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The good and the bad of chemokines/chemokine receptors in melanoma

Ann Richmond, **Jinming Yang**, and **Yingjun Su**
Department of Veterans Affairs and Department of Cancer Biology, Vanderbilt University School of Medicine, Nashville, TN, USA

Summary

Chemokine ligand/receptor interactions affect melanoma cell growth, stimulate or inhibit angiogenesis, recruit leukocytes, promote metastasis, and alter the gene expression profile of the melanoma associated fibroblasts. Chemokine/chemokine receptor interactions can protect against tumor development/growth or can stimulate melanoma tumor progression, tumor growth and metastasis. Metastatic melanoma cells express chemokine receptors that play a major role in the specifying the organ site for metastasis, based upon receptor detection of the chemokine gradient elaborated by a specific organ/tissue. A therapeutic approach that utilizes the protective benefit of chemokines involves delivery of angiostatic chemokines or chemokines that stimulate the infiltration of cytotoxic T cells and natural killer T cells into the tumor microenvironment. An alternative approach that tackles the tumorigenic property of chemokines uses chemokine antibodies or chemokine receptor antagonists to target the growth and metastatic properties of these interactions. Based upon our current understanding of the role of chemokine-mediated inflammation in cancer, it is important that we learn to appropriately regulate the chemokine contribution to the tumorigenic `cytokine/chemokine storm', and to metastasis.

Keywords

melanoma; chemokine; chemokine receptor; metastasis; angiogenesis; tumor microenvironment; inflammation

Introduction

Chemokines are secreted, low molecular weight proteins (~8–30 kDa) that bind to seven transmembrane G protein-coupled receptors. There are approximately 50 chemokines characterized to date and over 20 chemokine receptors (Zlotnik et al., 2006) (Figure 1). Chemokines have conserved cysteine residues that play an important role in their structural conformation and function. Depending upon whether there are intervening amino acids between the first two conserved cysteines, chemokines are classified into subfamilies denoted as CXC, CC, CX3C and C, where the CXC chemokines have one amino acid between the first two cysteine residues, CC chemokines lack this intervening amino acid, CX3C have three intervening amino acids between the first two cysteines, and the C subfamily lacks one cysteine in the first pair of cysteine residues. Figure 1 shows the chemokines that bind to each chemokine receptor. For example CXCR1 binds CXCL6 and CXCL8, while CXCR2 binds CXCL1–3, 5–8. CXCR4 only binds CXCL12, CX3CR1 binds only CX3CL1, and CCR9 only binds CCL25. CCR7 binds CCL19 and 21, while CCR2 binds CCL2, 7, 8, 13 and 16.

CORRESPONDENCE Ann Richmond, ann.richmond@vanderbilt.edu.

Though chemokines were first characterized in reference to their role in leukocyte trafficking, over time we have come to understand that chemokines are produced by most tissues and chemokine receptors are expressed not only on leukocytes, but on most cell types. Chemokine/chemokine receptor interactions are important in development, wound healing, infection, and tissue maintenance. The expression of chemokines and chemokine receptors are often strongly up-regulated during tumorigenesis (Figure 2). This is true in cancers of the breast, lung, prostate, colon, ovary, bladder, pancreas, liver, skin (including melanocytes), head and neck, cervix, kidney, brain, and blood cells including multiple myeloma and leukemia (Raman et al., 2007). Tumor cells take advantage of the expression of chemokines and chemokine receptors to either stimulate the immune response to the tumor or to induce tumor angiogenesis and tumor growth, alter the tumor microenvironment, and facilitate metastasis to specific target organs (Figure 3). A number of reviews have been written previously attesting to the role of chemokines in melanoma tumor growth and metastasis (Dhawan and Richmond, 2002;Dhawan et al., 2008;Kakinuma and Hwang, 2006;Luan et al., 1997;Murakami et al., 2004;Payne and Cornelius, 2002;Raman et al., 2007;Zigler et al., 2008) and signal transduction pathways associated with chemokine receptor activation that modulate cell motility, cell growth, and chemotaxis have been recently reviewed (Thelen and Stein, 2008). Therefore, this review will provide an update on chemokine/chemokine receptors as mediators of melanoma tumor progression/growth, tumor angiogenesis, metastasis, alterations in the tumor microenvironment, or as regulators of the innate immune response to the tumor. Finally, chemokines and chemokine receptors as targets for melanoma therapy and prognosis will be discussed.

Melanoma tumor cells have been reported to produce CXCL1–3, CXCL5–8, CXCL10, CCL2, and CCL5. Interestingly, chemokine receptors have also been reported to be expressed by melanoma cells, including CXCR1, CXCR2, CXCR3, CXCR4, CXCR6, CXCR7, CCR1, CCR2, CCR5, CCR7, CCR9, CCR10 (Navarini-Meury and Conrad, 2008; Raman et al., 2007; Richmond, 2008). CCR5 and CCR7 are expressed in primary melanoma lesions and some metastatic lesions, while CXCR4 and CCR1 are expressed in melanocytes, melanoma cell lines, primary and metastatic melanomas (Seidl et al., 2007). Expression of CXCR3 by melanoma tumor cells has been correlated with absence of the tumor infiltrating lymphocytes and a poorer prognosis in melanoma (Monteagudo et al., 2007). The mechanism for this quite interesting observation is unclear.

Chemokines/chemokine receptors in melanoma tumor progression and tumor growth

CXCL8 as well as CXCR1 and CXCR2 are associated with melanoma tumor progression by affecting growth of the tumor cells, angiogenesis and metastasis (Bar-Eli, 1999; Norgauer et al., 1996; Ramjeesingh et al., 2003; Schadendorf et al., 1993; Varney et al., 2006; Wang et al., 1990). During melanoma tumor progression, chemokine expression progressively increases, in part due to the deregulation of the NF-κB family of transcription factors (Richmond, 2002; Yang and Richmond, 2001). In fact, blocking NF-κB inhibits the endogenous production of angiogenic chemokines and inhibits melanoma tumor growth in mice (Yang et al., 2006; Yang et al., 2007).

Gene expression profiling studies show that CXCL1 mRNA is highly upregulated in three dimensional cultures of melanoma cells and in human melanoma tumors (Ghosh et al., 2005; Haqq et al., 2005; Taxman et al., 2003). CXCL1–3 can act as oncogenes in immortalized murine melanocytes. Over-expression of CXCL1, 2 or 3 in spontaneously immortalized melanocytes (Bennett et al., 1987) confers the malignant phenotype on these cells based upon enhanced growth in soft agar and tumor formation in mice (Balentien et al., 1991; Owen et al., 1997). Moreover, antibodies to CXCL1, 2, or 3 inhibit the growth of melanoma tumors expressing

each of these respective chemokines in mice (Haghnegahdar et al., 2000; Owen et al., 1997). Additional evidence in support for the transforming properties of CXCL1 comes from genetically modified mice. INK4a/ARF null transgenic mice expressing MIP-2 (murine ortholog of CXCL1) under the transcriptional direction of the tyrosinase promoter/enhancer, exhibited increased melanoma tumor incidence after treatment of the skin with the chemical carcinogen, DMBA (Yang et al., 2001). Altogether, these data point to an important role for this chemokine in the promotion of melanocyte transformation.

Angiogenesis

A number of angiogenic chemokines are produced by malignant melanoma cells. The major ones are CXCL1–3, CXCL5–8. These ligands bind to CXCR2, which is expressed on endothelial cells, and promote the growth and migration of these endothelial cells to facilitate tumor angiogenesis. CXCL8 and CXCL6 also bind CXCR1 which functions much like CXCR2. Varney et al. have shown that CXCR1 is expressed ubiquitously in melanoma tumor specimen but the CXCR1 expression does not correlate with the Clark level (Varney et al., 2003). In contrast, expression of CXCL8 and CXCR2 increased between thin and thick melanoma as well as with metastatic lesions. CXCL8 and CXCR2 expression increase during the transition from radial to vertical growth phase is thought to be important in the transition to increased aggressiveness of tumors and the metastatic properties of the tumor (Varney et al., 2006).

Melanoma cells exhibit the capacity to form tube-like structures that mimic endothelial cells, and this is termed `vasculogenic mimicry' (Maniotis et al., 1999). CXCL8 and several other genes co-expressed with galectin-3 have been linked to melanoma cell vascular mimicry (Mourad-Zeidan et al., 2008). Silencing of galectin-3 reduces the transcription of vascular endothelial-cadherin and CXCL8 and results in a loss of vascular mimicry properties of human melanoma cells. Though the data do not point to a direct link between chemokine expression and vascular mimicry, the association is quite interesting.

Thrombin induction of angiogenesis has been shown to up-regulate CXCL1 expression, which in turn stimulates angiogenesis around melanoma tumors, MMP1, MMP2, VEGF, angiopoietin-2, CD31 and KDR as well as CXCR2 expression in HUVECs. The effects of thrombin on the induction of gene expression and angiogenesis in B16 melanoma cells were blocked with shRNA for CXCL1, strongly supporting a major role for CXCL1 in melanoma tumor angiogenesis (Caunt et al., 2006). In addition, there are reports that activation of CXCR2 leads to an up-regulation of VEGF, which can in turn further promote tumor angiogenesis (Caunt et al., 2006).

Several chemokines outside the CXC group are elaborated by melanoma cells and affect the tumor vasculature, including CCL2 (Graves et al., 1992), CX3CL (Ren et al., 2007) and CCL5 (Mrowietz et al., 1999). CCL2 has been implicated in arteriogenesis (Keeley et al., 2008). Fractalkine (CX3CL) is expressed on the surface of B16 melanoma cells and promotes cell-cell adhesion. When CX3CL1 expression is targeted by sh-RNA vectors, the growth of the B16 melanoma tumors is slowed and there is reduced tumor angiogenesis (Ren et al., 2007). The full mechanism for this reduced angiogenesis has not yet been determined.

In addition to angiogenic chemokines, melanoma cells also produce the angiostatic chemokines CXCL9, CXCL10, and CXCL11. These angiostatic chemokines bind CXCR3 and inhibit angiogenesis (Keeley et al., 2008; Mehrad et al., 2007). In addition, a variant of another angiostatic chemokine, CXCL4, is reported to block angiogenesis and inhibit melanoma tumor growth (Struyf et al., 2007). CXCL11 binds to both CXCR3 and CXCR7, but the functional significance of the CXCR7 interaction remains unclear. CXCR7 is expressed on both endothelial cells and tumor cells. There are reports that CXCR7 competes with CXCR4 for

CXCL12 binding and in this way serves as a `decoy' receptor (Boldajipour et al., 2008). Other reports argue that CXCR7 is capable of stimulating the growth of tumors (Meijer et al., 2008; Miao et al., 2007; Wang et al., 2008). CXCR2, CXCR3, CXCR4 and CXCR7 are expressed on melanoma cells and to a lesser extent on endothelial cells (Schutyser et al., 2007). These data argue that each of these chemokine receptors are available in vivo for modulation of melanoma tumor cell growth, regulation of angiogenesis, or migration.

CXCR4 expression on vascular endothelial cells is reported to be pro-angiogenic (Chen et al., 2006; Guleng et al., 2005; Liang et al., 2007; Petit et al., 2007; Salcedo and Oppenheim, 2003; Zagzag et al., 2006), though controversy remains as to whether this is a direct or indirect effect. Hypoxia has been shown to induce the expression of CXCR4 on HMEC-1 microvascular endothelial cells and on SK-Mel5 melanoma cells, but hypoxia does not induce the expression of CXCR2 or CXCR3 (Schutyser et al., 2007). Since hypoxia induces new angiogenesis, perhaps induction of CXCR4 on endothelial cells facilitates this.

Metastasis

Perhaps the most progress in understanding the role of chemokines in melanoma in the last 5 yrs has been made in the area of metastasis. We have come to understand that CXCR4, CCR7 and CCR9 are major determinates of melanoma metastasis to specific target organs. CXCR4 expressing melanomas tend to metastasize to the lung, bone marrow and liver, while CCR7 and CXCR3 expressing melanoma lesions metastasize to the lymph node (Cardones et al., 2003; Kawada et al., 2004; Kim et al., 2006; Murakami et al., 2004; Murakami et al., 2002; Scala et al., 2007). CXCR4 is the most widely expressed chemokine receptor in melanoma based upon microarray studies and qRT (Kim et al., 2006). CXCL12 expression by liver, lung and bone marrow promote metastasis of melanoma tumor cells to these organs (Kim et al., 2006). For uveal melanoma, both CXCR4 and CCR7 are reported to be associated with liver metastasis (Li et al., 2008; Scala et al., 2007). CCR9 expressing melanomas have a very high probability of metastasizing to the small intestine (Amersi et al., 2008). In addition, there is preclinical evidence that CXCR2 is important for melanoma metastasis to the lung (Singh et al., 2009).

CXCR4 is important for early steps in melanoma metastasis to the lung. Knocking down CXCR4 in melanoma cells impairs melanoma metastasis to the lung in a murine xenograft model (Bartolome et al., 2009). CXCR4 activation by CXCL12 also induces expression of the MT1 membrane-bound matrix metalloproteinase (MT1-MMP), but this matrix metalloproteinase (MMP) remains intracellular until the cells come in contact with the appropriate basement membrane proteins, then MT1-MMP moves to the plasma membrane in a PI-3K dependent manner (Bartolome et al., 2009). Tumor cell expression of CXCR4, down regulation of E-cadherin, and elevations in activated Cdc42 are associated with poor prognosis in melanoma. Inhibition of the CXCR4/CXCL12 pathway by pretreatment of melanoma cells with CTCE-9908, a small peptide antagonist of CXCR4, reduces pulmonary metastasis in a melanoma mouse tail vein injection model (Kim et al., 2008). This inhibition did not occur unless the melanoma cells were pretreated with CTCE-9908, indicating the antagonist was disrupting the interaction of tumor cell CXCR4 with ligand and was not due to an effect on the tumor infiltrating lymphocytes or endothelial cells.

CCL21, one of the ligands for CCR7, is produced by lymphatic endothelial cells and the release of this chemokine by the lymphatic endothelial cells is associated with the metastatic properties of CCR7 expressing malignant melanoma cells (Shields et al., 2007; Wiley et al., 2001). CCL27 is produced by the skin and when melanoma cells express its receptor, CCR10, this facilitates skin metastasis (Ben-Baruch, 2008; Forster et al., 2001; Simonetti et al., 2006; Takeuchi et al., 2004; Takeuchi et al., 2007). A study of 59 cutaneous melanocytic lesions demonstrated that

CCR10 is expressed in both benign and malignant melanoma lesions and the CCR10 expression level correlates with the Breslow depth (Simonetti et al., 2006). Interestingly, in that study CCL27 expression levels correlated with reduced levels of $CD3^+$ and $CD8^+$ lymphocytes in the lesions, suggesting that CCR10/CCL27 may facilitate escape from host immune surveilance and facilitate tumor growth.

CCR9 has been described as a `homing receptor' for melanoma metastasis to the small bowel. CCL25, the ligand for CCR9 is highly expressed in small bowel and thymus. It has been demonstrated that 102 out of 198 melanomas metastasize to the small bowel and 88 of 102 with small bowel metastasis expressed CCR9. Thus CCR9 expression on melanoma carries a greater than 60% chance that there will be small bowel metastasis (Amersi et al., 2008).

Expression of CCL5 by melanoma cells has been linked to tumor progression. Melanomas that express CCL5 are reported to be more aggressive, though receptors for this chemokine are not usually expressed by melanoma cells. However CCR5 expression on stromal cells has been reported to aid lung metastasis for melanoma in a murine model (van Deventer et al., 2005). The mechanism for this is unclear, but probably involves recruitment of macrophages into the tumor microenvironment that subsequently facilitate metastasis. Interestingly, the expression of the mutant form of CCR5, CCR5Δ32, in stage IV melanoma patients was associated with a decreased survival following immunotherapy, suggesting that patients with CCR5Δ32 mutations are less prone to benefit from immunotherapy (Ugurel et al., 2008).

Ultraviolet-B irradiation stimulates the production of CXCL8 which in turn enhances the migration of metastatic melanoma cells in vitro (Gebhardt et al., 2007). One of the receptors for CXCL8, CXCR1, is reported to be important for trans-endothelial migration of melanoma cells (Ramjeesingh et al., 2003). In vivo murine studies show that CXCR2 plays a major role in melanoma metastasis to the lung. In a very elegant series of experiments the $CXCR2^{-/-}$ Balb/C mouse was bred onto a Balb/C nude background. B16 melanoma xenografts exhibited inhibition of melanoma tumor growth and inhibition of lung metastasis on $CXCR2^{-/-}$ nude mice. This was accompanied by reduction in tumor cell proliferation, angiogenesis and reduced inflammation (Singh et al., 2009).

Alterations in the tumor microenvironment

Mantovani has recently reviewed the role that inflammatory cytokines play to facilitate the metastatic capacity of tumor cells. Included are interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNFα) and receptor activator of nuclear factor-kappa B ligand (RANKL). Each of these cytokines is regulated by the nuclear factor-kappa B (NF-κB) family of transcription factors (Mantovani, 2009). Toll-like receptors (TLRs) also can play a role in this NF-κB activation that induces expression of chemokines and chemokine receptors to augment the inflammatory process and produce the `cytokine storm', a never ending cycle of macrophage recruitment, release of cytokines, and activation of signal transduction pathways that can facilitate tumor growth and metastasis. These chemokines, cytokines and growth factors released by macrophages subsequently affect the stromal cells as well and activate expression of a number of genes that can predispose the microenvironment to enhanced tumor growth.

Macrophages recruited into the tumor microenvironment are known as tumor associated macrophages or TAMs. There are two types of TAMs, M1 and M2 (Hagemann et al., 2008). The M1 macrophages are the TAMs classically activated by microbial products or IFN-γ. M1 TAMs are characterized by high levels of production of IL-12, high production of pro-inflammatory cytokines and high levels of MHC molecules. When NF-κB is activated in these macrophages, they convert from the classically activated and cytotoxic M1 to the alternatively activated M2 phenotype. M2 macrophages exhibit a change in cytokine production from IL-12 and IL-10 with high levels of MHC molecules (M1) to high production of TGFβ and IL-10 but

not IL-12, and there is low production of MHC. M2 macrophages with their respective cytokine profile promote tumorigenesis, while M1 macrophages are thought to be more anti-tumorigenic. M2 macrophages release CCL2, which can stimulate metalloproteinase production. Inhibition of IKKβ in bone marrow derived macrophages or tumor associated macrophages enhanced M1 macrophage mediated tumoricidal activity in vitro (Hagemann et al., 2008). This enhanced tumoricidal activity was accompanied by increased production of IL-12, decreased arginase-1 expression and elevated iNOS2 expression, all traits of M1 macrophages.

In vivo, melanoma produced chemokines also recruit tumor-specific T cells. Production of CCL2 by melanoma cells is important for recruitment of cytotoxic T lymphocytes (CTLs) to the tumor microenvironment through interaction with lymphocyte CCR4 expression (Ugurel et al., 2008; Zhang et al., 2006a). Depending upon the level of expression of CCL2, this chemokine can either promote or reduce melanoma tumor growth (Bottazzi et al., 1992). In elegant experiments CCL2 was targeted with shRNA in B16 melanoma cells and the transfected melanoma cells were injected into the pleural cavity of syngeneic immunocompetent mice (Stathopoulos et al., 2008). Mice injected with the B16 melanoma cells expressing shRNA against CCL2 exhibited reduced malignant pleural effusions and enhanced survival, compared to control shRNA transfectants. If CCL2 was over-expressed, there was an increase in malignant pleural effusions, enhanced vascular permeability, mononuclear recruitment, angiogenesis and decreased survival. CCL2 also plays a role in recruitment of M2 macrophages which subsequently stimulate massive tumor angiogenesis (Gazzaniga et al., 2007). However, these data are in direct conflict with another report that over-expression of CCL2 in B16 melanoma cells resulted in enhanced production of Th2 cytokines and reduced melanoma tumor growth in syngenic C57Bl/6 mice (Hu et al., 2007). This reduction in tumor growth with over-expression of CCL2 was not observed in the CCL2 expressing B16 melanoma cells xenografted into nude mice. Since nude mice with human melanoma xenografts that do not express CCL2 exhibit reduced T cell homing to the tumor after adoptive transfer of human cytotoxic lymphocytes (CTLs), it appears that CCL2 produced by tumor cells can boost the recruitment of adoptively transferred human CTLs to the tumor microenvironment and suppress tumor growth (Brown et al., 2007). This type of dichotomy in the role of chemokines on the immune system may be tightly linked to the tumor microenvironment. There will be different outcomes depending upon whether the T cells that are recruited are capable of tumor cell killing, or whether they promote tumor metastasis through release of factors that facilitate intravasation of tumor cells into the vascular system.

Recruitment of $CD8^+$ T cells and natural killer T cells (NKT) to the tumor microenvironment is very important for immune surveillance and anti-tumor defense processes. Parovirus delivery of CCL7 (MCP3) has been shown to boost T and NKT cell responses to melanoma tumors (Wetzel et al., 2007). Interestingly, expression of CXCR3 by $CD8^+$ $CD45RO^+$ T cells is associated with enhanced survival of stage III, but not stage IV, melanoma patients. This parameter may prove to be important for prognosis (Mullins et al., 2004). When melanoma cells were transfected to express CCL21, mice bearing these tumors survived longer after adoptive T cell therapy than mice bearing melanoma tumors that did not express CCL21 (Thanarajasingam et al., 2007). These data show that CCL21 primed antitumor immunity following adoptive T cell transfer. Additional data support for use of CCL21 to boost the immune response to the tumor by recruiting T cells and dendritic cells into the tumor microenvironment. Administering CCL21 prior to delivery of a plasmid DNA melanoma cancer vaccine induced strong immunity against the melanocyte antigen, TRP2 (Yamano et al., 2006). This observation could prove very useful in the clinic in the treatment of metastatic melanoma.

CXCR4 also is important for cytotoxic lymphocyte trafficking to melanoma tumor cells. As tumor cells secrete CXCL12 into the tumor microenvironment, CXCR4 expressing cytotoxic lymphocytes (Zhang et al., 2006b) and dendritic cells (Fushimi et al., 2006) move into the tumor microenvironment. This can be blocked with antibodies to CXCL12, CXCR4, very high concentrations of CXCL12, or a small molecule antagonist of CXCR4 (Zhang et al., 2006a; Zhang et al., 2005; Zhang et al., 2006b).

Chemokine serum levels are also reported to be biomarkers for inflammatory diseases as well as cancer. CXCL8 is a potential clinical biomarker for mutant B-RAF (V600E) mediated tumor growth since inhibition of B-RAF in melanoma tumor bearing mice reduces CXCL8 plasma levels (Crawford et al., 2008). Production of CXCL8 by human melanoma cells induces MMP-2 activity and increases both tumor growth and metastasis (Luca et al., 1997). Extensive data show that tumor cells also stimulate the production of CXCL1 and CXCL2 chemokines in fibroblasts, and these cancer associated fibroblasts play an important role in tumor progression and metastasis (Gallagher et al., 2005). Neutrophils recruited into the tumor microenvironment also are induced to produce CXCL8, which in turn promotes a microenvironment that is supportive of metastasis (Peng et al., 2007). Chemokines produced by tissues in predicted sites for metastasis are reported to be important in establishing the `premetastatic niche' through the recruitment of bone marrow derived progenitor stem cells (Croker and Allan, 2008; Kucia et al., 2005; Shiozawa et al., 2008).

Angiostatic chemokines as a therapeutic modality

Delivery of angiostatic chemokines to melanoma inhibits the growth of melanoma xenografts in mice. For example, xenograft models using human melanoma cells transfected to express CXCL10 grew more slowly than parental or vector control cells (Yang and Richmond, 2004). Moreover, expression of a mutant form of CXCL10 that cannot bind to CXCR3 in the melanoma cells resulted in tumor growth at a rate equivalent to that of parental melanoma cells. Mutation of the GAG binding domain alone did not reduce the CXCL10 growth inhibitory capacity (Yang and Richmond, 2004). These data argue that CXCL10 activation of the CXCR3 expressed on melanoma cells, endothelial cells, or immune cells is an important negative regulator of melanoma tumor growth. Interestingly, when B16 melanoma cells are given a vaccine strain encoding flk1 and one encoding CXCL10, the tumor growth was reduced, CTL responses were enhanced, angiogenesis and cell proliferation were reduced, and apoptosis was enhanced (Lu et al., 2008). Potentially, delivery of CXCL10 or factors that induce CXCL10 can be used for melanoma therapy. IL-12 biotherapy provides a potential example of this type of therapy, since IL-12 induces IFNγ, which in turn can induce CXCL9, 10 and 11 which are angiostatic (Lesinski et al., 2004; Okada et al., 2004; Palmer et al., 2001). Though a number of problems have arisen with IL-12 biotherapy, it is proposed that delivery of the IL-12 directly to the tumor cells may offer therapeutic potential through induction of an anti-angiogenic program (Airoldi et al., 2007). How much this is dependent upon induction of angiostatic chemokines remains in question, however.

Delivery of mutant CCL2 to melanoma bearing mice also inhibits tumor angiogenesis and tumor growth (Koga et al., 2008). The mutant CCL2 reduced TNFα, IL-1 and VEGF in the tumor microenvironment, likely due to the reduction in recruitment of tumor associated macrophages.

Therapy for melanoma based upon antagonizing chemokines

It has been postulated that therapies which can antagonize CXCR4, CCR7, CCR9, CCR10, CXCR2, CCL5, or CXCL8 will be useful for melanoma therapy. A number of peptide antagonists and small molecule inhibitors of chemokines and chemokine receptors are already being tested in preclinical models for therapeutic effects on melanoma tumors and other tumors

as well. CXCR4 has been targeted by a number of antagonists. One example of this is single subcutaneous delivery of the 4F-bTE peptide antagonist for CXCR4 in microcapsules of biodegradable poly D,L-lactic acid blocks lung metastasis of melanoma (Takenaga et al., 2004). Another example is the T22 peptide antagonist of CXCR4, which has also been shown to block lung metastasis of B16 melanoma cells expressing CXCR4. However, the T22 antagonist did not block the tumor growth of the CXCR4 expressing B16 melanoma cells (Lee et al., 2006). This suggests that combinational therapies will be needed to be used to block both growth and metastasis of melanoma tumors. The bicyclam AMD3100 is still another antagonist that targets CXCR4. AMD3100 has been successfully tested in patients with multiple myeloma, leukemia, non-Hodgkin's lymphoma to mobilize $CD34^+$ cells from the bone marrow (Devine et al., 2004; Fierro et al., 2008; Fruehauf et al., 2006). Though caution is needed with regard to detrimental cardiovascular effects based upon our knowledge from $CXCR4^{-/-}$ mice, CXCR4 may prove to be a useful target for melanoma and other cancer types.

The Duffy antigen receptor for chemokines (DARC) acts in part as a decoy receptor, binding CXC chemokines CXCL1, CXCL5, CXCL8, CCL5 and other chemokines. There are reports that persons who are $Dfy^{-/-}$ exhibit increased incidence of prostate cancer, suggesting this receptor may play a role as a negative regulator of tumor progression (Lentsch, 2002; Shen et al., 2006; Stephan et al., 2005). To determine whether DARC could serve as a negative regulator of melanoma tumor growth by providing a ligand sink for angiogenic chemokines and chemokines that recruit macrophages to the tumor microenvironment, we developed transgenic mice over-expressing DARC on the endothelial cells under the transcriptional regulation of the preproendothelin promoter/enhancer (PPEP). These PPEP-mDARC transgenic mice exhibited reduced melanoma tumor growth and reduced angiogenesis in association with the tumor (Horton et al., 2007). These data support the concept that targeting ligands for CXCR2 (CXCL1–3, and CXCL5–8) or the ligand CCL5 may offer therapeutic benefit for melanoma patients.

Antibodies to chemokines offer promise as a therapeutic modality for treatment of malignant melanoma. Humanized antibodies to CXCL8 have been shown to inhibit melanoma tumor growth, angiogenesis and metastasis (Melnikova and Bar-Eli, 2006; Zigler et al., 2008). Antagonists for CXCR2 are also under consideration for melanoma therapy. In vitro studies show that chemotherapy with paclitaxel dangerously induces CXCL1 and CXCL8 expression, but fortunately this induction can be inhibited with MEK inhibitors (Taxman et al., 2003). Schering Plough, AstraZeneca and Glaxo-Smith-Kline have developed CXCR2 antagonists that show some promise for cancer treatment (Gonsiorek et al., 2007; Wang et al., 2006; Wilson et al., 2008). Thus there is hope for utilizing CXC chemokine/chemokine receptor antagonist for future melanoma therapy.

Clearly we need drugs that appropriately and specifically target chemokines and we need appropriate molecular diagnostics on each melanoma patient tumor. With these in hand, we should be able to better predict the specific pathways affected in the patient tumor so that therapy can be individualized for each patient based upon the molecular genotype of the tumor. However, these genetic profiles will prove to be complex and depending upon the combination of genetic abnormalities any one patient has, therapeutic response will need to be differentially targeted. The complexity of the tumor profile is a direct consequence of mutations and loss of tumor suppressors. For example, mutation of BRAF, loss of P53, amplification of aurora kinases, loss of PTEN, mutation of N-Ras, loss of MITF, loss of INK4a/ARF are a few of the types of genetic alterations that often occur in melanoma. As research efforts continue toward improved diagnostics and development of drugs that specifically target the affected genes or pathways, there is hope for improved treatment of advanced melanoma patients.

New horizons for examining chemokines as prognostic indicators in cancer

We are learning that inflammation plays a key role in the development of many cancers. It remains unclear exactly how chronic inflammation is involved in initiation or progression of cancer, but recent work has shown that epigenetic processes may be involved (Hahn et al., 2008). Chemokine amplification leads to persistent leukocyte infiltration which is associated with tumorigenesis in a number of cancers including cancers of the lung, colon, liver, cervix, prostate, bladder, ovary, esophagus, skin and lymphatics (Balkwill et al., 2005; Balkwill and Mantovani, 2001; Mantovani, 2009; Mantovani et al., 2008).

Chemokine expression profiles have been shown to predict recurrence and disease free survival in many non-melanoma cancers. In prostate cancer, one study shows CX3CL1 and IL-15 expression in prostate tissue were associated with recurrence-free survival following prostatectomy, while CCL4 expression was associated with recurrence (Blum et al., 2008). Another study examining cancer cell expression of the receptor for CX3CL1, CX3CR1, shows that high expression of CX3CR1 in pancreatic ductal adenocarcinoma is associated with neurotropism of these cancer cells, likely due to the expression of CX3CL1 by neurons (Marchesi et al., 2008).

CXCR4 expression in primary tumors after neo-adjuvant chemotherapy has been demonstrated to predict poor outcome for patients with locally advanced breast cancer (Holm et al., 2009). Elevation in CXCR4 predicts recurrence in HER-2 negative breast cancer (Holm et al., 2007). Over-expression of CXCR4 and VEGF predict early distant relapse in stage II–III colorectal cancer patients (Ottaiano et al., 2006).

CCR7 expression in metastatic squamous cell carcinoma (SCC) is associated with lower incidence of disease free survival and lower overall survival (Tsuzuki et al., 2006). CCR7 positive SCCs were associated with large lymph node metastases, progressive disease, local recurrence and death due to cancer. Learning how to prevent this process will be important for future therapies (Tsuzuki et al., 2006).

Polymorphisms in VEGF (C936T and CXCL8 T251A) are shown to predict tumorrecurrence in stage III colon cancer, even though these mutations are silent mutations (Lurje et al., 2008). Polymorphism of CXCL8 is also associated with risk of recurrence for patients with rectal cancer (Gordon et al., 2006).

In melanoma patients, tumor burden, VEGF and CXCL8 serum levels are independent predictive factors of progression-free survival, where elevations in the serum levels of these angiogenic factors strongly correlate with a poor overall prognosis and reduced progression-free survival (Ugurel et al., 2001). Moreover, serum levels of CXCL8 are reported to fall as stage IV melanoma patients respond to chemotherapy or immune-chemotherapy, while persistence of elevated VEGF and bFGF serum levels is indicative of treatment resistance (Brennecke et al., 2005). These studies did not examine the expression of CXCR1 or CXCR2 on the melanoma cells, so it is unclear whether the effects of CXCL8 on the tumor growth are due to an autocrine or paracrine loop.

Though the serum level of CXCL8 is associated with prognosis of melanoma patients, there are not clear studies showing that tumor CXCL8, CCR7 or CXCR4 mRNA levels correlate with metastasis. This could be due to failure to appropriately micro-dissect samples prior to mRNA isolation and amplification (Otto et al., 2007).

Conclusions

Clearly much more work and better techniques are needed to adequately characterize the prognostic value of determining chemokine and chemokine receptor expression in melanoma tumor cells and patient serum. Studies thus far show that the major chemokine/chemokine receptor interactions that appear to be important in melanoma development and progression include CXCR4/CXCL12, CXCR2/CXCL1, 5 and 8, CCL2/CCR2, CCR7/CCL19 and 21, CCR9/CCL25. The status of a number of chemokine/chemokine receptor antagonists and a number of inhibitors in Phase I, II or III clinical trials in a variety of diseases has recently been reviewed (Wells et al., 2006). Though the pharmaceutical companies are reluctant to launch clinical trials with these reagents in cancer, as they are approved for other disorders, perhaps the door will open for targeting chemokine/chemokine receptor interactions in cancer. Hopefully new methods for appropriately intervening in these interactions will bring improved therapy for melanoma patients.

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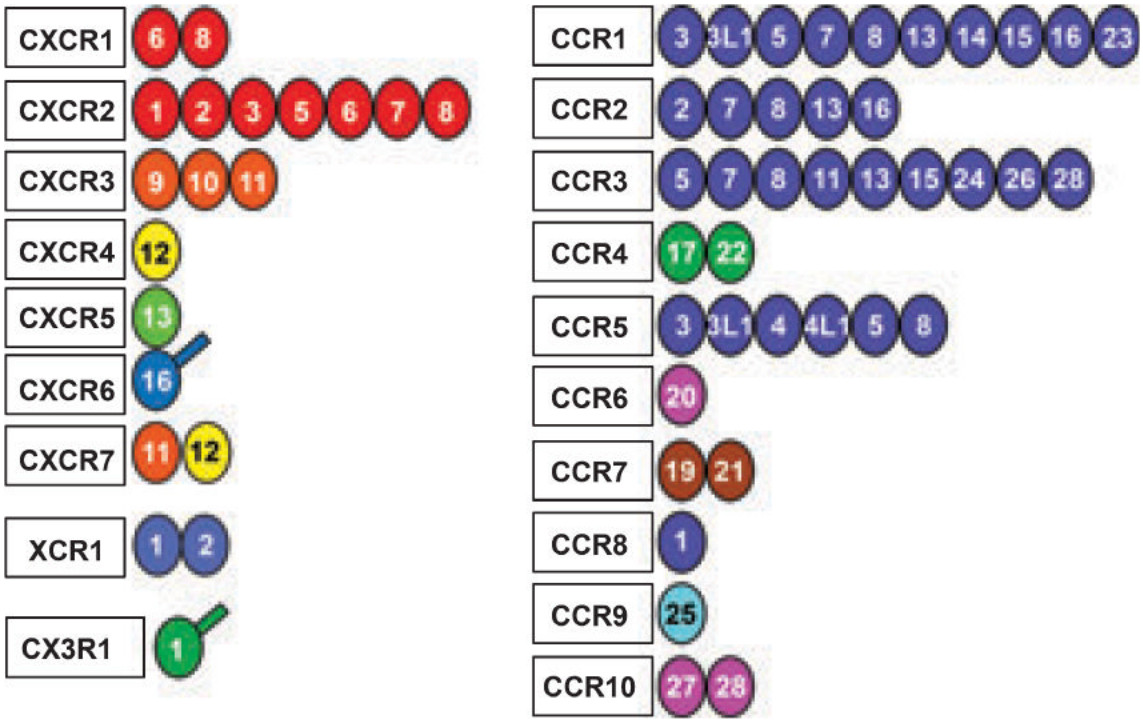

Figure 1.

Diagramic representation of the family of human chemokines and their receptors. The chemokines are represented by the ligand number and the receptors to which they bind are indicated according to their classification of CXC, C, CX3C or CC. Accordingly the ligand number beside the receptor indicates CC or CXC chemokine. For example, the `8' adjacent to `CXCR1' represents CXCL8. The colors are an indication of different chromosomal localization of the genes for that ligand such that if the color for all the ligands for one receptor is the same, then they are all found on the same chromosome. Two chemokines, CXCL16 and CX3CL1, are transmembrane proteins and the extra line on the ligand circle indicates this. [This figure is reprinted with permission from Zlotnik et al. (2006), Biomed Central.]

TUMOR TYPE	CHEMOKINE/CHEMOKINE RECEPTOR
Breast Cancer	CCL2,5; CXCL8, CXCL10
	CXCR4, CCR7, CXCR3
Melanoma	CXCL1-3, 5-8, CCL2,5
	CXCR4, CCR7, CCR9, CCR10
Lung Cancer	CXCL 4,5,6,8,12
	CXCR4, CXCR3
Prostate Cancer	CCL2,5,25; CXCL1,8, 10; CX3CR
	CXCR2, CCR9, CX3CR1,
Multiple Myeloma	CCL2,3,4; CXCL8, CXCL12
	CXCR3,4; CCR1,5,6
Colorectal Cancer	CCL1, CXCL1, 10
	CXCR3,
Ovarian Cancer	CXCL12, CXCL1,8; CCL2
	CCR2, CXCR4

Figure 2.
Cancer cells display a variety of chemokine receptors and produce multiple chemokines. This figure lists the chemokine receptors and ligands reported to be produced in a number of types of cancer.

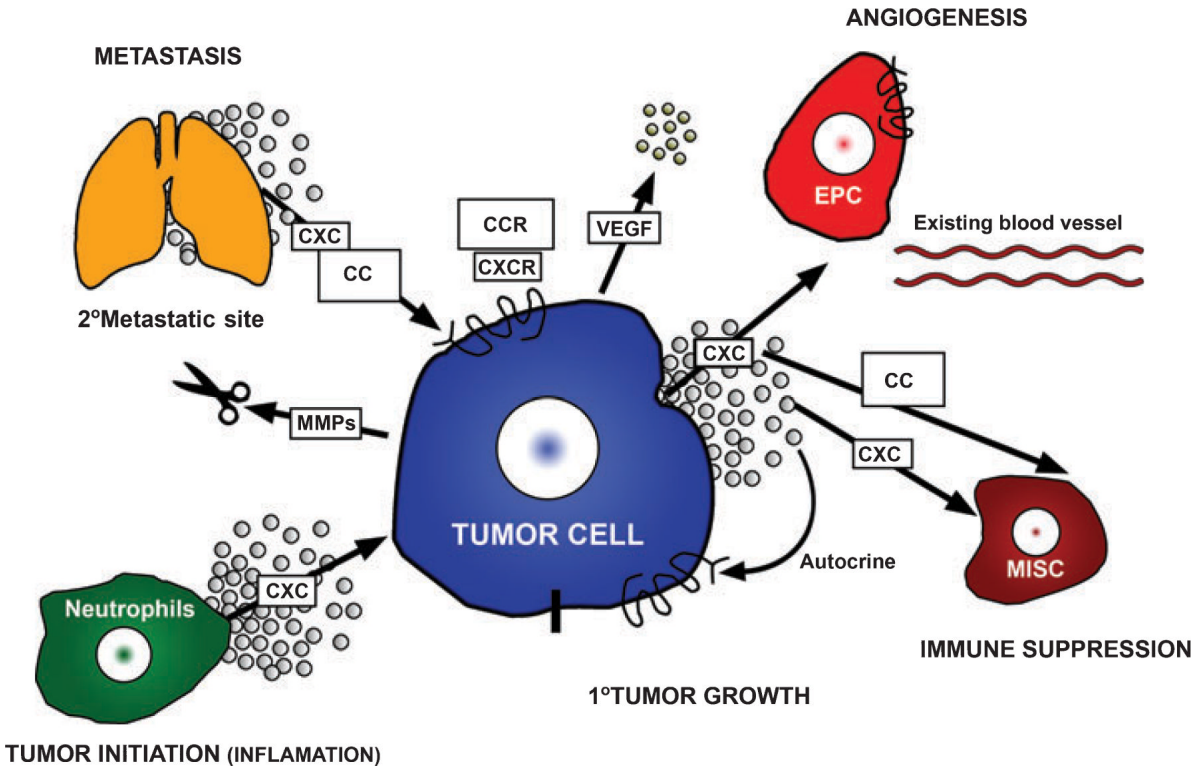

Figure 3.

Melanoma tumor cells elaborate CXC and CC chemokines that affect angiogenesis, inflammation, the tumor microenvironment, immune cell response, recruitment of leukocytes, tumor growth and metastasis. The melanoma cells also express chemokine receptors that direct metastasis to specific target organs that elaborate a chemokine gradient that binds and activates these melanoma cell chemokine receptors. Chemokines also stimulate production of metalloproteinases that degrade the matrix and facilitate melanoma metastasis. CXC, CXC chemokine ligands; CXCR, CXC chemokine ligand receptor; CC, CC chemokine ligands; CCR, CC chemokine ligand receptor; EPC, endothelial progenitor cell; MISC, myeloid immune suppressor cells; MMPs, matrix metalloproteinases; VEGF, vascular endothelial growth factor.