induced by either LPS or IL-4.[52]

Tregs

Tregs can promote the differentiation of monocytes to tumor-promoting M2 macrophages. The interaction of Tregs and M2 macrophages represents a mutually beneficial effect, as the M2 macrophages directly induce Tregs, which then suppresses tumor specific cytotoxic T-cells.[53] Human malignant melanoma cells with monosomy of chromosome 3 can produce chemokines such as macrophage-derived chemokine, thymus- and activation-regulated chemokine and MCP-1, all of which contribute to Treg migration and can also be produced by M2 macrophages.[54]

Macrophages promote tumorigenesis of melanoma by cytokines

Macrophages recruited to the melanoma can, in turn, produce melanoma-stimulating molecules such as IFN-γ, angiotensin, cyclooxygenase-2 (COX-2) and IL-1β to support the growth and metastasis of melanoma.

IFN-γ

Interferon gamma (IFNγ) is a dimerized soluble cytokine that is the only member of the type II class of interferons.[55] IFN-γ has also been reported that IFN-γ may have pro-tumorigenic effects in solid tumors under certain conditions.[55,56] Although interferon γ, reduces cellular growth *in vitro*, when inoculated with B16 melanoma cells intravenously, it stimulates lung colonization along with an enhanced expression of class I major histocompatibility complex antigens.[55,56] These effects are more frequently observed in advanced melanoma and are related to an increased risk of metastasis in primary melanoma.[56] Elevated levels of IFN-γ show promise as being an independent predictor of disease recurrence. In addition, they may serve as a means for identifing early-stage melanoma patients that are more vulnerable to disease recurrence and who may then benefit from adjuvant therapies, such as immunotherapies.[57] Indeed, a randomized clinical trial by the Southwest Oncology Group observed an adverse effect of IFN-γ on melanoma relapse and mortality rates.[58] Moreover, in a mouse skin cancer model, as induced by ultraviolet B, macrophage-produced IFN-γ promoted melanoma growth by inhibiting apoptosis.[59] Pro-tumorigenic effects of IFN-γ may, in part, be due to a pro-expression of CD74 in melanoma.[60]

Angiotensin

Macrophages express angiotensin II type 1 and type 2 receptors during the process of monocyte differentiation to macrophages.[61] Tumors produce angiotensin II to enhance the amplification of macrophages to stimulate cancer-promoting immunity.[62] Angiotensin-converting enzyme (ACE) is a peptidase which is responsible for the cleavage of angiotensin I. Mice with enhanced macrophage ACE levels show increased production of interleukin-12 and nitric oxide but reduced interleukin-10, and are resistant to melanoma.[63] However, ACE inhibitors as a pharmacological tool to inhibit tumor angiogenesis is controversial.[64] Angiotensin II type 1 receptor expression on TAM is related with increased melanoma tumor growth.[64] It is possible that the angiotensin II type 1 receptor pathway may play an important role in promoting tumor angiogenesis and growth via a macrophage and VEGF-dependent mechanism.[64]

COX-2

Cyclooxygenase-2 (COX-2), also known as prostaglandin-endoperoxide synthase 2, is involved in the conversion of arachidonic acid to prostaglandin H2.[65] As shown with use of immunohistochemical analysis, COX-2-positive macrophages, are rare in common nevi and "dysplastic nevi," but found in high levels *in situ* and in thin melanoma. COX-2-positive macrophages were also found in more advanced tumors and metastatic melanoma, although at much lower levels than that observed *in situ* or in thin melanoma. As demonstrated *in vitro*, COX-2 has been shown to be expressed in peritoneal macrophages when exposed to B16 murine melanoma cells, but not following exposure to normal murine fibroblasts. Taken together, results obtained from both *in vivo* and *in vitro* studies indicate that not only may COX-2 expressed in macrophages have the potential to provide a valid and reliable biomarker of melanoma progression, but also the possibility that melanoma cells themselves might stimulate COX-2 in macrophages.[65]

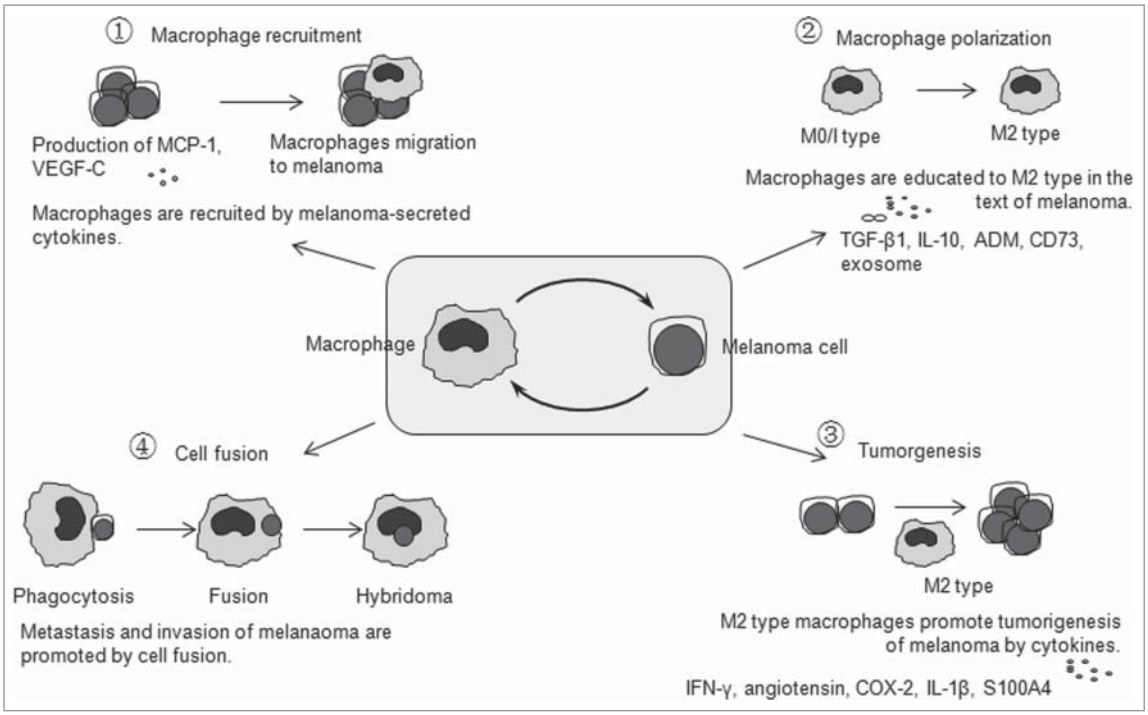

Figure 1. Melanoma progression and pro-tumor activities of macrophages. ① Macrophage recruitment to melanoma. Melanomas release many different types of macrophage-recruiting molecules, such as MCP-1 and VEGF-C, to attract macrophage migration to melanoma sites. ② Polarization to pro-tumor M2 type. Macrophages can be induced and educated to adopt a protumoral phenotype (M2) in the text of melanoma, which is co-made up by both melanoma cells and macrophages. ③ Cytokines by macrophages promote tumorigenesis of melanoma. Macrophages recruited to the melanoma can produce melanoma-stimulating molecules such as IFN-γ, angiotensin, COX-2, IL-1β and S100A4 to support the growth and metastasis of melanoma. ④ The cancer cell fusion theory. Macrophages in the melanoma microenvironment can devour melanoma cells, if digestion fails, then would likely form a hybridoma of the macrophage-melanoma cell, that results in metastasis of the melanoma.

IL-1β

IL-1β as a pleiotropic pro-inflammatory cytokine contributes to cell growth, differentiation and regulation of immune responses.[66] The IL-1β gene or its protein expression are associated with the degree of invasiveness and metastasis of melanoma.[67] Interestingly, although metastatic melanoma cell lines do not secrete IL-1β, they do promote IL-1β production from macrophages.[68] Therefore, more work directed toward revealing the mechanisms and consequences of IL-1β production by infiltrating macrophages may be of interest for the development of IL-1β targeted therapy, such as an anti-IL-1β antibody (Canakinumab) of metastatic melanoma.[68] Moreover, IL-1β as generated from tumor cells may be considered as a threat to the host's immune system. In this regard, IL-1β-producing melanoma cells can induce reduced tumor growth by recruiting immune cells.[69]

The cancer cell fusion theory

The cancer cell fusion theory initially proposed by Prof. Aichel,[70] and currently accepted by many investigators, states that the fusion of cancer cells with macrophages or other phagocytes could underlie cancer metastasis. The Aichel's hypothesis can be simplified by the model — white blood cell + non-metastatic cancer cell = metastatic cancer cells.[70] Cancer cells, especially non-adherent ones, favor destruction by white blood cells, preferably by phagocytes like macrophages or neutrophils. If a captured cancer cell escapes to be digested by a predator phagocyte, the 2 cells would fuse, pooling their chromosomes to form a white blood cell–tumor cell hybrid. At least some of these hybrids become metastatic, exhibiting both motility and continuous cell division.[70] It has been demonstrated that hybrids of weakly metastatic Cloudman S91 mouse melanoma cells and normal mouse or human macrophages can be created *in vitro* with use of polyethylene glycol -induced fusion.[71] Compared to the parental melanoma cells, most daughters of the 35 hybrids tested were found to be more aggressive, with the result being that metastases onset was more rapid and observed in greater numbers of mice.[71] The majority of these hybrid clones showed markedly enhanced chemotactic motility toward a variety of attractants in 2-chambered culture systems, a hallmark of metastatic cells.[72] Most notably, the expression of macrophage-like glycosylation patterns showed an increase in oligosaccharide chains conjugated with β1,6-branched oligosaccharides and the responsible glucosyltransferase, β1,6-N-acetylglucosaminyltransferase (GNT-V).[73] The significance of this finding is twofold: 1) β1,6-branched oligosaccharides and GNT-V are highly associated with malignant transformation as has been shown in rodent and human cells and 2) these melanoma patients show a poor prognosis.[74]

Conclusion

Whether macrophages in the tumoral microenvironment are anti- or pro-tumor has been enigmatic. The primary reason for this controversy regarding macrophages in tumor progression can be traced to the contrasting results obtained regarding the effects of macrophages in the literature. It remains, however, widely accepted that macrophages, owing to their malleability, can be domesticated as a tumor's handyman, as summarized in

Fig. 1. Interactions between 2 categories of cells often can offer mutual benefits and achieve the common goal of melanoma progression. It is anticipated that the application of such interactions could be used to develop a feasible anti-melanoma strategy which incorporates a combination of macrophage recruitment inhibition and the "re-education" of macrophage polarization.

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