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Review

Myeloid regulatory cells in tumor spreading and metastasis

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ABSTRACT

Development of metastasis is determined by both the accretion of essential changes in cancerous cells and by their communications with different stromal elements in the tumor microenvironment. Specifically, inflammatory response and emergence of immune regulatory cells, such and myeloid regulatory cells (macrophages, dendritic cells, neutrophils, myeloid-derived suppressor cells) and lymphoid regulatory cells (regulatory T, B and NK cells) to the tumor site have been reported to support tumor growth in addition to spreading and metastasis. Every phase of tumor progression, from its initiation through metastatic expansion, is endorsed by interaction between malignant and immune cells mediated by a number of growth factors, cytokines, proteases and other molecules that modify the tumor microenvironment. Invasion and metastasis depend on intratumoral vascularization, alterations of the basement membrane and degradation of the extracellular matrix for tumor cell spreading, invasion and extravasation into the blood and lymphatic vessels. The consequent dissemination of cancerous cells to distant tissues and organs necessitates a trafficking through the vasculature, which is promoted by further interactions with cells of the immune system, including myeloid regulatory cells. Moreover, the formation of the pre-metastatic niche and specific metastasis organ tropism is also regulated and controlled by bone marrow-derived hematopoietic immune progenitor cells, immature myeloid cells and certain cytokines, chemokines and growth factors derived from tumor and immune cells, which amend the local microenvironment of the organ or tissue to promote adhesion and survival of circulating cancerous cells. Although the potential role for myeloid regulatory cells in tumor spreading and development of pre-metastatic niche has been suggested, the concept still requires further supportive experimental and clinical data, as well as data related to specific factors and mechanisms responsible for myeloid regulatory cell functioning at malignant sites.

Contents

Abbreviations: BM, bone marrow; CCL2, chemokine (C-C motif) ligand 2; COX2, cyclooxygenase-2; CXCR4, C-X-C chemokine receptor type 4; DC, dendritic cells; ECM, extracellular matrix; FKBP51, FK506 binding protein 51; G-MDSCs, granulocytic MDSCs; GPCRs, G protein coupled receptors; HIF, hypoxia-inducible factor; iNOS, inducible nitric oxide synthase; MCP-1, monocyte chemoattractant protein-1; MDSC, myeloid-derived suppressor cells; MHC, major histocompatibility complex; MMP9, matrix metalloproteinase 9; Mo-MDSCs, monocytic MDSCs; MyD88, myeloid differentiation antigen 88; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; NGP, neutrophilic granule protein; NO, nitric oxide; PGE-2, prostaglandin E2; ROS, reactive oxygen species; RTKs, receptor tyrosine kinases; S1PR1, sphingosine-1-phosphate receptor 1; STAT3, signal transducer and activator of transcription 3; TLR/IL1Rs, Toll-like receptor/interleukin1 receptor family members; VCAM-1, vascular cell adhesion molecule 1.

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Introduction

Myeloid regulatory cells have been reported to play a key pro-tumorigenic and immunosuppressive role in tumor development, growth and progression (Gutkin and Shurin, 2014; Zhong et al., 2014). The cells include myeloid-derived suppressor cells (MDSCs), type 2 or M2 tumor-associated macrophages, regulatory dendritic cells (DCs), type 2 or N2 tumor-associated neutrophils and a subset of mast cells. MDSCs represent a heterogenic population of mixed immature bone marrow-derived myeloid cells, including myeloid progenitors and precursors of macrophages, granulocytes and DCs. MDSCs are characterized by a combination of certain phenotypic markers and a strong ability to suppress various T cell functions (Gabrilovich and Nagaraj, 2009). In normal conditions, they usually quickly differentiate into mature granulocytes, macrophages and DCs. In contrast, in case of pathological conditions, such as cancer, infectious diseases, sepsis, trauma, bone marrow transplantation or some autoimmune disorders, a partial block in the differentiation of immature myeloid cells into mature myeloid cells results in an expansion of this population in different lymphoid and non-lymphoid tissues.

MDSCs can be found in the bone marrow (BM), spleen, liver and tumor sites and have been identified in most patients and in experimental animals with cancer based on their ability to suppress T cell activation and proliferation. It has been shown that in solid tumors infiltration of MDSCs is associated with poor prognosis (Gabrilovich and Nagaraj, 2009; Marigo et al., 2008) and MDSC levels are elevated in peripheral blood of certain categories of cancer patients. For instance, $Lin^{neg}CD14+HLA\text{-}DR^{neg}$ monocytic MDSCs were enriched in peripheral blood of melanoma patients compared to healthy donors (Meyer et al., 2014). Circulating $CD14+CD11b+HLA\text{-}DR^{-/low}$ MDSCs have a negative impact on survival and inversely correlate with the presence of functional antigen-specific T cells in patients with advanced melanoma (Weide et al., 2014). Interestingly, clinical responders to ipilimumab therapy in melanoma patients showed significantly less $Lin^{neg}CD14+HLA\text{-}DR^{neg}$ cells as compared to non-responders, suggesting that the frequency of monocytic MDSCs may be used as predictive marker of response, as low frequencies identify patients more likely benefitting from ipilimumab treatment (Meyer et al., 2014). Characterization of peripheral $CD14+HLA\text{-}DR^{-/low}$ MDSC subsets in patients with non-small cell lung cancer (NSCLC) revealed that both frequency and absolute number of MDSCs were significantly increased in the peripheral blood of NSCLC patients compared with that of the healthy controls and indicated an association with metastasis, response to chemotherapy and progression-free survival (Huang et al., 2013). Cancer stage and shorter median overall survival time correlated with higher MDSC blood levels in solid tumor patients (Diaz-Montero et al., 2009; Gabitass et al., 2011; Solito et al., 2011). Another study of patients with terminal cancer investigated the overall survival time according to the numbers of granulocytic MDSCs. Patients with low levels of peripheral blood $CD15+CD16^{low}$ cells had significantly longer survival times than those with high levels ($p = 0.0011$, median survival time was 2.6 *versus* 0.8 months). Moreover, patients with high levels of $CD15+CD16^{low}$ cells tended to have poor performance status ($p = 0.05$) (Choi et al., 2012).

Although the tumor-supporting role of tumor-associated MDSCs has been well documented, their role in the induction of pre-metastatic niche and tumor spreading is not completely understood.

Immunobiology of tumor-associated MDSCs

Originally described as CD11b/Gr-1 double-positive cells in mice, the Gr-1 antigens Ly-6G and Ly-6C now distinguish G-MDSCs and Mo-MDSCs, respectively. The difference between MDSC subsets lies in morphology/phenotype and in the mechanisms by which they conduct immune function suppression (Youn and Gabrilovich, 2010). In mice, the minimum definition for the phenotype of monocytic MDSCs (Mo-MDSCs) is the co-expression of CD11b and Ly-6C, whereas granulocytic MDSCs (G-MDSCs) co-express CD11b and Ly-6G. More recently, a number of additional markers have been associated with the MDSC phenotype (Youn et al., 2012).

Phenotypically human MDSCs consist of a mixture of monocytic (expressing CD14) and granulocytic cells (expressing markers such as CD15, CD66b, CD33). Human Mo-MDSCs are mostly referred to as being CD14+ with negative or low expression of HLA-DR. Mo-MDSCs express high amounts of CD11b and CD33. Human G-MDSCs are mostly defined as CD11b+ and CD15+ or CD66b+. G-MDSCs are negative for HLA-DR, display an intermediate expression of CD33 and a variable expression of CD11b, depending on their maturation stage (Peiyuan Zhu et al., 2013). It is likely that the frequency of MDSCs subset may be different in different types of cancer (Zhang et al., 2013). For instance, patients with renal cancer display immunosuppressive CD14-CD15+CD11b+CD66b+ granulocytic MDSCs (Rodriguez et al., 2009), whereas $CD14+HLA\text{-}DR2^{low}$ monocytic MDSCs are found in melanoma, multiple myeloma, prostate cancer, hepatocellular carcinoma or head and neck cancer patients (Hoechst et al., 2008; Poschke et al., 2010; Serafini et al., 2006; Vuk-Pavlovic et al., 2010). Despite sharing similar phenotype and morphology MDSCs have been shown to display functional differences dependent on their location at either the primary tumor site or peripheral lymphoid organs (Corzo et al., 2010). These finding may, at least in part, be explained by differential role of adhesion molecules and cytokines in recruitment, homing and trafficking of MDSCs.

The integrin adhesion molecule family is an extensive group of structurally related receptors for extracellular matrix (ECM) proteins and immunoglobulin superfamily molecules. Integrins at bone marrow-derived immune cells promote tumor inflammation by facilitating myeloid cell trafficking to the tumor microenvironment as myeloid cells express a number of functional integrins, including $\alpha2\beta1$, $\alpha4\beta1$, $\alpha5\beta1$, $\alpha v\beta3$, $\alpha v\beta5$, $\alpha M\beta2$ (CD11b) and $\alpha X\beta2$ (CD11c). Recent studies by Schmid and Varner (2012) indicate that integrin $\alpha4\beta1$, a receptor for vascular cell adhesion molecule 1 (VCAM-1) and CS-l fibronectin, selectively promotes the homing of myeloid cells to the tumor microenvironment. It is known that human and murine myeloid cells adhere to endothelial cells *in vitro* and to tumor endothelium *in vivo via* integrin $\alpha4\beta$. In fact, genetic and pharmacological blockade of integrin $\alpha4\beta$l significantly suppressed tumor inflammation, growth and metastasis. In addition, combination of anti-integrin $\alpha4$ antibody and chemotherapeutic agents markedly reduced tumor burden compared to chemotherapeutic treatment alone (Schmid and Varner, 2012).

Lechner et al. (2011) identified IL-6, IL-1β and GM-CSF as the major inducing factors of CD33+ MDSCs and FLT3L and TGF-β as major contributors to CD11b+ MDSCs development (Lechner et al., 2011). Considering the possible molecular targets of GM-CSF and IL-6, the C/EBP family of transcription factors is one of particular application points. Whereas C/EBPa is the "master regulator" of the steady-state granulopoiesis, C/EBPb controls the emergency granulopoiesis induced by cytokines and infections (Hirai et al., 2006). The ablation of C/EBPb in the myeloid compartment led to a reversal of tolerance in tumor-antigen specific CD8+ T cells and revealed a full therapeutic activity of tumor-specific CTLs on established tumors. Moreover, in tumor-bearing mice, C/EBPb ablation increased the number of monocytes–macrophages and DCs with a concomitant reduction in mature granulocytes, suggesting that lack of C/EBPb might also lead to an altered

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