

Review

The CXCR4/CXCL12 axis in cutaneous malignancies with an emphasis on melanoma

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Summary. The highly metastatic and variable behavior of melanoma has accentuated the need for early detection and targeted therapy. Putative targets identified include those belonging to the extensive network of chemokines and their receptors. One such target is the chemokine receptor CXCR4, a G protein-coupled receptor with a 34 amino acid extracellular N-terminus, the primary ligand of which is CXCL12 (SDF-1, stromal derived factor-1). The ligand uniquely utilizes the N-terminus of CXCR4 for signal transduction and stimulates the protein kinase B (AKT)/mitogen activated protein kinase (MAPK) pathway.

Functionally, the CXCR4/CXCL12 axis is believed to play a key role in cell migration and proliferation. Upregulation of CXCR4 and consequently dysregulation of the CXCR4/CXCL12 axis has been implicated in the progression of several lineage-unrelated malignancies including melanoma. The contributions of the CXCR4/CXCL12 axis in melanomagenesis are well documented. More recently, the potential cooperativity between the mutational status of BRAF and the CXCR4/CXCL12 axis has been shown, lending credence to the concept that both CXCR4 and CXCL12 may be putative targets for therapy in melanoma.

In this review, we summarize the role of the CXCR4/CXCL12 axis in cancer progression and metastasis, with an emphasis on cutaneous malignancy, melanoma in particular. Furthermore, we discuss the effects of CXCL12 on CXCR4 expressing malignant cells *in vitro* and the potential prognostic utility of both CXCR4 and CXCL12 expressions. Lastly, we highlight

the therapeutic potential of targeting this axis and the unique response of CXCR4 expression to anti-cancer treatments with an emphasis on melanoma.

Key words: CXCR4, CXCL12, Melanoma

Introduction

Members of the chemokine superfamily are small molecular weight signaling proteins (Payne and Cornelius, 2002). Chemokines are classified into four groups based on the position of conserved cysteine residues—CC (2 adjacent N-terminal cysteines) CXC (2 N-terminal cysteines separated by 1 amino acid), C (1 N-terminal cysteine), and CX₃C (two N-terminal cysteines separated by 3 amino acids)—and for each family of ligand there is a corresponding receptor (Payne and Cornelius, 2002).

Chemokines and their receptors play an integral role in the immune system, directing leukocyte migration and regulating cellular proliferation (Balkwill, 2004). Chemokines and their receptors—in particular CXCL12 and CXCR4—are believed to play a key role in cell migration and proliferation—two processes relevant in the progression and metastasis of cancer (Sehgal et al., 1998; Müller et al., 2001). The ligand and receptor are two key components in the cross talk between malignant cells and their microenvironment, yielding this axis a great influence on malignant cell phenotype.

CXCR4 and CXCL12—structure and binding interaction

To date, known ligands for CXCR4 are CXCL12 (stromal derived factor-1, SDF-1) and ubiquitin (Saini et

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al., 2011). Ubiquitin does not bind the same site as CXCL12 and its effects are poorly understood (Saini et al., 2011). The structure of CXCL12 has been visualized in nuclear magnetic resonance (NMR) studies, showing a 67 residue peptide consisting of an N-terminal strand and a core globular domain (Crump et al., 1997). With the use of NMR spectroscopy, migration assays, and calcium measurements Crump et al. demonstrated in 1997 that binding of CXCR4 and CXCL12 involves all extracellular domains of the receptor and both the core globular domain and N-terminus of the ligand resulting in a two site mechanism of binding for signaling transduction (Crump et al., 1997).

The CXCR4/CXCL12 axis—downstream pathways and cellular effects

Stimulation of CXCR4 by CXCL12 can activate four downstream signaling pathways leading to four distinct cellular responses (Drury et al., 2011). Induction of the phospholipase C pathway results in the release of intracellular calcium stores and cellular migration (Kremer et al., 2011). Induction of the RhoA pathway leads to an increase in production of the collagenase membrane type-1 matrix metalloproteinase (MMT-1 MMP) (Bartolomé et al., 2004). Cross-talk between CXCR4 and MMT-1 MMP increases the invasiveness of malignant cells and enables them to migrate through membranes and extracellular matrices, thus enhancing the metastatic potential of the malignant cells (Bartolomé et al., 2009). Activation of the PI3K/AKT pathway enhances tumor cell survival (Luo et al., 2013). Lastly, stimulation of the MAPK pathway enhances tumor cell proliferation (Robledo et al., 2001; Sun et al., 2002; Alsayed et al., 2007; Heinrich et al., 2012). A study on the BLM melanoma cell line demonstrated that phosphorylation of the MAP kinases p44/42 (ERK1/2) and p38 (downstream of the Raf protein) upon *in vitro* CXCL12 administration, indicating enhanced MAPK pathway activation (Robledo et al., 2001).

These pathways belonging to the CXCR4/CXCL12 axis induce malignant cell migration, invasion, proliferation, and survival, all of which contribute to the progression and metastasis of cancer.

Normal functions of the CXCR4/CXCL12

Beginning as early as in embryogenesis, CXCR4 and CXCL12 play a role in directing cellular migration in all mammals (Domanska et al., 2013). In 1996 Nagasawa et al. demonstrated that loss of function of the CXCR4/CXCL12 axis results in defects in lymphopoiesis and bone marrow myelopoiesis while knockout studies of CXCR4 and CXCL12 in mice demonstrated a loss in stem cell migration and perinatal death due to failed embryonic development of the hematopoietic, renal, cardiovascular and nervous systems (Nagasawa et al., 1996; Sierro et al., 2007; Takabatake et al., 2009; Bonig and Papayannopoulou, 2013; Domanska et al., 2013; Mithal et al., 2013). The

CXCR4/CXCL12 axis is also important in adults as it directs immune responses, induces neovascularization and promotes cellular proliferation after injury (Bollag and Hill, 2013; Fu et al., 2013; Liu et al., 2013). Varied expression of CXCL12 throughout the body allows for the establishment of CXCL12 gradients, which help to direct the migration of CXCR4 expressing cells (Loetscher et al., 2000). In 2000 Loetscher et al. demonstrated that constitutive CXCL12 expression at sites of inflammation results in the establishment of a CXCL12 gradient, which directs CXCR4 expressing phagocytic cells of the innate immune system (Loetscher et al., 2000). A 2001 study showed that elevated CXCL12 expression was not limited to sites of inflammation, but was also observed at the lymph nodes, lung, liver and bone marrow, whereas comparatively lower expression was demonstrated in the small intestine, kidney, skin, brain and skeletal muscle (Müller et al., 2001). This pattern of expression is necessary for normal function of the immune system, directing lymphocyte trafficking, and also helps explain the increased prevalence of metastasis at lymph nodes, lungs, liver, and bone marrow—sites of higher relative CXCL12 expression (Müller et al., 2001; Stein and Nombela-Arrieta, 2005).

CXCR4/CXCL12 in cancer biology—relevance to tumor growth and metastatic development

In vitro migration and invasion assays confirm that murine CXCL12 enhances migration and invasion in human breast carcinoma MDA-MB-231 cells and it has been shown that *in vivo* inhibition of the CXCR4/CXCL12 axis can block the development of metastasis, demonstrating for the first time the contribution of this axis to the metastatic cascade (Müller et al., 2001). To date, CXCR4 expression has been identified in several lineage-unrelated malignancies of which a third are cutaneous. Studies identifying CXCL12 expression are limited in number, particularly at the primary site; however, many studies have focused on the effects of CXCL12 on tumor cells *in vitro*, demonstrating the ligand's proliferative, invasive, and migratory effects. To date, the utility of CXCL12 expression as a prognosticator of patient outcome has been demonstrated in only eleven lineage-unrelated malignancies of which three are cutaneous (Uchida et al., 2007; Clatot et al., 2011; Toyozawa et al., 2012).

CXCR4/CXCL12 in cutaneous non-melanoma malignancies

Both CXCR4 and its ligand CXCL12 appear to be involved in the progression and metastasis of several lineage-unrelated cutaneous malignancies.

Epithelial tumors

In one study encompassing actinic keratosis, basal cell carcinoma and squamous cell carcinoma, compared

CXCR4/CXCL12 axis in cutaneous malignancies

to normal skin, expression of CXCR4 appeared to be downregulated in actinic keratosis and basal cell carcinoma, and upregulated in squamous cell carcinoma (Basile et al., 2008). Given the limited metastatic potential of basal cell carcinoma compared to squamous cell carcinoma, this study supports the notion that CXCR4 expression is associated with neoplasms with the ability to metastasize (Basile et al., 2008). In the same year, a study showed the presence of CXCR4 protein expression in head and neck squamous cell carcinoma and noted the absence of expression in adjacent normal tissue (Ou et al., 2008). This study also demonstrated a correlation between CXCR4 protein expression and tumor staging, supporting a role for the predictive value of CXCR4 for poor patient outcome (Ou et al., 2008). In 2003 Uchida et al. demonstrated enhanced expression of CXCR4 mRNA and protein in primary oral squamous cell carcinoma and metastases as compared to adjacent normal tissues; however, CXCR4 expression could not be correlated to patient prognosis or tumor size (Uchida et al., 2003). In a 2007 follow-up study Uchida et al. noted a statistically significant decrease in the 5-year survival of oral squamous cell

carcinoma patients exhibiting high CXCR4 and/or CXCL12 protein expression at primary tumor sites, linking the CXCR4/CXCL12 axis to poor patient outcome (Uchida et al., 2007). In 2011 Uchida et al. demonstrated that the development of metastases in nude mice injected with B88 oral squamous cell carcinoma cells could be inhibited by inoculation with the CXCR4 antagonist AMD3100, thus elucidating the potential therapeutic value of targeting the CXCR4/CXCL12 axis (Uchida et al., 2011).

Fibrohistiocytic tumors

Expression of CXCR4 has also been demonstrated in fibrohistiocytic tumors such as dermatofibrosarcoma protuberans, malignant fibrous histiocytoma, and dermatofibroma (Toyozawa et al., 2010). In this study, significantly higher CXCR4 protein expression was noted in tumors with an aggressive biologic behavior such as malignant fibrous histiocytoma compared to dermatofibroma, suggesting that CXCR4 protein expression marker may be diagnostic utility as a histopathologic adjunct in predicting tumor

Table 1. Chronologic historical overview of studies on the CXCR4/CXCL12 axis in cutaneous non-cutaneous melanoma malignancies.

REFERENCE	STUDY DESIGN	FINDINGS	CONCLUSION
Uchida et al., 2003, 2007, 2011	Sample studied: Oral squamous cell carcinoma Methods: RT-PCR for CXCR4 mRNA, IHC for CXCR4 and CXCL12 protein	Enhanced expression of CXCR4 mRNA and protein in primary oral squamous cell carcinoma and metastases as compared to adjacent normal tissues; decrease in the 5-year survival in patients with high CXCR4 and/or CXCL12 protein expression at primary tumor sites; AMD3100 inhibits metastasis in mice	CXCR4 and CXCL12 protein expression may be of utility as a prognosticator for poor patient outcome oral squamous cell carcinoma. The CXCR4/CXCL12 axis contributes to the etiopathogenesis of oral squamous cell carcinoma and may be a putative target for treatment.
Basile et al., 2008	Samples studied: actinic keratosis, basal cell carcinoma, cutaneous squamous cell carcinoma Method: IHC for CXCR4 protein	Compared to normal skin, CXCR4 protein expression was downregulated in actinic keratosis and basal cell carcinoma, while expression was upregulated in cutaneous squamous cell carcinoma	CXCR4 protein expression may be associated with cutaneous malignancies with potential for metastasis.
Ou et al., 2008	Samples studied: squamous cell carcinoma (head and neck area) Method: IHC for CXCR4 protein	CXCR4 protein expression correlates with clinical stage and presence of lymph node metastasis.	CXCR4 protein expression may be of utility as a prognosticator for poor patient outcome in head and neck squamous cell carcinoma.
Toyozawa et al., 2010	Samples studied: dermatofibrosarcoma protuberance, malignant fibrous histiocytoma, dermatofibroma Method: IHC for CXCR4 protein	CXCR4 protein expression higher in dermatofibrosarcoma and malignant fibrous histiocytoma compared to dermatofibroma; CXCR4 protein expression higher in dermatofibrosarcoma patients who relapsed	CXCR4 protein expression may be of utility as a predictor of phenotypically aggressive cutaneous fibrohistiocytic tumors.
Knapp et al., 2012	Samples studied: Merkel cell carcinoma, local nodal metastasis and distant metastasis Method: IHC for CXCR4 protein	Higher expression in local nodal metastasis compared to primary and distant metastasis	CXCR4 expression may be associated with the progression of Merkel cell carcinoma, particularly early stages of metastasis.
Dobner et al. 2012	Sample studied: uveal melanoma Method: IHC for CXCR4 protein	CXCR4 protein expression correlates with presence of liver metastasis	CXCR4 protein expression may be of utility as a prognosticator for poor patient outcome in uveal melanoma.
Mo et al., 2013	Samples studied: malignant peripheral nerve sheath tumor (MPNST) cell lines and primary tumor Methods: <i>In vivo</i> murine injection of MPNST cells, RT-PCR for CXCL12 mRNA and IHC for CXCR4 protein	MPNST cells with CXCR4 knockdown displayed impaired <i>in vivo</i> tumor cell proliferation and attenuated tumorigenesis, as compared to injection with CXCR4 positive MPNST cells in murine models; administration of CXCR4 antagonist elicits abrogation of MPNST proliferation and metastasis in murine models; CXCR4 protein present in human MPNSTs	The CXCR4/CXCL12 axis may play a role in MPNST progression and metastasis. CXCR4 may be a putative target in treatment of MPNST.

aggressiveness (Toyozaawa et al., 2010). Further in support of this, the same study showed significantly great CXCR4 protein expression in relapsed dermatofibrosarcoma protuberans cases compared to non-relapsed cases (Toyozaawa et al., 2010).

Neuroectodermal tumors

In a study of cells derived from malignant peripheral nerve sheath tumors (MPNST), researchers demonstrated that the CXCR4/CXCL12 axis promotes cell survival and metastasis (Mo et al., 2013). Briefly, murine injection of CXCR4-negative MPNST cells resulted in impaired tumor cell proliferation and attenuated tumorigenesis compared to injection with CXCR4-positive MPNST cells (Mo et al., 2013). Interestingly, co-injection of the CXCR4 antagonist AMD3100 and CXCR4-positive MPNST cells resulted in tumorigenesis similar to that of the CXCR4-negative MPNST cells (Mo et al., 2013). This same study demonstrated CXCR4 protein expression in human MPNSTs using immunohistochemistry (Mo et al., 2013). Based upon these findings Mo et al. hypothesized that the CXCR4/CXCL12 axis may be relevant in the progression of MPNST and may be of utility as a therapeutic target (Mo et al., 2013).

Neuroendocrine tumors

Earlier studies failed to demonstrate CXCR4 protein expression in Merkel cell carcinoma; contrasting with more recent ones including a 2012 study by Knapp et al. who successfully demonstrated CXCR4 protein expression in the same (Tucci et al., 2006; Knapp et al., 2012). Of note, they found statistically significant greater CXCR4 immunoreactivity in local nodal Merkel cell carcinoma metastasis biopsies compared to primary and distant metastatic biopsies, leading the authors to conclude that CXCR4 is particularly crucial in the early stages of Merkel cell carcinoma progression (Tucci et al., 2006; Knapp et al., 2012).

CXCR4/CXCL12 in cutaneous melanoma

The highly metastatic and variable behavior of melanoma has accentuated the need for early detection and targeted therapy. The relevance of the CXCR4/CXCL12 axis has been highlighted in both uveal and cutaneous melanoma.

In 2007 protein expression of CXCR4 was initially demonstrated in uveal melanoma biopsies in a study using immunohistochemistry (Scala et al., 2007). In this study by Scala et al. expression of CXCR4 protein correlated with epithelioid phenotype—a subgroup with poor clinical outcome—alluding to the potential prognostic utility of CXCR4 expression (Scala et al., 2007). The same year Cesare et al. demonstrated CXCR4 protein expression on five uveal melanoma cell lines and showed that CXCL12 promoted migration of the cell lines *in vitro* (Cesare et al., 2007). A 2012 study

highlighted the prognostic value of CXCR4 protein expression in uveal melanoma by correlating expression with presence of hepatic metastasis (Dobner et al., 2012).

In 2001 expression of CXCR4 in biopsies of primary cutaneous melanoma, melanoma metastatic to the lymph node and melanoma cell lines was demonstrated using multiple techniques, including immunohistochemistry and flow cytometry (Robledo et al., 2001). In this study CXCR4 protein expression was noted in 100% of biopsies of primary cutaneous melanoma and melanoma metastasis (n=7 and 5 respectively) as well as in the melanoma cell lines MeWo, A375 and BLM, indicating that the CXCR4/CXCL12 axis may be relevant in melanoma. This same study showed that CXCL12 greatly enhanced MeWo cell adhesion to the extracellular matrix protein fibronectin, facilitating the invasion of normal tissue by malignant cells (Robledo et al., 2001). In 2001 Robledo et al. also showed that *in vitro* administration of CXCL12 induced phosphorylation of the MAP kinases p44/42 and p38 (downstream of the Raf protein) indicating that CXCL12 could potentially be relevant in melanomagenesis (Robledo et al., 2001). A year later Murakami et al. similarly demonstrated CXCR4 expression in 100% (n=3) of primary cutaneous melanoma biopsies and 40% (n=5) of pulmonary metastasis biopsies (Murakami et al., 2002). In the same study Murakami et al. transduced CXCR4 cDNA into B16 melanoma cells and demonstrated that increasing CXCR4 protein expression resulted in significantly enhanced development of pulmonary metastasis in mice (Murakami et al., 2002). Murakami et al. showed that administration of the CXCR4 antagonists T22 inhibited the formation of metastasis, highlighting the potential therapeutic value of targeting the CXCR4/CXCL12 axis (Murakami et al., 2002). This study also showed that *in vitro* administration of CXCL12 enhanced B16 melanoma cell adhesion to pulmonary endothelial cells and promoted cell growth, identifying the CXCR4/CXCL12 axis as a potential contributor to melanomagenesis and the development of metastasis (Murakami et al., 2002).

Following the early findings of Robledo et al. and Murakami et al. were a number of studies demonstrating the prognostic utility of CXCR4 protein expression in primary cutaneous melanoma (Longo-Imedio et al., 2005; Scala et al., 2005; Tucci et al., 2007; Toyozaawa et al., 2012). Briefly, Longo-Imedio et al., demonstrated CXCR4 protein expression in 35% (n=40) of primary cutaneous melanoma and showed that CXCR4 expression correlated with ulceration, increased tumor thickness, development of metastases, and patient morbidity (Longo-Imedio et al., 2005). This same year Scala et al., correlated CXCR4 expression (56% positive, n=71) with shorter disease free survival and greater risk of morbidity in study patients with primary cutaneous melanoma (Scala et al., 2005). Two years later Tucci et al. identified CXCR4 protein expression in 100% (n=30) of nodular melanoma biopsies and showed that expression correlated with greater Breslow depth and patient morbidity, supporting a role for the CXCR4/CXCL12 axis in melanoma growth and, the utility of CXCR4 as a

CXCR4/CXCL12 axis in cutaneous malignancies

prognosticator for poor patient outcome (Tucci et al., 2007). Most recently, Toyozawa et al. demonstrated CXCR4 protein expression in 100% (n=19) of primary cutaneous melanoma cases and noted an association between expression and tumor thickness >2 mm as well as the development of distant metastasis, delineating the contribution of the CXCR4/CXCL12 axis to melanoma progression and development of metastases (Toyozawa et al., 2012). Of note, a 2012 study by Kühnelt-Leddihn et al. noted CXCR4 protein expression in 82% (n=38) of

primary cutaneous melanoma, but failed to demonstrate a correlation with established histopathologic prognosticators or patient morbidity (Kühnelt-Leddihn et al., 2012). Our own experience has shown CXCR4 and CXCL12 appear to be biomarkers associated with established prognosticators of good and poor clinical outcome respectively in primary cutaneous melanoma as we have noted that CXCR4 correlates with absence of mitoses and low AJCC staging, while CXCL12 correlates with ulceration and vascular invasion

Table 2. Chronologic historical overview of studies on the CXCR4/CXCL12 axis in melanoma.

REFERENCE	STUDY DESIGN	FINDINGS	CONCLUSION
Robledo et al., 2001	Samples studied: Melanoma cell lines, primary cutaneous melanoma and metastasis Method: Flow cytometry for CXCR4 protein, IHC for CXCR4 protein, <i>in vitro</i> cell adhesion assay and proliferation assay	CXCR4 protein is present on melanoma cell lines and melanoma metastases; CXCL12 induces enhanced fibronectin adhesion and phosphorylation of MAPK kinases.	The CXCR4/CXCL12 axis may stimulate melanoma cell proliferation and invasion, contributing to melanoma progression and the development of metastases.
Murakami et al., 2002	Samples studied: B16 melanoma cell line, primary cutaneous melanoma and pulmonary metastases Methods: Murine injection with CXCR4 positive and CXCR4 negative melanoma cells and IHC for CXCR4 protein	CXCR4 expression enhances pulmonary metastatic potential of melanoma cells in a murine model; CXCR4 is expressed on primary cutaneous melanoma and pulmonary metastasis	The CXCR4/CXCL12 axis contributes to the metastatic capacity of melanoma cells in mice and expression is present in human melanoma.
Longo-Imedio et al., 2005	Sample studied: Primary cutaneous melanoma Method: IHC for CXCR4 protein	CXCR4 protein expression correlates to the following: presence of ulceration, tumor thickness, development of lymph node metastasis, presence of distant metastasis, patient morbidity	CXCR4 protein expression may be of utility as a prognosticator for poor patient outcome.
Scala et al., 2005	Sample studied: Primary cutaneous melanoma with Breslow thickness >1 mm Method: IHC for CXCR4 protein	CXCR4 protein expression correlates to presence of sentinel lymph node metastasis and patient morbidity	CXCR4 protein expression may be of utility as a prognosticator for poor patient outcome.
Scala et al., 2006	Sample studied: Melanoma cell lines and melanoma metastases Methods: RT-PCR for CXCR4 mRNA, <i>in vitro</i> proliferation assays and IHC for CXCR4 protein	CXCR4 mRNA expressed in melanoma cell lines; CXCL12 induces melanoma cell proliferation and these effects can be abrogated by AMD3100; CXCR4 protein expressed in melanoma metastasis	The CXCR4/CXCL12 axis may stimulate melanoma growth and metastasis. CXCR4 may be a putative target for therapeutic treatment.
Kim et al., 2006	Sample studied: primary cutaneous melanoma Method: RT-PCR for CXCR4 mRNA	No significant correlation observed between overexpression of CXCR4 protein and survival	CXCR4 protein expression does not appear to have prognostic utility in primary cutaneous melanoma.
Tucci et al., 2007	Sample studied: Primary cutaneous melanoma Method: IHC for CXCR4 protein	CXCR4 protein expression correlates to Breslow thickness and patient morbidity	CXCR4 protein expression may be of utility as a prognosticator for poor patient outcome in primary cutaneous melanoma.
Franco et al. 2010	Sample studied: Primary cutaneous melanoma Methods: RT-PCR for CXCR4 mRNA and IHC for CXCR4 protein	CXCR4 mRNA and protein expression correlate to patient morbidity	CXCR4 protein expression may be of utility as a prognosticator for poor patient outcome.
Kühnelt-Leddihn et al. 2012	Sample studied: Primary cutaneous melanoma Method: IHC for CXCR4	CXCR4 protein expression does not correlate with prognosis or survival.	CXCR4 protein expression does not appear to have prognostic utility.
Monteagudo et al. 2012	Samples studied: Primary cutaneous melanoma and melanoma metastasis Methods: RT-PCR for CXCR4 and CXCL12 mRNA	Low CXCL12/CXCR4 ratio correlates with tumor thickness >1 mm and the development of metastasis	The CXCR4/CXCL12 axis may contribute to melanoma progression and metastasis. CXCL12/CXCR4 mRNA ratio expression may be of utility as a prognosticator for poor patient outcome.
Toyozawa et al. 2012	Sample studied: Primary cutaneous melanoma Method: IHC for CXCR4 and CXCL12 protein	CXCR4 protein expression correlates to the following: tumor thickness, development of distant metastasis, and CXCL12 protein expression.	CXCR4 and CXCL12 protein expressions may be of utility as prognosticators for poor patient outcome.
O'Boyle et al. 2013	Samples studied: BRAFWT and BRAFV600E melanoma cell lines Method: <i>In vitro</i> migration assay	Cell migration enhanced by BRAFV600E transfection and inhibited by the CXCR4 antagonist AMD11070	CXCR4 may be a putative target for therapeutic treatment.

(manuscript in preparation).

Higher expression of CXCR4 mRNA, like that of the protein, has also been identified as a prognosticator of poor outcome in melanoma in two studies (Franco et al., 2010; Monteagudo et al., 2012). In 2010 Franco et al. demonstrated high CXCR4 mRNA expression in 91% (n=23) of melanoma metastases to the lymph node and demonstrated an association between expression and shorter disease free survival (Franco et al., 2010). Of note, this same study evaluated CXCR4 protein expression in all cases and observed a perfect overlap of mRNA and protein expressions (Franco et al., 2010). More recently, Monteagudo et al. found that lower expression of CXCL12 mRNA compared to that of CXCR4 was a valuable prognosticator for the development of metastasis in primary cutaneous melanoma (Monteagudo et al., 2012). The same study showed that CXCL12 mRNA expression, as compared to that of CXCR4 was four times greater in thin (≤ 1 mm) than thick (> 1 mm) primary cutaneous melanomas (Monteagudo et al., 2012). Of note, a 2006 study by Kim et al. demonstrated CXCR4 mRNA expression in 89% (n=27) of melanoma biopsies, but noted the absence of a correlation with patient outcome (Kim et al., 2006).

The aforementioned studies highlight the relevance of the CXCR4/CXCL12 axis in the progression of melanoma as well as its prognostic value. The therapeutic value of targeting this chemokine axis has also been observed in three studies (Scala et al., 2006; Liang et al., 2012; O'Boyle et al., 2013). In 2006 Scala et al. demonstrated *in vitro* abrogation of CXCL12 induced melanoma cell proliferation by administering the CXCR4 antagonist AMD3100 (Scala et al., 2006). In 2012 Liang et al. administered the small molecule CXCR4 inhibitor MSX-122 and observed significant inhibition in uveal melanoma liver metastasis in mice, supporting that the previously observed *in vitro* effects of blocking the CXCR4/CXCL12 axis were maintained in an *in vivo* model (Liang et al., 2012). A year later O'Boyle et al. compared the migratory response to CXCL12 of CHL-1 melanoma cells transfected with either *BRAF*^{WT} or *BRAF*^{V600E} and found that migration was enhanced by *BRAF*^{V600E}, but could be inhibited by the newly synthesized CXCR4 antagonist AMD11070, indicating that targeting of CXCR4/CXCL12 axis may be therapeutically relevant (O'Boyle et al., 2013). Our own experience lends credence to the hypothesis that CXCR4 may be an ancillary molecule to explore as a putative target in primary cutaneous melanoma as we have found higher CXCR4 mRNA expression in patients with a BRAF mutation (manuscript in preparation).

Chemotherapy and the CXCR4/CXCL12 axis

Previous studies have indicated the potential role of the CXCR4/CXCL12 axis in establishing chemoresistance and even enhanced signaling in response to chemotherapy. In 2005, Mori et al. showed CXCR4 upregulation on melanoma cell lines in response

to treatment with trichostatin A (TSA), a histone deacetylase inhibitor, and 5-Aza-2-deoxycytidine (5-Aza), a demethylating agent (Mori et al., 2005). Mori et al. noted both increased CXCR4 mRNA and protein expression, as well as enhanced cellular migration towards CXCL12 (Mori et al., 2005). In 2008, Shaked et al. showed that the CXCR4/CXCL12 axis could also be dysregulated in response to chemotherapeutic treatments through the upregulation of CXCL12 (Shaked et al., 2008). In this melanoma murine study it was demonstrated that CXCL12 was released by platelets in response to treatment with paclitaxel-based chemotherapy (Shaked et al., 2008). Shaked et al. observed enhanced tumor vascularization and endothelial progenitor homing at the primary tumor in response to the increase in circulating CXCL12 (Shaked et al., 2008). The same results were observed in patients treated with paclitaxel-based chemotherapy, but not other chemotherapies, indicating that certain chemotherapies may lead to melanoma progression due to dysregulation of the CXCR4/CXCL12 axis (Shaked et al., 2008). In 2010, using an orthotopic murine melanoma model, Kim et al. showed that treatment with the chemotherapeutic agent, dacarbazine, lead to enrichment of CXCR4-expressing chemoresistant tumor cells by 500-700% (Kim et al., 2010). Furthermore, concomitant use of the CXCR4 antagonist AMD3100 and dacarbazine treatment on the chemoresistant melanoma cells effectively blocked tumor metastasis in mice to a much greater extent than dacarbazine alone (Kim et al., 2010).

These studies highlight the clinical relevance of the CXCR4/CXCL12 axis and its potential targeting for the treatment of cutaneous malignancies, particularly melanoma.

Conclusions

The CXCR4/CXCL12 axis has been defined as a contributor to the metastatic cascade and progression of various malignancies. Both CXCR4 and CXCL12 appear to be of utility as prognosticators in cutaneous malignancies and with further study may prove to be biomarkers of potential clinical use. Targeting of CXCR4 is a promising therapeutic approach that has shown improvement of both murine and clinical trials. Additionally, the targeting of CXCL12 has been shown effective in murine models and may yield clinical efficacy. Lastly, this axis appears to be of great relevance in the development of chemoresistant malignancies and both may prove to be putative targets for overcoming observed clinical chemoresistance. Further studies are necessary in order to affirm their prognostic and therapeutic utility.

References

- Alsayed Y., Ngo H., Runnels J., Leleu X., Singha U.K., Pittsillides C.M., Spencer J.A., Kimlinger T., Ghobrial J.M., Jia X., Lu G., Timm M., Kumar A., Côté D., Veilleux I., Hedin K.E., Roodman D.G., Witzig

CXCR4/CXCL12 axis in cutaneous malignancies

- T.E., Kung A.L., Hideshima T., Anderson K.C., Lin C.P. and Ghobrial I. (2007). Mechanisms of regulation of CXCR4/SDF-1 (CXCL12)-dependent migration and homing in multiple myeloma. *Blood* 109, 2708-2717.
- Balkwill F. (2004). Cancer and the chemokine network. *Nat. Rev. Cancer* 4, 540-550.
- Bartolomé R.A., Gálvez B.G., Longo N., Baleux F., Muijen G.N.P. van, Sánchez-Mateos P., Arroyo A.G. and Teixidó J. (2004). Stromal Cell-Derived Factor-1 α Promotes Melanoma Cell Invasion across Basement Membranes Involving Stimulation of Membrane-Type 1 Matrix Metalloproteinase and Rho GTPase Activities. *Cancer Res.* 64, 2534-2543.
- Bartolomé R.A., Ferreira S., Miqulena-Colina M.E., Martínez-Prats L., Soto-Montenegro M.L., García-Bernal D., Vaquero J.J., Agami R., Delgado R., Desco M., Sánchez-Mateos P. and Teixidó J. (2009). The chemokine receptor CXCR4 and the metalloproteinase MT1-MMP are mutually required during melanoma metastasis to lungs. *Am. J. Pathol.* 174, 602-612.
- Basile J., Thiers B., Maize J. and Lathers D.M.R. (2008). Chemokine receptor expression in non-melanoma skin cancer. *J. Cutan. Pathol.* 35, 623-629.
- Bollag W.B. and Hill W.D. (2013). CXCR4 in Epidermal Keratinocytes: Crosstalk within the Skin. *J. Invest. Dermatol.* 133, 2505-2508.
- Bonig H. and Papayannopoulou T. (2013). Hematopoietic stem cell mobilization: updated conceptual renditions. *Leukemia* 27, 24-31.
- Cesare S.D., Marshall J.C., Fernandes B.F., Logan P., Anteckka E., Filho V.B. and Burnier M.N. (2007). *In vitro* characterization and inhibition of the CXCR4/CXCL12 chemokine axis in human uveal melanoma cell lines. *Cancer Cell Int.* 7, 17.
- Clatot F., Picquenot J.M., Choussy O., Gouérand S., Moldovan C., Schultheis D., Cornic M., François A., Blot E. and Laberge-Le-Couteux S. (2011). Intratumoural level of SDF-1 correlates with survival in head and neck squamous cell carcinoma. *Oral Oncol.* 47, 1062-1068.
- Crump M.P., Gong J.H., Loetscher P., Rajarathnam K., Amara A., Arenzana-Seisdedos F., Virelizier J.L., Baggiolini M., Sykes B.D. and Clark-Lewis I. (1997). Solution structure and basis for functional activity of stromal cell-derived factor-1; dissociation of CXCR4 activation from binding and inhibition of HIV-1. *EMBO J.* 16, 6996-7007.
- Dobner B.C., Riechardt A.I., Jousen A.M., Englert S. and Bechrakis N.E. (2012). Expression of haematogenous and lymphogenous chemokine receptors and their ligands on uveal melanoma in association with liver metastasis. *Acta Ophthalmol. (Copenh.)* 90, e638-e644.
- Domanska U.M., Kruizinga R.C., Nagengast W.B., Timmer-Bosscha H., Huls G., de Vries E.G.E. and Walenkamp A.M.E. (2013). A review on CXCR4/CXCL12 axis in oncology: No place to hide. *Eur. J. Cancer* 49, 219-230.
- Drury L.J., Ziarek J.J., Gravel S., Veldkamp C.T., Takekoshi T., Hwang S.T., Heveker N., Volkman B.F. and Dwinell M.B. (2011). Monomeric and dimeric CXCL12 inhibit metastasis through distinct CXCR4 interactions and signaling pathways. *Proc. Natl. Acad. Sci. USA* 108, 17655-17660.
- Franco R., Cantile M., Scala S., Catalano E., Cerrone M., Scognamiglio G., Pinto A., Chiofalo M.G., Caracò C., Anniciello A.M., Abbruzzese A., Caraglia M. and Botti G. (2010). Histomorphologic parameters and CXCR4 mRNA and protein expression in sentinel node melanoma metastasis are correlated to clinical outcome. *Cancer Biol. Ther.* 9, 423-429.
- Fu X., Yang B., Lao S., Fan Y. and Wu C. (2013). Human memory-like NK cells migrating to tuberculous pleural fluid via IP-10/CXCR3 and SDF-1/CXCR4 axis produce IFN- γ in response to Bacille Calmette Guerin. *Clin. Immunol.* 148, 113-123.
- Heinrich E.L., Lee W., Lu J., Lowy A.M. and Kim J. (2012). Chemokine CXCL12 activates dual CXCR4 and CXCR7-mediated signaling pathways in pancreatic cancer cells. *J. Transl. Med.* 10, 68.
- Kim J., Mori T., Chen S.L., Amersi F.F., Martinez S.R., Kuo C., Turner R.R., Ye X., Bilchik A.J., Morton D.L. and Hoon D.S.B. (2006). Chemokine receptor CXCR4 expression in patients with melanoma and colorectal cancer liver metastases and the association with disease outcome. *Ann. Surg.* 244, 113-120.
- Kim M., Koh Y.J., Kim K.E., Koh B.I., Nam D.H., Alitalo K., Kim I. and Koh G.Y. (2010). CXCR4 Signaling regulates metastasis of chemoresistant melanoma cells by a lymphatic metastatic niche. *Cancer Res.* 70, 10411-10421.
- Knapp C.F., Sayegh Z., Schell M.J., Rawal B., Ochoa T., Sondak V.K. and Messina J.L. (2012). Expression of CXCR4, E-cadherin, Bcl-2, and survivin in Merkel cell carcinoma: an immunohistochemical study using a tissue microarray. *Am. J. Dermatopathol.* 34, 592-596.
- Kremer K.N., Clift I.C., Miamen A.G., Bamidele A.O., Qian N.X., Humphreys T.D. and Hedin K.E. (2011). Stromal cell-derived factor-1 signaling via the CXCR4-TCR heterodimer requires phospholipase C- β 3 and phospholipase C- γ 1 for distinct cellular responses. *J. Immunol. Baltim. Md 1950* 187, 1440-1447.
- Kühnelt-Leddihn L., Müller H., Eisendle K., Zelger B. and Weinlich G. (2012). Overexpression of the chemokine receptors CXCR4, CCR7, CCR9, and CCR10 in human primary cutaneous melanoma: a potential prognostic value for CCR7 and CCR10? *Arch. Dermatol. Res.* 304, 185-193.
- Liang Z., Zhan W., Zhu A., Yoon Y., Lin S., Sasaki M., Klapproth J.M.A., Yang H., Grossniklaus H.E., Xu J., Rojas M., Voll R.J., Goodman M.M., Arrendale R.F., Liu J., Yun C.C., Snyder J.P., Liotta D.C. and Shim H. (2012). Development of a unique small molecule modulator of CXCR4. *PLoS One* 7, e34038.
- Liu X., Liang F., Yang J., Li Z., Hou X., Wang Y. and Gao C. (2013). Effects of stromal cell derived factor-1 and CXCR4 on the promotion of neovascularization by hyperbaric oxygen treatment in skin flaps. *Mol. Med. Rep.* 8, 1118-1124.
- Loetscher P., Moser B. and Baggiolini M. (2000). Chemokines and their receptors in lymphocyte traffic and HIV infection. *Adv. Immunol.* 74, 127-180.
- Longo-Imedio M.I., Longo N., Treviño I., Lázaro P. and Sánchez-Mateos P. (2005). Clinical significance of CXCR3 and CXCR4 expression in primary melanoma. *Int. J. Cancer* 117, 861-865.
- Luo D.H., Chen Q.Y., Liu H., Xu L.H., Zhang H.Z., Zhang L., Tang L.Q., Mo H.Y., Huang P.Y., Guo X. and Mai H.Q. (2013). The independent, unfavorable prognostic factors endothelin A receptor and chemokine receptor 4 have a close relationship in promoting the motility of nasopharyngeal carcinoma cells via the activation of AKT and MAPK pathways. *J. Transl. Med.* 11, 203.
- Mithal D.S., Ren D. and Miller R.J. (2013). CXCR4 signaling regulates radial glial morphology and cell fate during embryonic spinal cord development. *Glia* 61, 1288-1305.
- Mo W., Chen J., Patel A., Zhang L., Chau V., Li Y., Cho W., Lim K., Xu J., Lazar A.J., Creighton C.J., Bolshakov S., McKay R.M., Lev D., Le L.Q. and Parada L.F. (2013). CXCR4/CXCL12 mediate autocrine cell- cycle progression in NF1-associated malignant peripheral nerve

- sheath tumors. *Cell* 152, 1077-1090.
- Monteagudo C., Ramos D., Pellín-Carcelén A., Gil R., Callaghan R.C., Martín J.M., Alonso V., Murgui A., Navarro L., Calabuig S., López-Guerrero J.A., Jordá E. and Pellín A. (2012). CCL27-CCR10 and CXCL12-CXCR4 chemokine ligand-receptor mRNA expression ratio: new predictive factors of tumor progression in cutaneous malignant melanoma. *Clin. Exp. Metastasis* 29, 625-637.
- Mori T., Kim J., Yamano T., Takeuchi H., Huang S., Umetani N., Koyanagi K. and Hoon D.S.B. (2005). Epigenetic up-regulation of C-C chemokine receptor 7 and C-X-C chemokine receptor 4 expression in melanoma cells. *Cancer Res.* 65, 1800-1807.
- Müller A., Homey B., Soto H., Ge N., Catron D., Buchanan M.E., McClanahan T., Murphy E., Yuan W., Wagner S.N., Barrera J.L., Mohar A., Verástegui E. and Zlotnik A. (2001). Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410, 50-56.
- Murakami T., Maki W., Cardones A.R., Fang H., Kyi A.T., Nestle F.O. and Hwang S.T. (2002). Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells. *Cancer Res.* 62, 7328-7334.
- Nagasawa T., Hirota S., Tachibana K., Takakura N., Nishikawa S., Kitamura Y., Yoshida N., Kikutani H. and Kishimoto T. (1996). Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. 382, 635-638.
- O'Boyle G., Swidenbank I., Marshall H., Barker C.E., Armstrong J., White S.A., Fricker S.P., Plummer R., Wright M. and Lovat P.E. (2013). Inhibition of CXCR4-CXCL12 chemotaxis in melanoma by AMD11070. *Br. J. Cancer* 108, 1634-1640.
- Ou D.L., Chien H.F., Chen C.L., Lin T.C. and Lin L.I. (2008). Role of twist in head and neck carcinoma with lymph node metastasis. *Anticancer Res.* 28, 1355-1359.
- Payne A.S. and Cornelius L.A. (2002). The role of chemokines in melanoma tumor growth and metastasis. *J. Invest. Dermatol.* 118, 915-922.
- Robledo M.M., Bartolomé R.A., Longo N., Rodríguez-Frade J.M., Mellado M., Longo I., Muijen G.N.P. van, Sánchez-Mateos P. and Teixidó J. (2001). Expression of functional chemokine receptors CXCR3 and CXCR4 on human melanoma cells. *J. Biol. Chem.* 276, 45098-45105.
- Saini V., Staren D.M., Ziarek J.J., Nashaat Z.N., Campbell E.M., Volkman B.F., Marchese A. and Majetschak M. (2011). The CXC chemokine receptor 4 ligands ubiquitin and stromal cell-derived factor-1 α function through distinct receptor interactions. *J. Biol. Chem.* 286, 33466-33477.
- Scala S., Ottaiano A., Ascierto P.A., Cavalli M., Simeone E., Giuliano P., Napolitano M., Franco R., Botti G. and Castello G. (2005). Expression of CXCR4 predicts poor prognosis in patients with malignant melanoma. *Clin. Cancer Res.* 11, 1835-1841.
- Scala S., Giuliano P., Ascierto P.A., Ieranò C., Franco R., Napolitano M., Ottaiano A., Lombardi M.L., Luongo M., Simeone E., Castiglia D., Mauro F., Michele I.D., Calemma R., Botti G., Caracò C., Nicoletti G., Satriano R.A. and Castello G. (2006). Human melanoma metastases express functional CXCR4. *Clin. Cancer Res.* 12, 2427-2433.
- Scala S., Ieranò C., Ottaiano A., Franco R., Mura A.L., Liguori G., Mascolo M., Staibano S., Ascierto P.A., Botti G., Rosa G.D. and Castello G. (2007). CXC chemokine receptor 4 is expressed in uveal malignant melanoma and correlates with the epithelioid-mixed cell type. *Cancer Immunol. Immunother.* 56, 1589-1595.
- Sehgal A., Keener C., Boynton A.L., Warrick J., and Murphy G.P. (1998). CXCR-4, a chemokine receptor, is overexpressed in and required for proliferation of glioblastoma tumor cells. *J. Surg. Oncol.* 69, 99-104.
- Shaked Y., Henke E., Roodhart J.M.L., Mancuso P., Langenberg M.H.G., Colleoni M., Daenen L.G., Man S., Xu P., Emmenegger U., Tang T., Zhu Z., Witte L., Streiter R.M., Bertolini F., Voest E.E., Benezra R. and Kerbel R.S. (2008). Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: Implications for antiangiogenic drugs as chemosensitizing agents. *Cancer Cell* 14, 263-273.
- Sierro F., Biben C., Martinez-Munoz L., Mellado M., Ransohoff R.M., Li M., Woehl B., Leung H., Groom J., Batten M., Harvey R.P., Martinez A.C., Mackay C.R. and Mackay F. (2007). Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. *Proc. Natl. Acad. Sci. USA* 104, 14759-14764.
- Stein J.V. and Nombela-Arrieta C. (2005). Chemokine control of lymphocyte trafficking: a general overview. *Immunology* 116, 1-12.
- Sun Y., Cheng Z., Ma L. and Pei G. (2002). β -Arrestin2 Is Critically Involved in CXCR4-mediated Chemotaxis, and This Is Mediated by Its Enhancement of p38 MAPK Activation. *J. Biol. Chem.* 277, 49212-49219.
- Takabatake Y., Sugiyama T., Kohara H., Matsusaka T., Kurihara H., Koni P.A., Nagasawa Y., Hamano T., Matsui I., Kawada N., Imai E., Nagasawa T., Rakugi H. and Isaka Y. (2009). The CXCL12 (SDF-1)/CXCR4 axis is essential for the development of renal vasculature. *J. Am. Soc. Nephrol.* 20, 1714-1723.
- Toyozawa S., Yamamoto Y., Ishida Y., Kondo T., Nakamura Y. and Furukawa F. (2010). Immunohistochemical analysis of CXCR4 expression in fibrohistiocytic tumors. *Acta Histochem. Cytochem.* 43, 45-50.
- Toyozawa S., Kaminaka C., Furukawa F., Nakamura Y., Matsunaka H. and Yamamoto Y. (2012). Chemokine receptor CXCR4 is a novel marker for the progression of cutaneous malignant melanomas. *Acta Histochem. Cytochem.* 45, 293-299.
- Tucci M., Lucarini G., Giangiacomi M., Zizzi A., Criante P., Ricotti G. and Biagini G. (2006). Immunohistochemical study of apoptosis markers and involvement of chemokine CXCR4 in skin Merkel cell carcinoma. *J. Eur. Acad. Dermatol. Venereol.* 20, 1220-1225.
- Tucci M., Lucarini G., Brancorsini D., Zizzi A., Pugnali A., Giacchetti A., Ricotti G. and Biagini G. (2007). Involvement of E-cadherin, beta-catenin, Cdc42 and CXCR4 in the progression and prognosis of cutaneous melanoma. *Br. J. Dermatol.* 157, 1212-1216.
- Uchida D., Begum N.M., Almofti A., Nakashiro K., Kawamata H., Tateishi Y., Hamakawa H., Yoshida H. and Sato M. (2003). Possible role of stromal-cell-derived factor-1/CXCR4 signaling on lymph node metastasis of oral squamous cell carcinoma. *Exp. Cell Res.* 290, 289-302.
- Uchida D., Onoue T., Tomizuka Y., Begum N.M., Miwa Y., Yoshida H. and Sato M. (2007). Involvement of an autocrine stromal cell-derived factor-1/CXCR4 system on the distant metastasis of human oral squamous cell carcinoma. *Mol. Cancer Res.* 5, 685-694.
- Uchida D., Onoue T., Kuribayashi N., Tomizuka Y., Tamatani T., Nagai H. and Miyamoto Y. (2011). Blockade of CXCR4 in oral squamous cell carcinoma inhibits lymph node metastases. *Eur. J. Cancer* 47, 452-459.