Interleukin 8 - Major Player in Cutaneous Melanoma Metastatic Process

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Abstract
Cutaneous melanoma remains a major health problem, having a worldwide increased incidence, still lacking an efficient therapy for the advanced stages. Intense research is seeking to establish new immune-related therapeutic approaches and to unveil new biomarkers that can orient these therapies. Complex mechanisms of intracellular signaling guide tumorigenesis, while inter-cellular communications sustained by circulating molecules promote advanced processes like progression and metastasis. Among the chorus of immune-related molecules, interleukin 8 has its voice heard especially in processes like angiogenesis, hypoxia, and this array of events leading to the advanced metastasis. Renal metastasis of melanoma accounts for an important percentage of late stage melanoma patients and new biomarkers can pinpoint the appearance of this metastasis.

Keywords: cutaneous melanoma, metastasis, chemokine, interleukin 8, angiogenesis, hypoxia, renal metastasis

1. Introduction
The one of the most aggressive forms of human cancer (CHIN et al [1]), although represents only 4% of all skin cancers, it accounts for 80% of skin cancer deaths being placed second after adult leukemia in terms of potential productive life-years loss (NEAGU et al [2]). The up-dated figures show that 132,000 melanoma skin cancers occur globally each year, and is estimated that only in USA 63,770 melanoma in situ will be newly diagnosed in 2014 (AMERICAN CANCER SOCIETY [3]). As recently shown, the neoplastic process can be an immune-mediated disease. Out of many other forms of cancers, melanoma has a clear immune background and can be generated by defective immune responses. Pro-tumor immune processes converge, the neoplastic transformed cells are not eliminated due to the activation of immune suppressive functions, tumor initiation and progression is sustains by maintaining a chronic inflammatory state and generating a pro-tumor cellular microenvironment through polarized immunosuppressive regulatory cells (SHURIN [4]). Advanced melanoma remains a continuous
clinical provocation for the physicians due to the fact that available therapeutic approaches produce low response rates, un-manageable toxicities, and short clinically response duration. Early-stage melanoma is resolved mainly by surgery and large margin excision, but for advanced stages, systemic therapies, whether chemotherapy, immune-therapy or combined ones, have had very low efficacy (NEAGU & CONSTANTIN [5]).

2. IL-8 in the immune system picture. Interleukin 8 (IL-8) or CXCL8 is an-immune related molecule that has gained in the last years a growing interest in melanoma research domain. It is actually a chemokine produced by a large array of cells: macrophages, epithelial cells, airway smooth muscle cells and endothelial cells that can deposit IL-8 in their storage vesicles (HEDGES et al [6], WOLFF et al [7]). In humans, IL-8 is initially produced as a precursor peptide of 99 amino acids long which then undergoes cleavage in order to obtain several active IL-8 isoforms. In culture, a 72 amino acid peptide is the major form secreted by macrophages (BRAT et al [8]). Monomeric and dimeric forms of IL-8 were recognized in the early nineties, then in early 2000, it was shown that IL-8 dimerization is a negative regulator for the receptor function and as a positive regulator for binding to glycosaminoglycans and that both forms have their role in the neutrophil recruitment process (FERNANDO et al [9]). This chemokine can be bound by many cellular receptors, the most frequently published specific receptors are G protein-coupled serpentine receptors CXCR1 and CXCR2. CXCR1 has increased affinity for IL-8 in comparison to CXCR2. Toll-like receptors (TLR), characterizing cells appending to innate immune system, can regulate IL-8 secretion. Neutrophil elastase (NE) can induce IL-8 expression using the IL-1 receptor-associated kinase signalling pathway and TLR4. Human embryonic kidney (HEK) 293 cells manipulated to express TLR4 were found having IL-8 promoter activity as response to NE action (DEVANEY et al [10]). Hence secreted IL-8 is an important mediator of innate immune system. IL-8, also known as neutrophil chemotactic factor, can induce chemotaxis mainly in neutrophils and migrate toward the infection site. IL-8 can be a potent promoter of angiogenesis, inducing migration, phagocytosis and intracellular Ca2+ increase. IL-8 has been reported to be potent inducers of the chemokine receptors CXCR1 and CXCR2. Being a member of the CXC chemokine family, the genes encoding it as for the other ten members of the CXC form a cluster in a region mapped to chromosome 4q (MODI et al [11]). In this paper’s context the most interesting cytokine cascade witch accodates IL-8 is the one generated by the hindrance of human skin’s barrier function. Immediately upon stratum corneum removal, keratinocytes became activated within hours, up-regulate their keratin-16 expression and when keratinocyte’s proliferation initiated, generates an array of cytokine, chemokines and adhesion molecules, in both epidermal and dermal skin compartments. Within 6 hours of skin injury, is described an over-expression of mRNAs coding for tumor necrosis factor-α (TNF-α), IL-8, IL-10, interferon-γ (IFN-γ), intercellular adhesion molecule-1 (ICAM-1), transforming growth factor-α (TGF-α) and –β. Keratinocyte’s ICAM-1 is accompanied by over-expression of other molecules on endothelial cell, such as E-selectin and vascular cell adhesion molecule-1 (VCAM-1). After these 6 hours cell motility was induced, namely inflammatory cells extravasated from circulation into dermis / epidermis. The results highlight that to a skin injury the immune response is rapid and IL-8 action is congruent to the cytokine/chemokines secreted panel that protects the epidermal barrier (NICKOLOFF & NAIDU [12]. A diagram that shows the physiological functions of IL-8 in both immune-related cells (polymorphonuclear cells, macrophages, lymphocytes) and non-immune cells, like endothelial cells and fibroblasts is depicted in Figure 1. Without being
comprehensive this diagram highlights several aspects regarding IL-8 biological functions: it has an ubiquitary action, its main role is to induce chemotaxis as an important player in inflammation and it is related with a vast array of cytokines/chemokines appending to major cellular physiological tasks.

**Figure 1.** Biological function of IL-8. This chemokine acts on major immune population whether adaptive or innate cells (LTB4-leukotriene B4, MMP-9-matrix metalloproteinase-9) (adapted from MUKAIDA [13]).

**Figure 2.** IL-8 involvement in various tumorigenesis–related processes (adapted from YUAN et al [14]).

3. **IL-8 as a circulatory biomarker.** IL-8 is circulating in isoforms, as such, an endothelial-derived [ala-IL-8] (77) isoform and a more potent one, [ser-IL-8] (72) are the largely secreted isoforms. Isoform [ala-IL-8] (77) can be converted into [ser-IL-8] (72) by proteolytic removal of pentapeptide spanning the N-terminal sequence. A study published several years ago showed that [ala-IL-8] (77) is the predominant circulating isoform of IL-8 in premature neonates, while [ser-IL-8] (72) is predominant in normal term neonates/adults. The authors highlight that developmental changes in IL-8 isoforms are a physiological process to minimize its inflammatory effects in the fetus restoring its full activity after birth (MAHESHWARI et al [15]). IL-8 can be produced as well by malignant cells from various sources. Its functions in tumorigenesis are mainly related to neo-vascularization and inflammation processes. In *in vitro* cellular models, IL-8 concentrations correlate with the number of IL8-producing tumor cells. Serum IL-8 concentrations correlated with tumor burden, stage, survival and objective responses to therapy. From this point of view IL-8 can become a useful biomarker (SANMAMED et al [16]). Our hand-on experience has shown that while IL-8 is 2.5 fold enhanced in advanced stages it did not correlate statistically with overall survival, or relapse-free survival (NEAGU et al [17]). Looking for correlations between IL-8 serum level and the cells that are expected to secrete them, we found that only for patients in stage I a slight correlation of IL-8 level with the circulatory percentage of CD16+ and CD8+ exists. Overall, high IL-8 levels have been registered in patients with metastatic melanoma, and a decrease of serum IL-8 level has been described as a result of chemotheraphy or immune-therapy (SCHEIBENBOGEN et al [18], BRENNECKE et al [19]). Besides IL-8 serum levels decreased elevated serum levels of VEGF and FGF-2 that persisted after the initial cytostatic administration. Recently myeloid-derived suppressor cells (MDSC) have been characterized as a heterogeneous cell population with clear immunosuppressive activity. When investigating the frequency of MDSC in the melanoma patient’s peripheral blood, an accumulation of CD11b+ CD33+ CD14+ HLA-DRlow MDSC in all stages of disease (I-IV) was reported. One of the cytokines that is associated to MDSC is IL-8. Indeed, the finding
that IL-8 is increased in all stages of disease can be due to this enhanced circulatory immune-suppressive immune population (RUDOLPH et al [20]). IL-8, thoroughly revised (DEWING et al [21]), accompanies the angiogenesis process in many inflammatory and malignant disease states. Circulatory levels of pro-angiogenic factors strongly correlate with cutaneous melanoma progression and poor prognosis. Circulatory vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), platelet growth factor (PGF-1), IL-8 and TGFs modulate the angiogenic switch, toward metastatic growth.

4. IL-8 in angiogenesis. Melanoma angiogenesis was one of the first reported processes and it is still a subject of intense research. In skin melanoma this is the crucial event triggering metastasis, mainly in young adults (STREIT & DETMAR [22]). IL-8 is one of the main promoters of angiogenic activity in endothelial cells, increases their proliferation and survival, sustains the migration of cancer cells, endothelial cells, and activates infiltrating inflammatory cells at the tumor site. The array of processes in which IL-8 is involved in the tumorigenesis complex pathways is depicted in Figure 2. As IL-8 is incriminated as one of the main angiogenesis promoters, in xenograft and orthotopic in vivo models, IL-8 expression was shown to be correlated with the angiogenesis, tumorigenicity, and metastasis of experimental tumors (RIBATTI et al [23]). Its specific receptors, CXCR1 and CXCR2, were also found increased in cancer cells, infiltrating neutrophils, tumor-associated macrophages and endothelial cells, that means that in the tumor microenvironment there is regulatory loop sustained by IL-8 (WAUGH&WILSON [24]). This regulatory loop is sustained not by all melanoma cell line, hence primary and metastatic melanoma cells constitutively secrete IL-8, while non-metastatic cells are not active regarding IL-8 expression (WESTHAPL et al [25] SINGH et al [26]). The fact that IL-8 over expression along with its specific receptors induce tumor progression, metastasis and angiogenesis in human melanoma (GABELLINI et al [27], SINGH et al [28]), was again proven when specific antibodies were used for IL-8 and its receptors. This antibodies could inhibit melanoma angiogenesis (HUNANG et al [29]). In animal model with mCXCR2 (−/−), mCXCR2 (+/−), and wild-type phenotype it was demonstrated a significant lower number of microvessels in tumors from double negative CXCR2 and CXCR2 (+/−) mice in comparison to wild-type mice (SINGH et al [30]). Multi-approach technologies were reported in 2014 studying the secreted IL-8 and its receptors while melanoma cells are in different cellular stages, anchored or not, hence during initiating metastasis or not. Thus various melanoma cell suspensions were generated and followed by in vivo xenograft experiments. Complex evaluation was performed starting from classical proliferation/migration tests to microarray for adherent and suspended melanoma cells. Suspended melanoma cells were anoikis resistant proving an elevated malignancy in both in vivo and in vitro experiments. Gene expression showed alteration correlated with cell survival/death and cell signaling. Out of all the tested genes enhanced expression of IL-8 was found in suspended melanoma cells. Upregulated IL-8 induced chemotaxis mediated by MEK/ERK signaling upon cell suspension. Alteration in JNK phosphorylation status induced CXCR1 down regulation in suspension, but when the cell reattached the receptor was up regulated. This recent report highlights this inter-play between elevated IL-8 secretion and modification in CXCR expression that mediates melanoma malignancy and hence de- and re-attachment of tumoral cells. In this inter-play the status of the tumoral cells, suspended or attached is critical in promoting melanoma malignancy in vivo and in vitro [31]. As established in several other cancers, the first step is the VEGF secretion performed by proliferating tumour cells; this growth factor links to its specific receptor (VEGF-R)
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expressed by endothelial cells residing in tumoral and peritumoral sites. To be noted that VEGF-R is not expressed by healthy tissues. One of the earliest studies has shown that VEGF-A secretion is correlated with the melanoma growth from the horizontal to the vertical phase, and moreover to the metastatic process (ERHARD et al. [32]). Further correlation with increased tumoral microvasculature density, tumor VEGF-A positivity, high Breslow index (>3.6 mm) was established with VEGF-A hypersecretion and tumor infiltrating cells (SALVEN et al. [33]). In normal skin and in benign proliferating melanocytes IL-8 expression is reduced, while in the malignant proliferation is enhanced. In this skin cancer it controls endothelial cells migration as the first step in angiogenesis. IL-8 over-expression is associated with adhesion molecule MCAM/MUC18. It seems that they are regulated at transcriptional level and develop a major role in melanoma growth, angiogenesis and metastasis. The importance of these molecules was reported several years ago when in animal models it was described that anti-MCAM/MUC18 and anti-IL-8, could reduce tumour growth and metastasis. Suppressing IL-8 could be a new therapeutic approach in combination with other immune-therapy agents (MELNIKOVA & BAR-ELI [34]).

4.1. IL-8 activated by an array of factors to induce angiogenesis. Metastasis is a multi-step process that involves an inter-cellular communication between tumor cells and endothelial cells. Despite this, the molecular mechanisms developed in the tumor microenvironment still remain elusive (ZHANG et al. [35]). New experimental co-cultivation data have shown that human umbilical endothelial cells (HUVECs) and human melanoma cell line (Lu1205) have an increased intercellular adhesion molecule 1 (ICAM-1) and E-selectin expression on HUVECs when this cells are cultivated in presence of melanoma cell line. When using a non-metastatic cell line (WM35) this increased expression on HUVECs was not found. In the co-culture, cell supernatant had increased levels of IL-8, IL-6, and growth-related oncogene α (Gro-α). Another growth factor that clearly influences IL-8 involvement in angiogenesis is TGF-β1. It was demonstrated that TGF-β1 selectively induces IL-8 expression in only in melanoma cell lines that are highly metastatic (A375SM), but not in non-metastatic parental cells (LIU et al [36]). Another factor, this time appending to platelets, platelet-activating factor (PAF) known as pro inflammatory factor, can mediate an array of processes from wound healing, to inflammation, apoptosis, angiogenesis, and reproduction. It was demonstrated that in tumorigenesis cancer cells and activated endothelial cells increase the expression of PAF specific receptor- PAF-R that triggers several pathways involved in the onset and development of angiogenesis and further metastasis (TSOUPRAS et al [37]). In cutaneous melanoma PAF and PAF-R act as important modulators of melanoma angiogenesis. Moreover, protease activated receptor-1 (PAR-1) was reported as over expressed in highly metastatic melanoma cell lines and in metastatic lesions of melanoma patients (TELLEZ et al [38], MASSI et al [39]). There are several genes that are over-expressed upon the direct influence of PAR-1 activation, such as IL-8, VEGF and platelet derived growth factor (PDGF); all these genes are clearly connected to the initiation of melanoma angiogenesis (MELNIKOVA et al [40]).CD44 antigen is a cell-surface glycoprotein involved in cell–cell interactions, in cell adhesion and migration and it was shown that CD44/selectin coupling was responsible for the ICAM-1 up-regulation on HUVECs. This process is maintained by the PKCα-p38-SP-1 signaling pathway, which further enhances melanoma cell adhesion to endothelial cells during metastasis. Another recent study was published in 2014 depicting the influence of cross-communication of tumour cells and endothelial cells having IL-8 as functional mould. MV3 melanoma cell lines were shown to trigger a prompt calcium-flux-dependent, a pro-coagulatory endothelial response accompanied by the release of von Romanian Biotechnological Letters, Vol. 20, No. 6, 2015
Willebrand factor (Ultra large VWF) that was immobilized to the endothelial cells. NFκB activation in endothelial cells in this system was dependent on IL-1α and IL-1β secreted from melanoma cells. Upon IL-1 secretion performed by melanoma cells, pro-inflammatory cytokines IL-6 and IL-8 were upregulated. Adhesion molecules, ICAM-1 and VCAM-1, along with the procoagulatory tissue factor (TF) were increased in endothelial cells as pro-inflammatory molecules. These recent clear cut data shows that melanoma cells activate endothelial cells directly and/or via other cytokines based on a IL-1-mediated NFκB activation. Through any pathways, direct or indirect, endothelial cells activation, leads to a pro-inflammatory and pro-coagulatory surface that supports tumor progression (STROZYK et al [41]). Neuropilin-2 (NRP-2), a cell surface receptor involved in angiogenesis and axonal guidance, was tested as being involved in metastasis. Using a heterotypic coculture melanoma and endothelial cells, the up-regulation of neuropilin-2 was confirmed as important in the melanoma-endothelial interactions. As it was reported that neuropilin-2 is increased as expression in primary and metastatic melanoma this tissue marker can be relevant in vivo (RUSHING et al [42]). As demonstrated in other types of cancers, exogenous IL-8 up-regulated the expression of VEGF and NRP-2 in cancer cell lines via ERK activation (LI et al [43]). Thus, studies must be done in the future to establish if in cutaneous melanoma the same relation can be demonstrated, hence targeting IL-8 along with other anti angiogenesis therapy could enhance the anti-angiogenic drug panel. We can conclude that the array of factors that influence, mediates and potentates IL-8 as angiogenesis trigger in melanoma is complex and we are convinced that due to its complexity only multi-technological approaches, such as microarray technology can depict the proper inter-relation.

5. IL-8 related to hypoxia and metastasis. Reports that link hypoxia and IL-8 in cutaneous melanoma are very scarce. One of the first reports showing this link was published more than 10 years ago (OLIVER et al [44]). Almost in the same year it was demonstrated a correlation between hypoxia, IL-8, angiogenesis and metastasis in human melanoma xenografts. Moreover, neutralizing antibodies against IL-8 reduced the vascular density and the incidence of metastases (ROFSTAD and HALSOR [45]). In an in vivo experimental model that tested carboxyamido-triazole (CAI), in A2058 human melanoma xenograft, demonstrated the reduction of circulatory VEGF and IL-8 in treated animals. Moreover HIF-1alpha (hypoxia-inducible factor 1) was found reduced at the level of both mRNA and protein. It is postulated that CAI inhibition of tumor cell VEGF production and endothelial cell response to VEGF disrupts the inter-cellular signaling between the tumor cell and its microenvironment, causing a net anti-angiogenic effect. Hence the few published reports have shown that besides IL-8 secreted by melanoma cells, through its transcriptional regulator HIF-1, VEGF is actively produced promoting angiogenesis and metastasis. Agents that address A (2B) receptor can impair IL-8 production, whereas blocking A(3) receptors induce further VEGF decrease in melanoma cells. Refining chemotherapeutical agents like DNA-damaging compounds with anti-angiogenic function can aid cutaneous melanoma therapy (MERIGHI et al [46]). Mechanisms that explain hypoxia inducing tumoral angiogenesis state that HIF-1 increases the resistance of tumor cell to programmed cell death that is induced by anchorage-dependent cells detaching from the surrounding extracellular matrix. This effect is obtained by integrin α5 inhibition. In the end the hypoxic milieu and HIF-1 are inducing metastasis preparing an environment favorable for tumor cells to thrive (ERLER [47]). In uveal melanoma HIF-1 is the critical biomarker for metastasis, where Siah2 regulated via Akt signaling is stabilizing HIF structure and hence favours metastasis (QI et al [48]. Overall,
although few studies relate IL-8 and hypoxia to melanoma metastasis, we can emphasize that tumorigenesis in cutaneous melanoma needs to further establish a clear link in this disease with the declared purpose of finding new molecular therapy targets.

6. IL-8 and renal metastasis of the cutaneous melanoma. As a target-organ for melanoma metastasis, the kidney can have around 18% of melanoma metastasis (ABRAMS et al [49]), and when the clinical symptomatology of renal metastasis is installed the mean survival of the patient is under 1 year (ABDULAKADIR et al [50]). In this case the rapid detection of metastasis indicating biomarkers is mandatory (DHANHA et al [51]). Recently, it was shown that quantification of circulating microRNAs (miRNAs), more specifically the loss of serum miR-29c and miR-324-3p, is an indication of metastatic melanoma, pinpointing to a colon and renal metastases (GREENBERG et al [52]). In a recent mouse model it was reported that the lack of proteinase-activated receptor 2 (PAR-2) induces mainly renal and lung metastases in this knock-out mice model. The absence of functional PAR-2 could be an important factor influencing the growth and spread of melanoma in vivo, probably associated with tumour cell migration, invasiveness and metastasis formation (MATEJ et al [53]). The link of PAR-2 with IL-8 was recently reported in human kidney tubular epithelial cells (HTEC) whereas an important cytokines release was noticed (GM-CSF, IL-6, IL-8, and TNF-α), after using a PAR-2 activator. These recent results suggest PAR2 antagonists as being anti-inflammatory agents (VESEY et al [54]). Probably renal metastasis in cutaneous melanoma has also IL-8 increment that activates signaling pathways upon binding to CXCR2, mechanisms that trigger this organ metastasis.

7. Conclusions
Taking into account the difficulties of cutaneous melanoma patients’management, the immune surveillance at the skin level is the element that harbours both the tumorigenesis in melanoma and the therapeutical targets for future immune-therapies. All the conditions that favor the tumor escape from the immunological arm, the immune pattern of skin melanoma with diagnostic/prognostic relevance, can be mirrored by the circulatory immune markers. IL-8 is a glimpse of the complex immune network that lies within tumor escape and where to search for immune-therapeutical targets in skin melanoma (NEAGU [55]). Therefore chemokines like IL-8 can be empowered with biomarker capability of pin-pointing disease progression but as well monitoring therapy efficacy.

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