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Invited Review

IL-8 expression in malignant melanoma: implications in growth and metastasis

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Summary. This review article has described briefly studies supporting the concept that IL-8 expression and its regulation by inflammatory cytokines like IL-1 may play an important role in controlling the phenotypes associated with melanoma progression and metastasis. It is clear from the experiments presented here that IL-8 is an important autocrine multifunctional cytokine that modulates melanoma/cell proliferation, migration by induction of extracellular matrix degradation enzymes and induces neovascularization, all of which are critical for melanoma growth and metastasis. In addition, their expression in melanoma tumor specimens suggests an association between IL-8 expression and tumor aggressiveness. Further, inflammatory cytokines produced by either tumor cells or stromal cells may regulate IL-8 expression, which can control melanoma growth and enhance our current knowledge regarding rnelanoma progression and metastasis. Understanding these events and their significance will allow us to design novel therapeutic approaches for treatment of melanoma.

Key words: IL-8, Melanoma, Metastasis

Introduction

Human cutaneous malignant melanoma is a highly aggressive form of skin cancer, the incidence of which is increasing at a greater rate than any other type of cancer, and for which there is no effective treatment for advanced disease (Lee, 1976; Clark et al., 1984; Franceschi et al., 1991). The dramatic increase in melanoma incidence has continued with 40,300 new invasive cases this year alone (Salopek et al., 1995; Rigel et al., 1996; Landis et al., 1998). The lifetime risk of an American developing an invasive melanoma had reached one in 87 (one in 70 for white males) (Rigel et al., 1996).

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The incidence of melanoma is currently rising faster than any other cancer in men and second only to lung cancer in women (Halaban, 1993; Barnhill et al., 1993; Kosary, 1995; Rigel, 1996). Further, the mortality rate of melanoma is rising at a rate second only to that of lung cancer (Henderson et al., 1991; Kosary, 1995; Rigel, 1996), and the American Cancer Society predicts that there will be 7300 deaths from the disease in 1998 (Landis et al., 1998).

There are a number of well-defined clinical and pathological stages of melanoma progression and metastasis. For example, normal melanocytes can give rise to precancerous lesions known as benign, atypical melanocytic nevi, the vast majority of which eventually regress (Clark et al., 1984; Clark, 1991; Herlyn, 1993). Occasionally, however, a nevus can progress to a radial growth phase (RGP) primary melanoma (Clark, 1991; Guerry et al., 1993). These lesions are rather indolent and confined primarily to the epidermis ('melanoma in situ'), as they tend to expand in a horizontal plaque manner (Clark, 1991; Guerry et al., 1993). RGP melanomas are almost always cured by surgical excision and presumably consist of tumors that are not competent for metastasis (Clark, 1991; Guerry et al., 1993). Slowly growing RGP melanomas can evolve over long periods of time and eventually change to the more rapidly growing vertical growth phase (VGP) primary melanoma (Clark, 1991). Such lesions penetrate the basement membrane separating the epidermis from underlying mesenchyme and contain progressively greater proportions of metastatically competent melanoma cells that can readily proliferate in different organ environments (Clark, 1991). The final phase of melanoma progression is the formation of distant organ metastases, particularly from primary lesions greater than 0.76 mm in thickness (Clark et al., 1984; Clark, 1991; Franceschi et al., 1991; Henderson et al., 1991; Barnhill et al., 1993; Halaban, 1993; Herlyn, 1993; Kosary, 1995; Salopek et al., 1995; Rigel, 1996; Rigel et al., 1996; Landis et al., 1998).

The etiology, progression, diagnosis and therapy of malignant melanoma have been intensely investigated (Herlyn et al., 1987; Holzmann et al., 1987; Herlyn,

1990; Kerbel, 1990). However, in contrast to our understanding of the steps of melanoma development, little is known about the molecular regulation of its metastatic ability. Improved diagnosis of melanoma has led to the treatment of earlier stages of malignancy, resulting in a higher survival rate in patients with early forms of the disease. Melanoma's lethality is primarily related to its propensity to metastasize. Unfortunately, the majority of patients with malignant melanoma will have metastasized by the time of diagnosis (Balch et al., 1985). The major obstacle to the effective treatment of metastatic melanoma is a lack of understanding of the mechanisms regulating the metastatic process. This review will focus on the significance of interleukin (IL)-8 expression and its regulation by inflammatory cytokines in regulating the process of melanoma growth and metastasis.

The pathogenesis of melanoma metastasis

The process of cancer metastasis consists of a series of sequential interrelated steps, each of which can be rate-limiting (Fidler, 1990). The major steps in the formation of a metastasis are as follows: 1) After the initial transforming event, either unicellular or multicellular growth of neoplastic cells must be progressive. Extensive vascularization must occur if a tumor mass is to exceed 2 mm in diameter. The synthesis and secretion of several angiogenesis factors play a key role in establishing a neocapillary network from the surrounding host tissue. Local invasion of the host stroma by some tumor cells could occur by several mechanisms that are not mutually exclusive. Thin-walled venules, like lymphatic channels, offer very little resistance to penetration by tumor cells and provide the most common pathways for tumor-cell entry into the circulation. 2) Detachment and embolization of small tumor-cell aggregates occur next; those that survive the circulation must then arrest in the capillary beds of organs. 3) Extravasation occurs next, probably by the same mechanisms that influence initial invasion. 4) Finally, proliferation within the organ parenchyma completes the metastatic process. To produce detectable lesions, these metastases must develop a vascular network, evade the host immune system, and respond to organ-specific factors that influence their growth. Once they do so, the cells can invade host stroma, penetrate blood vessels, and enter the circulation to produce secondary metastases, the so-called "metastasis of metastases". In malignant melanoma, neovascularization, invasion and migration of malignant cells to distinct organs and proliferation are crucial steps of metastasis that can be regulated by IL-8 and, thus, will be discussed in greater detail in this review.

IL-8 in melanoma growth and metastasis

Many molecular changes in tumor cells have been implicated as contributing factors to tumor progression and metastasis. These changes include phenotypic characteristics described as "gain of function" such as the autocrine expression of cytokines/growth factors and their receptors and expression of various cell adhesion molecules, or "loss of function" characteristics such as the inactivation of tumor suppressor genes and loss of responsiveness to inhibitory cytokines/growth factors (Kerbel, 1992). Aberrant production of autocrine cytokines/growth factors has been proposed as an essential element for tumor growth and metastasis (Nicolson, 1991). Thus, tumor cells can produce and secrete cytokines/growth factors that stimulate their own growth. Several lines of evidence suggest that in malignant melanoma, this autocrine loop confers a growth advantage to neoplastic cells (Herlyn et al., 1987; Rodeck et al., 1987; Herlyn, 1990). Recent reports suggest that human melanoma cells grown in culture express and secrete a variety of cytokines/growth factors either constitutively or upon induction (Herlyn, 1990). These include IL-1, IL-6, and melanoma growth-stimulating activity (MGSA), IL-8, platelet derived growth factor (PDGF)-A and PDGF-B, granulocyte macrophage colony-stimulating factor (GM-CSF), basic fibroblast growth factor (bFGF), transforming growth factor (TGF)-α and TGF-ß, and nerve growth factor (NGF) (Herlyn, 1990; Singh et al., 1995). The role of these cytokines/growth factors in the development of the malignant phenotype, perhaps by acting as autocrine growth factors, is under intense investigation. Incubation of melanoma cells with antisense oligonucleotides for bFGF inhibits their growth *in vitro* (Becker et al., 1989; Halaban, 1993). However, few studies have analyzed whether a population of melanoma cells produces multiple autocrine growth factors, and, if so, whether this has a critical role in the acquisition of the metastatic phenotype. For example, Pinchon and Largrade (Pichon et al., 1989) studied one melanoma cell line (MeWo) in detail and detected evidence of the concerted interaction of two intracellular autocrine growth factors, specifically bFGF and MGSA/gro-α. However, additional autocrine growth factors must be involved during progression of the malignant phenotype because MGSA/gro-α, like bFGF, is detected in melanoma cells at a very early stage (benign) lesions and even in dysplastic nevi (Richmond, 1991). Our analysis of the expression of autocrine growth factors (bFGF and IL-8) in the melanoma cell variants with different metastatic capabilities suggested that there was no correlation with the steady state expression of the bFGF gene and metastatic potential in nude mice (Singh et al., 1994a). In contrast, the expression of steady state levels of mRNA for IL-8 correlated with the metastatic capacity of all the human melanoma cell lines analyzed. Furthermore, no IL-8 mRNA was observed in non-tumorigenic, non-metastatic clones (Gutman et al., 1994; Singh et al., 1994b, 1995).

IL-8 is a member of the C-X-C family of chemokines, a set of related cytokines with inflammatory, chemotactic and growth-stimulating potential (Matsushima and Oppenheim, 1989;

Oppenheim et al., 1991; Baggiolini et al., 1994). Chemokines are members of a large superfamily of structurally and functionally related inflammatory cytokines that stimulate the migration of distinct sets of cells (Matsushima and Oppenheim, 1989), including neutrophils, monocytes, lymphocytes and fibroblasts. Different members of this family share conserved cysteine residues. Three subfamilies can be distinguished according to the positions of the first two cysteines which are either separated by one amino acid (C-X-C family, also called α-chemokines), are adjacent to each other (C-C family, or ß-chemokines) or contain one cysteine (-C- family, IL-16). The observation that IL-8 has produced a variety of tumor cells is important in understanding the potential role of this chemokine in the pathophysiology of neoplasia (Matsushima et al., 1989). Even though an increasing amount of information has become available on the role of chemotactic cytokines in host defense, their role in tumor growth has yet to be established. Nonetheless, recent studies suggest that this multifunctional cytokine may play an important role in tumor growth and metastasis. Recent studies have demonstrated differential expression of IL-8 in various tumor systems. Furthermore, it has been shown that inflammatory stimuli such as IL-1 and TNF can induce the synthesis of IL-8 mRNA in normal and human tumor cell lines (Kasahara et al., 1991; Baggiolini et al., 1994; Strieter et al., 1995).

The potential role of IL-8 in the pathogenesis of melanoma is suggested by an increased incidence of metastasis at sites of inflammation (Murphy et al., 1988). Further, enhanced expression of IL-8 by human melanoma cells after treatment with inflammatory cytokines like IL-1 and TNF has also been reported (Zachariae et al., 1991). Similar to other cytokines, IL-8 is multifunctional and an autocrine growth factor (Forster et al., 1991; Schadendorf et al., 1993; Singh et al., 1994a). IL-8 has also been shown to induce angiogenesis (Koch et al., 1992; Strieter et al., 1992) and migration of melanoma cells (Wang et al., 1990). Since angiogenesis, migration (invasion) and cell proliferation are important components of the metastatic process we hypothesize that IL-8 expression by tumor cells could influence their metastatic phenotype. Our preliminary data demonstrate a correlation between IL-8 expression and metastatic behavior in human melanoma cells (Singh et al., 1994a). In another study, the highly metastatic variant WM 98.1 (Herlyn, 1990) expressed 4.5 times higher levels of IL-8 protein as compared to the nonmetastatic variant, WM 1341 (Herlyn, 1990; Bani et al., 1996; Schadendorf et al., 1996). Elevated serum levels of IL-8 in patients with metastatic melanoma and hepatocellular carcinoma have also been reported to correlate with tumor burden and poor prognosis (Ueda et al., 1994; Scheibenbogen et al., 1995). A recent report also showed that the inhibition of IL-8 decreased the *in vitro* proliferation of human carcinoma cells of the lung, stomach, pancreas, liver, gall bladder, colon and gastric carcinoma (Ishiko et al., 1995; Kitadai et al., 1998). Attenuation of IL-8 expression and/or activity by antibodies to IL-8 decreased the angiogenesis and growth of bronchogenic carcinomas, squamous cell carcinoma, adenocarcinoma and non-small cells lung carcinoma (Smith et al., 1994). However, the precise role of these cytokines in melanoma growth and metastasis remains to be elucidated.

Recent observations from our laboratory suggest that the expression of IL-8 in tumor specimens from different stages of melanoma was found to be different in RGP (melanoma-in-situ) and VGP (invasive) primary malignant melanoma and subcutaneous, muscle and lymph node metastases. RGP tumors did not show any staining for IL-8. In contrast, 50% of the VGP tumors showed varying degrees of staining for IL-8. VGP tumors demonstrated a heterogeneous pattern of staining, and 50% were positive. None of the RGP tumors were positive for IL-8 immunoreactivity. In contrast, all the metastatic lesions analyzed from skin, muscle and lymph node demonstrated an intense IL-8 immunoreactivity. The staining pattern was homogenous within the metastatic lesions with no detectable 'hot spots' of IL-8 expression. Furthermore, the expression of IL-8 in metastatic lesions showed higher levels of staining as compared to VGP tumors. Similar results have been demonstrated in another study (Hensley et al., 1998) showing a correlation between IL-8 expression and tumor depth. These data suggest an association between the expression of IL-8 and metastasis in human cutaneous melanoma. Expression of IL-8 in metastatic lesions and VGP lesions may result in an increase in the serum IL-8 concentration (Herlyn, 1990) and may be of prognostic use and serve as a determinant of melanoma growth and metastasis. Whether expression of IL-8 predicts disease outcome and survival is currently under investigation.

One corollary to our primary hypothesis is that the over expression of receptors for cytokines/growth factors may also be a determinant of the malignant phenotype. Little is known about the role of receptors for autocrine cytokine/growth factor and their role in melanoma metastasis. Aberrant expression of EGF-R has been found in 50-60% of melanoma cell lines (Halaban, 1993). Similar observations have been made for other receptors (Herlyn et al., 1990; Halaban, 1993); however, an autocrine loop with these receptors has not been demonstrated. Two receptors for IL-8 have been cloned. The type A IL-8 receptor (IL-8RI or CXCR1) and type B IL-8 receptor (IL-8RII or CXCR2) both bind IL-8 with high affinity (Beckmann et al., 1991; Holmes et al., 1991; Murphy and Tiffany, 1991; Cerretti et al., 1993; Horuk et al., 1993). Studies have demonstrated the expression of IL-8RI and IL-8RII receptors in keratinocytes, fibroblasts, endothelial and melanoma cells (Horuk et al., 1993; Moser et al., 1993). These receptors have also been implicated in the angiogenic response to IL-8 and neutrophil and lymphocyte migration (Holmes et al., 1991; Murphy et al., 1991;

Horuk et al., 1993; Moser et al., 1993; Peiper et al., 1995). We speculate that the involvement of IL-8 receptors will demonstrate an autocrine role for IL-8 in the pathogenesis of melanoma growth and metastasis.

IL-8 expression in melanoma: potential role of inflammatory cytokines

The demonstration of an increased incidence of secondary tumor growth (metastasis) at sites of inflammation support the role of inflammatory cytokines in the pathogenesis of tumor growth and metastasis (Murphy et al., 1988; Zachariae et al., 1991). Increased serum levels of IL-8 can be detected in inflammatory conditions (Gross et al., 1992). In addition, an inflammatory cytokine like IL-1, which increases melanoma cell adhesion to cultured endothelium, could enhance the metastatic potential of human melanoma cells (Singh and Varney, 1998). Inflammatory cytokines, including IL-1 and TNF, are potent inducers of IL-8, and the local production of these inflammatory cytokines could enhance secondary implantation of melanoma cells (Murphy et al., 1988). This hypothesis is supported by the demonstration that injections of IL-1 and TNF augment the metastasis of human melanoma cells in nude mice (Matsushima and Oppenheim, 1989; Giavazzi et al., 1990).

The role of IL-1 in melanoma growth and metastasis remains to be clarified. It may have a direct effect on melanoma growth as an antiproliferative agent or by modulating the expression of other important regulatory molecules involved in melanoma growth and metastasis. Recent reports suggest the expression of IL-1 in surgical specimens from melanoma patients (Tyler et al., 1995). IL-1 has been shown to be expressed in metastatic tumors, but not in primary lesions (Tyler et al., 1995). The precise role of significance of IL-1 in metastatic melanoma lesions remains unclear. However, it is possible that IL-1 might also act as an autocrine regulator of melanoma cell production of molecules associated with metastasis. In addition, data suggests that advanced melanoma demonstrated resistance to the antiproliferative effect of IL-1 which also permits invasion of human melanoma cells *in vitro* (Hayashi et al., 1997). However, these cells failed to establish metastasis, suggesting that an intricate and complex interaction of IL-1 with other cytokines may be crucial in melanoma growth and metastasis.

Recent reports from our laboratory demonstrate that the treatment of human melanoma cells with IL-1ß or TNF-α enhanced the levels of IL-8 mRNA by increasing IL-8 gene transcription leading to the upregulation of IL-8 production in human melanoma cells. Further, IFN-ß and IFN-α treatment leads to a decrease in IL-1ß or TNF-α induced IL-8 mRNA levels due to a decrease in the IL-8 gene transcription. The mechanism responsible for this specific inhibitory effect of IFN-α or ß on IL-1 or TNF induced IL-8 expression in human melanoma cells might be due to a transient modification of preexisting factor(s) (Singh and Varney, 1998). We demonstrated an organ site-specific expression of IL-8, which might be due to either stimulatory (IL-1, TNF) or inhibitory factors (IFN-ß, TGF-ß) from the host stroma. IL-1 can also augment metastasis by human melanoma cells in nude mice (Murphy et al., 1988), suggesting that inflammatory stimuli, such as IL-1 and TNF, may induce the synthesis of IL-8 mRNA in normal and transformed human cells including melanoma (Matsushima et al., 1989; Hayashi et al., 1997). In addition, modulated expression of IL-8 in human melanoma cells affects their proliferation *in vitro* (Singh et al., 1998).

Cytokines are involved in a cascade of molecular interactions, including stimulatory and inhibitory, i.e., cytokines either stimulate production of cytokines/growth factors in target cells (Zachariae et al., 1991) or inhibit production as they can also downregulate the expression of other cytokines/growth factors (Lee et al., 1990; Thomson, 1991; Gusella et al., 1993). Reports from other laboratories have suggested positive or negative effects of IFNs (α and ß), inflammatory cytokines (IL-1 and TNF) or bacterial endotoxins in the regulation of IL-8 expression in monocytes, neutrophils, and diploid fibroblast cell lines (Oliveira et al., 1992; Gusella et al., 1993; Ohmori and Hamilton, 1994; Schnyder-Candrian et al., 1995).

In malignant melanoma, the most effective biological agent tested thus far is IFN-α (Sheridan and Hancock, 1992; Krasagakis et al., 1993). Although the response rate varies from 11-38%, all of the clinical trials report a therapeutic benefit of IFN-α in a proportion of melanoma patients. In addition high, local concentrations of IFN-α, after intralesional application to cutaneous melanomas, led to the regression of >50% of the injected lesions (Ishihara et al., 1983; von Wussow et al., 1988; Cascinelli et al., 1994). Cytogenetic analysis of both sporadic and familial melanoma shows a frequent loss of 9p13-22 (Cannon-Albright et al., 1992; Linge et al., 1995). The tumor suppressor gene p16, the IFN-α gene family and the single IFN-ß gene, are located within this region. Recent reports suggest that a defect in the IFN system occurs in melanoma cells and may be important in the pathogenesis of melanoma metastasis (Linge et al., 1995). Thus, IFNs may have a role in melanoma growth and metastasis based on their regulation of other cytokines such as IL-8. Recent reports from our laboratory and others suggest that IFNs significantly modulate the expression of growth factors and oncogenes that have a role in the pathogenesis of melanoma. Evidence also suggests that IFNs inhibit the expression of IL-8 by dermal fibroblasts. Indirect evidence suggests that IFNs can act as anti-inflammatory agents by inducing the expression of IL-1 receptor antagonists *in vivo*, which has been shown to suppress the IL-8 expression (Tilg et al., 1993).

These complex cytokine interactions *in vivo* may regulate the site-specific growth and metastasis of melanoma. Independent reports have established direct and indirect evidence suggesting the role of these

interactions in regulating the expression of growth-related genes (Van Damme and Opdenakker, 1990). The cellular localization of IL-8 and its inducers TNF and IL-1 in different pathological and physiological settings suggests that these cytokines act in concert. Recent studies have examined sites of inflammation, psoriatic lesions and wound repair, demonstrating the differential distribution of these interacting cytokines (Nickoloff et al., 1991, 1994). The role of inhibitory cytokines in regulating the expression of IL-8 is not yet clear. The inhibition of IL-1 or TNF-induced IL-8 expression in dermal fibroblast cells *in vitro* represent one such interaction associated with stimulatory (IL-1 and TNF) and inhibitory cytokines (IFNs) (Singh and Varney, 1998). Further, studies have demonstrated that this interaction exists at the transcriptional level mediated by the interaction of transcription factors (Nickoloff et al., 1994; Oliveira et al., 1994; Singh and Varney, 1998). At the systemic level, IFNs (IFN-α and IFN-ß) have been shown to block the effects of inflammatory signals by upregulating the secretion of IL-1 receptor antagonists (Aman et al., 1993; Oliveira et al., 1994). The induction of increased levels of IL-1 receptor antagonist (IL-1Ra) after IFN-α administration in healthy individuals provides a model whereby IFNs may modulate the effects of inflammatory cytokines (Tilg et al., 1993). Analysis of the interactions of different stimulatory and inhibitor cytokines in the regulation of IL-8 expression will be crucial in defining the role of organ-derived regulatory factor(s) in organ-specific expression of IL-8 and site-specific growth and metastasis of melanoma cells.

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