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FOCUSSED RESEARCH REVIEW

CCR5 in recruitment and activation of myeloid-derived suppressor cells in melanoma

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Abstract Malignant melanoma is characterized by the development of chronic inflammation in the tumor microenvironment, leading to the accumulation of myeloid-derived suppressor cells (MDSCs). Using *ret* transgenic mouse melanoma model, we found a significant migration of MDSCs expressing C-C chemokine receptor (CCR)5 into primary tumors and metastatic lymph nodes, which was correlated with tumor progression. An increased CCR5 expression on MDSCs was associated with elevated concentrations of CCR5 ligands in melanoma microenvironment. *In vitro* experiments showed that the upregulation of CCR5 expression on $CD11b^+Gr1^+$ immature myeloid cells was induced by CCR5 ligands, IL-6, GM-CSF, and other inflammatory factors. Furthermore, $CCR5^+$ MDSCs infiltrating melanoma lesions displayed a stronger immunosuppressive pattern than their $CCR5^-$ counterparts. Targeting CCR5/CCR5 ligand signaling via a fusion protein mCCR5-Ig, which selectively binds and neutralizes all three CCR5 ligands, increased the survival of tumor-bearing mice. This was associated with a reduced migration and immunosuppressive potential of tumor MDSCs. In melanoma patients, circulating $CCR5^+$ MDSCs were increased as compared to healthy donors. Like in melanoma-bearing mice, we observed an enrichment of these cells and CCR5 ligands in tumors as compared to the peripheral blood. Our findings define a critical role for CCR5 not only in the recruitment but also in the activation of MDSCs in tumor lesions, suggesting that novel strategies of melanoma treatment could be based on blocking CCR5/CCR5 ligand interactions.

Keywords Myeloid-derived suppressor cells · Chemokine receptors · Chemokines · Immunosuppression · Melanoma · Regulatory myeloid suppressor cells

Abbreviations

ARG	Arginase
BM	Bone marrow
CCL	C-C motif ligand
CCR	C-C motif receptor
CXCL	C-X-C motif ligand
CXCR	C-X-C motif receptor
DCs	Dendritic cells
GM–CSF	Granulocyte–macrophage colony-stimulating factor
HIF	Hypoxia-inducible factor
IFN	Interferon
IL	Interleukin
M	Monocytic
MDSCs	Myeloid-derived suppressor cells
NF-κB	Nuclear factor-κB
NO	Nitric oxide
PD-1	Programmed death receptor
PD-L1	Programmed death-ligand 1
PMN	Polymorphonuclear

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ROS	Reactive oxygen species
TGF	Transforming growth factor
TNF	Tumor necrosis factor
Tregs	Regulatory T cells
VEGF	Vascular endothelial growth factor

Introduction

Metastasizing malignant melanoma remains a disease with a rapid progression and dismal prognosis despite recent therapeutic improvements [1, 2]. Well-documented melanoma immunogenicity makes this tumor a preferred target for the application of different immunotherapeutic strategies in the last years, dealing with tumor antigen-specific and -nonspecific immunostimulation or adoptive transfer of melanoma-specific activated T cells [3, 4]. Moreover, recently approved novel treatments involving the blockade of molecules inducing T cell anergy (so called negative check points) has brought optimism into the field of melanoma immunotherapy. Antibodies against CTLA-4 (ipilimumab) or programmed death (PD)-1 receptor (pembrolizumab and nivolumab) have been demonstrated to induce durable responses with long-term survival in patients with metastatic malignant melanoma [5, 6]. However, such beneficial outcome could be achieved only in a subset of patients due to the ability of the tumor to "strike back" and induce the immunosuppression in non-responding cancer patients [7, 8].

The immune escape of melanomas is mediated by different mechanisms dealing with structural and functional changes both in tumor and stroma cells, leading finally to the inability of even activated effector immune cells to reject the tumor. One of these mechanisms has been recently shown to involve a rapid recruitment, expansion, and activation of MDSCs, representing a heterogeneous population of immature myeloid cells that fail to complete their differentiation under chronic inflammatory conditions that are typical for the tumor microenvironment and strongly accelerate tumor progression [9–12]. These cells are known to express in mice CD11b and Gr1 surface markers and are divided into two subsets: polymorphonuclear $Ly6G^{+}Ly6C^{lo}$ (PMN) and monocytic $Ly6G^{-}Ly6C^{hi}$ (M) cells [9, 10, 13]. In humans, MDSCs can be characterized as $Lin^{-}HLA\text{-}DR^{-/lo}CD33^{+}$ or $Lin^{-}HLA\text{-}DR^{-/lo}CD11b^{+}CD14^{-}CD15^{+}CD33^{+}$ PMN-MDSCs and $CD14^{+}HLA\text{-}DR^{neg/lo}$ or$Lin^{-}HLA\text{-}DR^{neg/lo}CD11b^{+}CD14^{+}CD15^{-}$M-MDSCs[13–15]. Both mouse and human MDSCs are able to inhibit the antitumor reactivity of T and NK cells due to different mechanisms, in particular, via reactive oxygen species (ROS), nitric oxide (NO), programmed death-ligand 1 (PD-L1), and arginase (ARG)-1 [9, 10, 13–16]. A long-term secretion of various chronic inflammatory mediators, including granulocyte–macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β), interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF-α), interferon (IFN)-γ, and chemokines (C-C motif) ligand (CCL) 2, CCL3, CCL4, and CCL5 produced by tumor and stroma cells has been reported to promote the generation, recruitment, and activation of MDSCs in tumor lesions [9–12].

Chemokines are small (8–14 kDa), structurally related chemotactic cytokines, which regulate the trafficking of lymphocytes and myeloid cells through interactions with specific transmembrane, G protein-coupled C-C chemokine receptors (CCR) [17]. Although CCR share a high degree of homology with up to 75% in case of CCR5 and CCR2 [18], they bind to different ligands. Few of the chemokines can bind more than one receptor. Chemokines are considered to be key drivers in the development of inflammatory diseases and cancer [17]. Chemokines are secreted by tumor and host cells to attract immune cells through binding onto their receptors, resulting in the signaling through the heterotrimeric G proteins [17]. Different chemokines were suggested to be involved in MDSC recruitment to melanoma microenvironment, however, the role of CCR5 and its ligands in such mobilization and in MDSC activation is poorly understood. In this review, we discuss the role of CCR5/CCR5 ligand interactions in the trafficking, expansion, and activation of MDSCs during tumor progression based on the data obtained in transgenic mouse melanoma model and in melanoma patients.

Chemokines and MDSC recruitment to the tumor site

The pattern of chemokines involved in MDSC migration to the tumor microenvironment seems to be dependent on the MDSC subset (monocytic or polymorphonuclear) and on the tumor model. The role of CCL2 and its receptors in the attraction of M-MDSCs has been well described. In particular, it has been reported that the trafficking of M-MDSCs in several mouse tumor models occurred via an interaction between CCL2 and its receptors CCR2, CCR4, and CCR5 [19]. Moreover, melanoma-infiltrating M-MDSCs have been shown to display CCR2-dependent immunosuppressive activities in the presence of GM-CSF [19]. In contrast, the enhanced production of C-X-C motif receptor (CXCR) 2 ligands supported the migration of PMN-MDSCs to the tumor site [20]. In the transplantable prostate cancer mouse model, it has been recently demonstrated using generated soluble CCR2-Ig fusion protein [21] that CCL2/CCR2 interaction plays a pivotal role in the recruitment of bone marrow-derived myeloid cells to the peripheral blood and their subsequent migration to the

tumor site [22, 23]. Moreover, the targeting of CCL2/CCR2 axis with antibody carlumab showed a therapeutic activity in patients with metastatic, castration-resistant prostate cancer [24].

The production of CCL2, chemokine (C-X-C motif) ligand (CXCL) 8 (also known as IL-8), and CXCL12 can be induced by prostaglandin E2 resulting in a strong enrichment of MDSCs in ovarian and gastric cancer microenvironment [25]. In contrast, the expression of CXCL12 has been demonstrated to decrease MDSC accumulation in a mouse model of breast cancer [26]. Other investigators, however, reported a dominating role of CXCL1, CCL5, and CCL7 but not CCL2 in the MDSC migration into mouse tumors [27].

The chemokine CCL5 has been recently described to activate hypoxia-inducible factor (HIF)-1α signaling cascades, leading to the upregulation of the VEGF expression [28]. Importantly, both HIF-1α and VEGF are considered to play a key role in MDSC generation and activation [9, 10]. It has been published that CCL5 supported the tumor growth, invasion, angiogenesis, as well as immune cell recruitment to the tumor microenvironment via the interaction with CCR5 [29]. This receptor is a member of the trimeric guanine nucleotide-binding-protein-coupled seven-transmembrane receptor superfamily that acts via G proteins. It is composed of 352 amino acids and has a molecular mass of 40.6 kDa [18]. Besides CCL5, CCR5 can bind chemokines CCL3 and CCL4 that are secreted by tumor cells, T cells, monocytes/macrophages, dendritic cells (DCs), and MDSCs [17]. CCR5 is mainly expressed on fibroblasts, myeloid cells, T cells, and vascular cells [18].

CCR5 in cancer progression

CCR5 has been found to be a key receptor for the entry of HIV. Interestingly, individuals, exhibiting a 32 pb deletion in the CCR5 gene (CCR5Δ32), produce a non-functional protein and are therefore resistant to HIV infection [30]. In addition, males bearing this functional mutation in CCR5 acquired resistance to the development of prostate cancer that indicates an importance of this chemokine receptor for cancer progression [31].

Mouse studies showed that CCR5 expression both in tumor cells and various host cells was important for tumor progression. Thus, breast cancer cells that express a functional CCR5 could display increased cell migration and invasiveness [32]. Furthermore, CCR5 deficiency caused apoptosis of melanoma cells through the inhibition of nuclear factor-κB (NF-κB) and upregulation of IL-1Ra [33]. Blocking CCR5 expression was reported to reduce the potential for gastric cancer cell dissemination [34], to suppress bone metastasis of prostate cancer cells [35] and to inhibit the proliferation and invasion of cervical cancer cells regulated by microRNA-107 [36]. Other publications demonstrated that CCR5 expression on stromal cells is necessary for the spread of melanoma cells to the lungs in a transplantable B16 melanoma model [37], and that CCR5-deficient mice displayed a delayed B16 melanoma growth and a better response to cancer vaccines [38, 39]. Furthermore, delayed growth of colon cancer and melanoma in CCR5 knockout mice was described to be associated with reduced tumor infiltration with regulatory T cells (Tregs) as compared to wild-type mice [40, 41].

Therefore, it was suggested that CCR5–CCR5 ligand interactions may favor tumor development in multiple ways: acting as growth factors, stimulating angiogenesis, modulating the extracellular matrix, inducing the recruitment of additional stromal and inflammatory cells, and taking part in immune evasion mechanisms [39].

To address the question whether MDSCs could be recruited to the melanoma microenvironment through CCR5 in more clinically relevant conditions, we used the *ret* transgenic mouse model of spontaneous skin melanoma, which resembles human melanoma with respect to histopathology and clinical development ensuring natural tumor–stroma interactions [42]. We found an increased frequency of $CCR5^+$ MDSCs among total MDSCs in melanoma lesions (primary skin tumors and metastatic lymph nodes) as compared to the BM and peripheral blood. Furthermore, the frequency of $CCR5^+$ MDSCs was significantly elevated in melanoma lesions in the course of melanoma progression. Interestingly, it has been recently described that a subset of $CCR5^+$ breast cancer cells showed increased invasion and migration capacity, promoting breast cancer metastasis [32].

The migration of $CCR5^+$ MDSCs to the tumor sites could be mediated by CCR5 ligands CCL3, CCL4, and CCL5 since we demonstrated that their concentrations were significantly increased in the lysates of primary tumors and metastatic lymph nodes as compared to serum in the same mice. These findings confirmed other reports demonstrating that melanoma and other tumors produced elevated amounts of CCR5 ligands [9, 39, 43, 44]. Interestingly, MDSCs infiltrating mouse melanoma may produce CCR5 ligands by themselves and attract high numbers of CCR5 expressing Tregs *in vitro* and *in vivo* [41]. However, the amount of tumor cells producing CCR5 ligands is much higher than that of infiltrating MDSCs, leading to an elevated ligand production in melanoma lesions as compared to the peripheral blood. Moreover, it has been found that these chemokines may not only induce trafficking of $CCR5^+$ cells but also upregulate the CCR5 expression on their surface [45]. In our *in vitro* experiments, CCR5 ligands were shown to enhance the trafficking of

$CD11b^+Gr1^+$ immature myeloid cells in the transwell assay that could provide an explanation for a profound accumulation of $CCR5^+$ MDSCs in skin tumors and metastatic lymph nodes.

Earlier we demonstrated that melanoma lesions from *ret* transgenic mice contained also elevated amounts of numerous cytokines and growth factors, including IL-6, GM-CSF, VEGF, IL-1β, and IFN-γ that were associated with the accumulation of tumor-infiltrating MDSCs and fast tumor progression [46, 47]. To address their potential effects on CCR5 expression, we incubated bone marrow (BM)-derived $CD11b^+Gr1^+$ immature myeloid cells with some of these factors alone or in combination with CCR5 ligands and found a strong stimulation of CCR5 expression. This suggests that not only CCR5 ligands but also other chronic inflammatory factors could mediate CCR5 upregulation and recruitment of MDSCs into melanoma lesions (Fig. 1). Other groups have recently presented similar observation on increased CCR5 expression induced by tumor-derived colony-stimulating factors and HIF-1α in breast cancer [48, 49].

Role of CCR5 in MDSC activation

Having shown that trafficking of $CCR5^+$ MDSCs into the tumor site was mediated by CCR5 ligands and other inflammatory factors, we addressed the question of their functionality. To this end, we analyzed the immunosuppressive pattern of $CCR5^+$ and $CCR5^-$ MDSC subsets in various lymphoid organs and melanoma lesions from the same tumor-bearing mice. In $CCR5^+$ MDSCs, we observed a significantly higher expression of ARG-1, ROS, PD-L1, and NO known to mediate MDSC immunosuppressive functions [9–16] than in their $CCR5^-$ counterparts. Importantly, this difference was especially pronounced between these MDSC subpopulations infiltrating skin tumors and metastatic lymph nodes. Moreover, an increasing production of all four immunosuppressive molecules by tumor-infiltrating $CCR5^+$ MDSCs (in contrast to $CCR5^-$ subpopulation) was shown to be significantly correlated with melanoma progression. In addition, *in vitro* incubation of BM-derived $CD11b^+Gr1^+$ immature myeloid cells with factors enriched in the tumor microenvironment (such as IL-6, GM-CSF, IL-1β, IFN-γ, IL-10, and CCR5 ligands) induced a significant upregulation of PD-L1 and ARG-1 expression on $CCR5^+$ MDSC. When investigating the impact of CCR5 expression on MDSC immunosuppressive activity, we observed that $CCR5^+$ MDSC isolated from the BM of tumor-bearing mice showed a tendency for stronger inhibition of T cell proliferation than their $CCR5^-$ counterparts. We had to use in this functional assay $CCR5^+$ and $CCR5^-$ MDSCs sorted from the BM due to insufficient numbers of these cells infiltrating tumors, which demonstrated stronger differences in the immunosuppressive pattern.

Taken together, we observed that $CCR5^+$ MDSCs could not only accumulate in melanoma lesions but also displayed an enhanced immunosuppressive capacity (Fig. 2). Recently, it has been reported that $CCR5^{high}$ Tregs infiltrating human colorectal tumors displayed stronger immunosuppressive activity than their $CCR5^{low}$ counterparts [50]. In addition, the blockade of CCR5 signaling could reduce the migration of Tregs into tumors in mouse colon

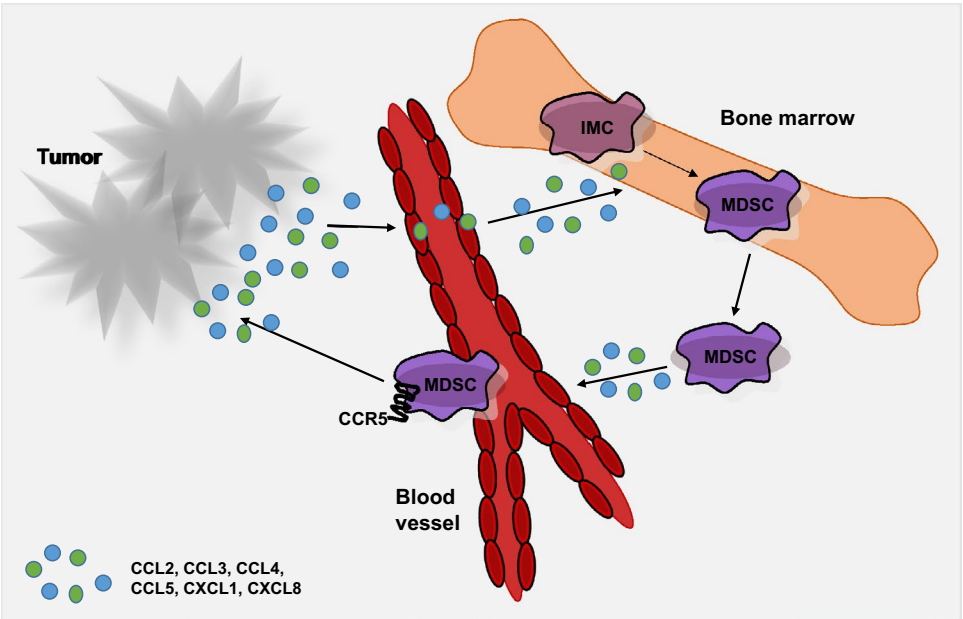

Fig. 1 Chemokines induce MDSC trafficking into melanoma microenvironment. Chemokines, including CCL2, CXCL1, CXCL8, and CCR5 ligands (CCL3, CCL4, and CCL5), stimulate migration of MDSCs from the bone marrow to peripheral blood and further to the tumor site. CCR5 ligands upregulate the expression of CCR5 on MDSCs, infiltrating tumor lesions

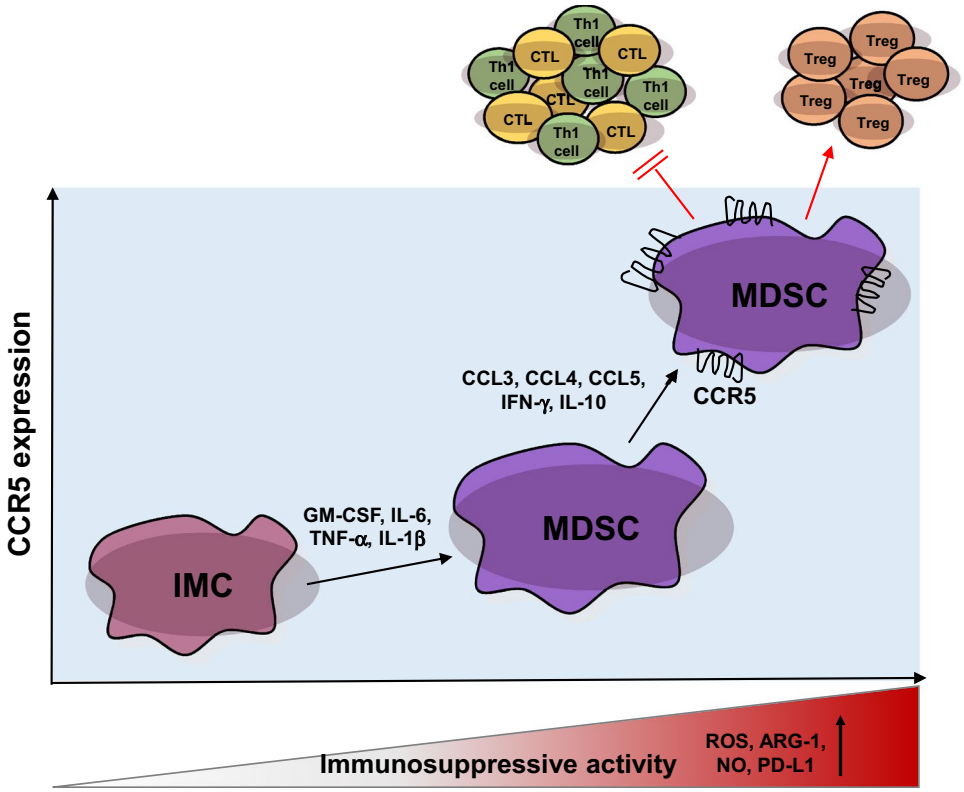

Fig. 2 Induction of CCR5 expression on MDSCs is associated with their activation. CCR5 ligands and other inflammatory factors (such as GM-CSF, IL-1β, IL-6, TNF-α, IL-10, IFN-γ) stimulate CCR5 expression on MDSCs. They displayed significantly stronger immunosuppressive pattern than their CCR5-negative counterparts reflected by higher expression ROS, ARG-1, NO, and PD-L1, allowing the inhibition of effector T cells (Th1 and CTL) and stimulation of Treg

carcinoma model and impair their *in vivo* suppression ability [40]. However, exact molecular mechanisms responsible for the stronger immunosuppression mediated by $CCR5^+$ MDSCs are not described and need to be investigated.

Targeting CCR5/CCR5 ligand interactions

Given a critical importance of CCR5 for cell migration and activation, this receptor and its ligands were considered as therapeutic targets. Human small molecular antagonist of CCR5, Maraviroc, which binds to CCR5, thereby blocking the receptor–ligand interaction, has been approved for the treatment of patients with HIV infection, acting by inhibition of viral entry through the CCR5 receptor [51]. However, it failed to show any activity against murine CCR5 [52]. Studies on cancer patients demonstrated that Maraviroc-mediated cytotoxic and apoptotic effects in colorectal cancer cells [53], reduced the potential for gastric cancer cell dissemination [34] and inhibited metastatic potential of prostate and breast cancer cells [32, 35]. Furthermore, the blockade of CCR5 by Maraviroc has been recently reported to induce the repolarization of tumor-associated macrophages and to result in beneficial clinical responses in colorectal cancer patients with liver metastases [54].

Another possibility includes an application of receptor-based fusion proteins or neutralizing antibodies to CCR5 or its ligands. In mice, CCR5 blockade with anti-CCR5 antibody has been recently reported to lead to the inhibition of B16 melanoma growth and MDSC accumulation in tumor tissues [55]. Furthermore, targeting of chemokine CCL5 could decrease apoptosis of tumor-infiltrated $CD8^+$ T cells in mouse colon tumor model [56] and reduce the immunosuppression activity of MDSCs in mouse mammary carcinoma [49], leading in both tumor models to the inhibition of tumor progression.

In our experiments in *ret* transgenic melanoma-bearing mice, we used a soluble receptor-based fusion protein mCCR5-Ig that was previously reported to selectively bind and neutralize all three CCR5 ligands (CCL3, CCL4, and CCL5) simultaneously [57]. We demonstrated that mice treated with the fusion protein displayed a significantly prolonged survival as compared to animals injected with non-related anti-mouse IgG (control group). Moreover, 25% of mice remained alive after 100 days of the treatment without any signs of tumor progression. Importantly, systemic injections of mCCR5-Ig resulted in a significant reduction in MDSC frequency among leukocytes and in the proportion of $CCR5^+$ subpopulation within total MDSCs infiltrating skin tumors as compared to the control group. In addition, tumor MDSCs from the mice treated with mCCR5-Ig displayed reduced immunosuppressive pattern reflected by a lower NO production than in MDSCs from control tumor-bearing mice. We observed also an inhibitory effect

of mCCR5-Ig on the recruitment of Tregs that are also characterized by the high expression of CCR5 [40, 41, 50]. Importantly, we failed to demonstrate a decreased accumulation of conventional $CD4^+$ and $CD8^+$ T cells in melanoma lesions since the expression of CCR5 on these cells was found to be significantly lower than on Tregs. This suggests that effector $CD4^+$ and $CD8^+$ T cells could use other chemokine receptors for their trafficking to the tumor microenvironment and were not negatively influenced by blocking CCR5/CCR5 ligand interactions.

CCR5 expression on MDSCs from melanoma patients

Numerous papers published during last years have documented an increase in the frequency of peripheral M-MDSCs and PMN-MDSCs in patients with malignant melanoma [14, 15, 58–60] and other tumors [14, 15, 61] that strongly correlated with tumor load. Furthermore, the frequency of circulating M-MDSCs has been demonstrated to correlate with reduced overall survival and decreased frequency of functionally active antigen-specific T cells in the peripheral blood of patients with advanced melanoma [60]. In addition, elevated amounts of circulating PMN-MDSCs have been reported to correlate with poor prognosis in patients with breast or colorectal cancer [61, 62]. Similar to findings of others, we observed a significant increase in the frequency of M-MDSCs in stage III–IV melanoma patients as compared to age- and gender-matched healthy donors [63]. Importantly, this enrichment was associated with elevated serum levels of chronic inflammatory factors such as IFN-γ, IL-1β, and CXCL10 that support MDSC accumulation and activation. Moreover, an enrichment of circulating M-MDSCs significantly correlated with a decreased progression-free survival of these patients [63].

Analyzing whether CCR5 could play a critical role for MDSC trafficking in melanoma patients, we detected a significant elevation of the frequency of $CCR5^+$ M- and PMN-MDSC subsets in the peripheral blood as compared to their counterparts in age- and gender-matched healthy donors. Interestingly, such increase was observed already in stage I–II patients. Moreover, directly comparing peripheral blood and skin melanoma samples from the same patients, we found a marked elevation of $CCR5^+$ M-MDSC frequencies in tumor tissues as compared to the peripheral blood. Similar to observations in tumor-bearing mice, we demonstrated increased concentrations of CCR5 ligands in melanoma lysates as compared to serum samples from the same patients. It has been demonstrated that several chemokines (including CCL5) could be involved in melanoma growth and progression [64]. In addition, the level of chronic inflammatory factors GM-CSF, IFN-γ, and IL-1β was found to be elevated in melanoma lesions that according to our mouse data [46] could create conditions for MDSC migration to the tumor site. Analysis of the immunosuppressive pattern of MDSC subsets from the peripheral blood of melanoma patients revealed higher expression of immunosuppressive molecules (such as ROS, ARG-1, PD-L1, and NO) in circulating $CCR5^+$ M-MDSCs and $CCR5^+$ PMN-MDSCs than in their $CCR5^-$ counterparts. Therefore, like in tumor-bearing *ret* transgenic mice, $CCR5^+$ MDSCs were found to be enriched in the peripheral blood and to be further accumulated in the tumor microenvironment of melanoma patients. In addition, they displayed an enhanced immunosuppressive capacity as compared to $CCR5^-$ MDSCs.

Conclusion

Taken together, CCR5–CCR5 ligand interactions could play a major role not only in driving MDSCs into the melanoma microenvironment but also in the stimulation of their immunosuppressive functions. Using transgenic mouse melanoma model and human melanoma samples, we demonstrated that melanoma lesions were enriched with $CCR5^+$ MDSCs showing enhanced immunosuppressive phenotype and function as compared to $CCR5^-$ cells. Importantly, the upregulation of CCR5 expression could be achieved not only by CCR5 ligands but also by other inflammatory factors accumulated in the tumor microenvironment (like GM-CSF, IL-6 etc.). The treatment of melanoma-bearing mice with the fusion protein mCCR5-Ig reduced MDSC migration and immunosuppressive activity, leading to a significant prolongation of mouse survival associated with the decrease in the frequency of MDSCs infiltrating skin tumors. Moreover, tumor MDSCs from the mice treated with mCCR5-Ig displayed reduced immunosuppressive pattern as compared to these cells from control tumor-bearing mice. Importantly, targeting CCR5/CCR5 ligand interactions inhibited the recruitment of Tregs without any changes in the migration of conventional $CD4^+$ and $CD8^+$ T cells in melanoma lesions. It is plausible that effector T cells use other chemokine receptors for their trafficking to the tumor microenvironment and are not negatively influenced by the treatment. We suggest that blocking CCR5/CCR5 ligand interactions could be combined with other melanoma immunotherapeutic strategies to enhance their efficiency by neutralizing immunosuppression in the tumor microenvironment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Eggermont AM, Spatz A, Robert C (2014) Cutaneous melanoma. Lancet 383:816–827
2. Stadler S, Weina K, Gebhardt C, Utikal J (2015) New therapeutic options for advanced non-resectable malignant melanoma. Adv Med Sci 60:83–88
3. Gogas H, Polyzos A, Kirkwood J (2013) Immunotherapy for advanced melanoma: fulfilling the promise. Cancer Treat Rev 39:879–885
4. Rosenberg SA, Restifo NP (2015) Adoptive cell transfer as personalized immunotherapy for human cancer. Science 348:62–68
5. Postow MA, Callahan MK, Wolchok JD (2015) Immune checkpoint blockade in cancer therapy. J Clin Oncol 33:1974–1982
6. Gebhardt C, Sevko A, Jiang H, Lichtenberger R, Reith M, Tarnanidis K, Holland-Letz T, Umansky L, Beckhove P, Sucker A, Schadendorf D, Utikal J, Umansky V (2015) Myeloid cells and related chronic inflammatory factors as novel predictive markers in melanoma treatment with ipilimumab. Clin Cancer Res 21:5453–5459
7. Umansky V, Sevko A, Gebhardt C, Utikal J (2014) Myeloid-derived suppressor cells in malignant melanoma. J Dtsch Dermatol Ges 12:1021–1027
8. Zimmer L, Eigentler TK, Kiecker F, Simon J, Utikal J, Mohr P, Berking C, Kämpgen E, Dippel E, Stadler R, Hauschild A, Fluck M, Terheyden P, Rompel R, Loquai C, Assi Z, Garbe C, Schadendorf D (2015) Open-label, multicenter, single-arm phase II DeCOG-study of ipilimumab in pretreated patients with different subtypes of metastatic melanoma. J Transl Med 13:351
9. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V (2012) Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 12:253–268
10. Parker KH, Beury DW, Ostrand-Rosenberg S (2015) Myeloid-derived suppressor cells: critical cells driving immune suppression in the tumor microenvironment. Adv Cancer Res 128:95–139
11. Kanterman J, Sade-Feldman M, Baniyash M (2012) New insights into chronic inflammation-induced immunosuppression. Semin Cancer Biol 22:307–318
12. Umansky V, Sevko A (2012) Overcoming immunosuppression in the melanoma microenvironment induced by chronic inflammation. Cancer Immunol Immunother 61:275–282
13. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, Mandruzzato S, Murray PJ, Ochoa A, Ostrand-Rosenberg S, Rodriguez PC, Sica A, Umansky V, Vonderheide RH, Gabrilovich DI (2016) Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun 7:12150
14. Solito S, Marigo I, Pinton L, Damuzzo V, Mandruzzato S, Bronte V (2014) Myeloid-derived suppressor cell heterogeneity in human cancers. Ann NY Acad Sci 1319:47–65
15. Filipazzi P, Huber V, Rivoltini L (2012) Phenotype, function and clinical implications of myeloid-derived suppressor cells in cancer patients. Cancer Immunol Immunother 61:255–263
16. Ostrand-Rosenberg S (2010) Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumor immunity. Cancer Immunol Immunother 59:1593–1600
17. Homey B, Muller A, Zlotnik A (2002) Chemokines: agents for the immunotherapy of cancer?. Nat Rev Immunol 2:175–184.
18. Combadiere C, Ahuja SK, Tiffany HL, Murphy PM (1996) Cloning and functional expression of CC CKR5, a human monocyte CC chemokine receptor selective for MIP-1(alpha), MIP-1(beta), and RANTES. J Leukoc Biol 60:147–152
19. Lesokhin AM, Hohl TM, Kitano S, Cortez C, Hirschhorn-Cymerman D, Avogadri F, Rizzuto GA, Lazarus JJ, Pamer EG, Houghton AN, Merghoub T, Wolchok JD (2012) Monocytic CCR2(+) myeloid-derived suppressor cells promote immune escape by limiting activated CD8 T-cell infiltration into the tumor microenvironment. Cancer Res 72:876–886
20. Sawanobori Y, Ueha S, Kurachi M, Shimaoka T, Talmadge JE, Abe J, Shono Y, Kitabatake M, Kakimi K, Mukaida N, Matsushima K (2008) Chemokine-mediated rapid turnover of myeloid-derived suppressor cells in tumor-bearing mice. Blood 111:5457–5466
21. Izhak L, Wildbaum G, Zohar Y, Anunu R, Klapper L, Elkeles A, Seagal J, Yefenof E, Ayalon-Soffer M, Karin N (2009) A novel recombinant fusion protein encoding a 20-amino acid residue of the third extracellular (E3) domain of CCR2 neutralizes the biological activity of CCL2. J Immunol 183:732–739
22. Izhak L, Wildbaum G, Weinberg U, Shaked Y, Alami J, Dumont D, Friedman B, Stein A, Karin N (2010) Predominant expression of CCL2 at the tumor site of prostate cancer patients directs a selective loss of immunological tolerance to CCL2 that could be amplified in a beneficial manner. J Immunol 184:1092–1101
23. Izhak L, Wildbaum G, Jung S, Stein A, Shaked Y, Karin N (2012) Dissecting the autocrine and paracrine roles of the CCR2–CCL2 axis in tumor survival and angiogenesis. PLoS ONE 7:e28305
24. Ugel S, De Sanctis F, Mandruzzato S, Bronte V (2015) Tumor-induced myeloid deviation: when myeloid-derived suppressor cells meet tumor-associated macrophages. J Clin Investig 125:3365–3376
25. Kalinski P (2012) Regulation of immune responses by prostaglandin E2. J Immunol 188:21–28
26. Williams SA, Harata-Lee Y, Comerford I, Anderson RL, Smyth MJ, McColl SR (2010) Multiple functions of CXCL12 in a syngeneic model of breast cancer. Mol Cancer 9:250
27. Connolly MK, Mallen-St Clair J, Bedrosian AS, Malhotra A, Vera V, Ibrahim J, Henning J, Pachter HL, Bar-Sagi D, Frey AB, Miller G (2010) Distinct populations of metastases-enabling myeloid cells expand in the liver of mice harboring invasive and preinvasive intra-abdominal tumor. J Leukoc Biol 87:713–725
28. Wang SW, Liu SC, Sun HL, Huang TY, Chan CH, Yang CY, Yeh HI, Huang YL, Chou WY, Lin YM, Tang CH (2015) CCL5/CCR5 axis induces vascular endothelial growth factor-mediated tumor angiogenesis in human osteosarcoma microenvironment. Carcinogenesis 36:104–114
29. Appay V, Rowland-Jones SL (2001) RANTES: a versatile and controversial chemokine. Trends Immunol 22:83–87
30. Harper AR, Nayee S, Topol EJ (2015) Protective alleles and modifier variants in human health and disease. Nat Rev Genet 16:689–701
31. Balistreri CR, Carruba G, Calabrò M, Campisi I, Di Carlo D, Lio D, Colonna-Romano G, Candore G, Caruso C (2009) CCR5 proinflammatory allele in prostate cancer risk: a pilot study in patients and centenarians from Sicily. Ann NY Acad Sci 1155:289–292

32. Velasco-Velazquez M, Jiao X, De La Fuente M, Pestell TG, Ertel A, Lisanti MP, Pestell RG (2012) CCR5 antagonist blocks metastasis of basal breast cancer cells. Cancer Res 72:3839–3850
33. Song JK, Park MH, Choi DY, Yoo HS, Han SB, Yoon DY, Hong JT (2012) Deficiency of C-C chemokine receptor 5 suppresses tumor development via inactivation of NF-kappaB and upregulation of IL-1Ra in melanoma model. PLoS ONE 7:e33747
34. Mencarelli A, Graziosi L, Renga B, Cipriani S, D'Amore C, Francisci D, Bruno A, Baldelli F, Donini A, Fiorucci S (2013) CCR5 antagonism by maraviroc reduces the potential for gastric cancer cell dissemination. Transl Oncol 6:784–793
35. Sicoli D, Jiao X, Ju X, Velasco-Velazquez M, Ertel A, Addya S, Li Z, Andò S, Fatatis A, Paudyal B, Cristofanilli M, Thakur ML, Lisanti MP, Pestell RG (2014) CCR5 receptor antagonists block metastasis to bone of v-Src oncogene-transformed metastatic prostate cancer cell lines. Cancer Res 74:7103–7114
36. Che LF, Shao SF, Wang LX (2016) Downregulation of CCR5 inhibits the proliferation and invasion of cervical cancer cells and is regulated by microRNA-107. Exp Ther Med 11:503–509.
37. van Deventer HW, O'Connor W Jr, Brickey WJ, Aris RM, Ting JP, Serody JS (2005) C-C chemokine receptor 5 on stromal cells promotes pulmonary metastasis. Cancer Res 65:3374–3379
38. Ng-Cashin J, Kuhns JJ, Burkett SE, Powderly JD, Craven RR, van Deventer HW, Kirby SL, Serody JS (2003) Host absence of CCR5 potentiates dendritic cell vaccination. J Immunol 170:4201–4208
39. Aldinucci D, Colombatti A (2014) The inflammatory chemokine CCL5 and cancer progression. Mediators Inflamm 2014:292376
40. Chang LY, Lin YC, Kang CW, Hsu CY, Chu YY, Huang CT, Day YJ, Chen TC, Yeh CT, Lin CY (2012) The indispensable role of CCR5 for in vivo suppressor function of tumor-derived CD103+ effector/memory regulatory T cells. J Immunol 189:567–574
41. Schlecker E, Stojanovic A, Eisen C, Quack C, Falk CS, Umansky V, Cerwenka A (2012) Tumor-infiltrating monocytic myeloid-derived suppressor cells mediate CCR5-dependent recruitment of regulatory T cells favoring tumor growth. J Immunol 189:5602–5611
42. Umansky V, Abschuetz O, Osen W, Ramacher M, Zhao F, Kato M, Schadendorf D (2008) Melanoma-specific memory T cells are functionally active in Ret transgenic mice without macroscopic tumors. Cancer Res 68:9451–9458
43. Zhu Z, Aref AR, Cohoon TJ, Barbie TU, Imamura Y, Yang S, Moody SE, Shen RR, Schinzel AC, Thai TC, Reibel JB, Tamayo P, Godfrey JT, Qian ZR, Page AN, Maciag K, Chan EM, Silkworth W, Labowsky MT, Rozhansky L, Mesirov JP, Gillanders WE, Ogino S, Hacohen N, Gaudet S, Eck MJ, Engelman JA, Corcoran RB, Wong KK, Hahn WC, Barbie DA (2014) Inhibition of KRAS-driven tumorigenicity by interruption of an autocrine cytokine circuit. Cancer Discov 4:452–465.
44. Richmond A, Yang J, Su Y (2009) The good and the bad of chemokines/chemokine receptors in melanoma. Pigment Cell Melanoma Res 22:175–186
45. Gao D, Rahbar R, Fish EN (2016) CCL5 activation of CCR5 regulates cell metabolism to enhance proliferation of breast cancer cells. Open Biol 6:160122
46. Meyer C, Sevko A, Ramacher M, Bazhin AV, Falk CS, Osen W, Borrello I, Kato M, Schadendorf D, Baniyash M, Umansky V (2011) Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model. Proc Natl Acad Sci USA 108:17111–17116
47. Sevko A, Michels T, Vrohlings M, Umansky L, Beckhove P, Kato M, Shurin GV, Shurin MR, Umansky V (2013) Antitumor effect of paclitaxel is mediated by inhibition of myeloid-derived suppressor cells and chronic inflammation in the spontaneous melanoma model. J Immunol 190:2464–2471
48. Lin S, Wan S, Sun L, Hu J, Fang D, Zhao R, Yuan S, Zhang L (2012) Chemokine C-C motif receptor 5 and C-C motif ligand 5 promote cancer cell migration under hypoxia. Cancer Sci 103:904–912
49. Zhang Y, Lv D, Kim HJ, Kurt RA, Bu W, Li Y, Ma X (2013) A novel role of hematopoietic CCL5 in promoting triple-negative mammary tumor progression by regulating generation of myeloid-derived suppressor cells. Cell Res 23:394–408
50. Ward ST, Li KK, Hepburn E, Weston CJ, Curbishley SM, Reynolds GM, Hejmadi RK, Bicknell R, Eksteen B, Ismail T, Rot A, Adams DH (2015) The effects of CCR5 inhibition on regulatory T-cell recruitment to colorectal cancer. Br J Cancer 112:319–328
51. Ray N (2009) Maraviroc in the treatment of HIV infection. Drug Des Dev Ther 2:151–161
52. Saita Y, Kondo M, Shimizu Y (2007) Species selectivity of small-molecular antagonists for the CCR5 chemokine receptor. Int Immunopharmacol 7:1528–1534
53. Pervaiz A, Ansari S, Berger MR, Adwan H (2015) CCR5 blockage by maraviroc induces cytotoxic and apoptotic effects in colorectal cancer cells. Med Oncol 32:158
54. Halama N, Zoernig I, Berthel A, Kahlert C, Klupp F, Suarez-Carmona M, Suetterlin T, Brand K, Krauss J, Lasitschka F, Lerchl T, Luckner-Minden C, Ulrich A, Koch M, Weitz J, Schneider M, Buechler MW, Zitvogel L, Herrmann T, Benner A, Kunz C, Luecke S, Springfeld C, Grabe N, Falk CS, Jaeger D (2016) Tumoral immune cell exploitation in colorectal cancer metastases can be targeted effectively by anti-CCR5 therapy in cancer patients. Cancer Cell 29:587–601
55. Tang Q, Jiang J, Liu J (2015) CCR5 blockade suppresses melanoma development through inhibition of IL-6-Stat3 pathway via upregulation of SOCS3. Inflammation 38:2049–2056
56. Chang LY, Lin YC, Mahalingam J, Huang CT, Chen TW, Kang CW, Peng HM, Chu YY, Chiang JM, Dutta A, Day YJ, Chen TC, Yeh CT, Lin CY (2012) Tumor-derived chemokine CCL5 enhances TGF-beta-mediated killing of CD8(+) T cells in colon cancer by T-regulatory cells. Cancer Res 72:1092–1102
57. Sapir Y, Vitenshtein A, Barsheshet Y, Zohar Y, Wildbaum G, Karin N (2010) A fusion protein encoding the second extracellular domain of CCR5 arrests chemokine-induced cosignaling and effectively suppresses ongoing experimental autoimmune encephalomyelitis. J Immunol 185:2589–2599
58. Jordan KR, Amaria RN, Ramirez O, Callihan EB, Gao D, Borakove M, Manthey E, Borges VF, McCarter MD (2013) Myeloid-derived suppressor cells are associated with disease progression and decreased overall survival in advanced-stage melanoma patients. Cancer Immunol Immunother 62:1711–1722
59. Weide B, Martens A, Zelba H, Derhovanessian E, Bailur JK, Kyzirakos C, Pflugfelder A, Eigentler TK, Di Giacomo AM, Maio M, Aarntzen EH, de Vries J, Sucker A, Schadendorf D, Büttner P, Garbe C, Pawelec G (2014) Myeloid-derived suppressor cells predict survival of advanced melanoma patients: comparison with regulatory T cells and NY-ESO-1- or Melan-A-specific T cells. Clin Cancer Res 20:1601–1609
60. Pico de Coaña Y, Poschke I, Gentilcore G, Mao Y, Nyström M, Hansson J, Masucci GV, Kiessling R (2013) Ipilimumab treatment results in an early decrease in the frequency of circulating granulocytic myeloid-derived suppressor cells as well as their Arginase1 production. Cancer Immunol Res 1:158–162
61. Zhang B, Wang Z, Wu L, Zhang M, Li W, Ding J, Zhu J, Wei H, Zhao K (2013) Circulating and tumor-infiltrating myeloid-derived suppressor cells in patients with colorectal carcinoma. PLoS ONE 8:e57114
62. Solito S, Falisi E, Diaz-Montero CM, Doni A, Pinton L, Rosato A, Francescato S, Basso G, Zanovello P, Onicescu G, Garrett-Mayer E, Montero AJ, Bronte V, Mandruzzato S (2011) A human promyelocytic-like population is responsible for the

immune suppression mediated by myeloid-derived suppressor cells. Blood 118:2254–2265
63. Jiang H, Gebhardt C, Umansky L, Beckhove P, Schulze TJ, Utikal J, Umansky V (2015) Elevated chronic inflammatory factors and myeloid-derived suppressor cells indicate poor prognosis in advanced melanoma patients. Int J Cancer 136:2352–2360
64. Payne AS, Cornelius LA (2002) The role of chemokines in melanoma tumor growth and metastasis. J Investig Dermatol 118:915–922