Perspective

The Potential Role of Neutrophils in Promoting the Metastatic Phenotype of Tumors Releasing Interleukin-8

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ABSTRACT

In the last decade, several groups have shown a direct correlation between the inappropriate or ectopic release of interleukin (IL)-8 by tumor cells *in vitro* and their growth and metastatic potential using *in vivo* models of tumor growth. IL-8 is a potent neutrophil chemoattractant. Neutrophils, as "early responders" to wounds and infections, release enzymes to remodel the extracellular matrix of the tissues through which they migrate to reach the site of the wound or infection. It is proposed that the host's cellular response to IL-8 released by tumor cells enhances angiogenesis and contributes to tumor growth and progression. The activities released by the responding neutrophils could serve as enablers of tumor cell migration through the extracellular matrix, helping them enter the vasculature and journey to new, metastatic sites. The reactive oxygen species produced by neutrophilic oxidases to kill invading organisms have the potential to interact with tumor cells to attenuate their apoptotic cascade and increase their mutational rate. It is proposed that the increase in metastatic potential of tumors ectopically releasing IL-8 is, in part, attributable to their ability to attract neutrophils. Discussed here are possible mechanisms by which the neutrophils responding to ectopic IL-8 contribute to the *in vivo* growth, progression, and metastatic potential of tumor cells. Possible targets are also presented for the development of therapies to attenuate the effects of the ectopic IL-8 release by tumor cells.

DISCOVERY OF INTERLEUKIN-8

The cytokine interleukin (IL)-8 is a small basic protein first purified on the basis of its neutrophil chemoattractant properties (1, 2). Soon after it was purified, a cDNA clone was obtained, and its gene was characterized (3, 4). IL-8 is a member of the α-chemokine family and a very potent neutrophil chemoattractant. It and its homologs are induced in wounds by the G_0 to G_1 transition, and its expression is greatly enhanced by the inflammatory mediators IL-1β and tumor necrosis factor (TNF) α (5, 6). IL-8 recruits neutrophils to the site of wounds to protect the tissue from invading microorganisms and enhance the healing process (7). However, the release of IL-8 by the wrong cells, at the wrong time, or at too high a concentration can lead to undesired pathologies, such as rheumatoid arthritis, inflammatory bowel disease, idiopathic pulmonary fibrosis, and cerebral and myocardial ischemia (8).

ECTOPIC INTERLEUKIN-8 EXPRESSION

In the last decade, several groups have observed a direct correlation between the level of ectopic IL-8 expression by individual clones of tumor cell lines and their *in vivo* growth rate and metastatic potential. This correlation has been shown for many tumor cell types (9–13) as well as for fresh breast tumor samples (14). Highly metastatic tumor cells produce more IL-8 constitutively than their poorly metastatic counterparts, and the amounts of IL-8 they release in response to the pro-inflammatory cytokines IL-1β and TNF-α are much greater (13, 15). Tumor-associated macrophages release these inflammatory mediators (16). Their release of these mediators may inadvertently induce the tumor cells to release even higher levels of IL-8. The mechanisms by which the ectopic expression of IL-8 contributes to an increase in metastatic potential are not fully appreciated or understood. In this review, we delineate a series of potential means by which the normal functions of IL-8 could contribute to the growth, progression, and metastatic potential of tumor cells.

Although it has been demonstrated for many tumor systems that the level of ectopic IL-8 released by tumor cells correlates with an enhanced *in vivo* progression and metastatic potential, ectopic IL-8 expression is neither essential nor sufficient for the expression of a metastatic phenotype. It is likely to act in conjunction with other phenotypic changes expressed by tumor cells to enhance their metastatic potential. Consequently, there is not a direct correlation between the level of ectopic IL-8 expression and the tumorigenic and metastatic potential when comparing tumor cell lines from different patients (17). There are metastatic tumor lines that produce no IL-8 (18), whereas other lines derived from human tumors produce large quantities of IL-8 but do not form tumors in nude mice (19).

A ROLE FOR NEUTROPHILS IN INTERLEUKIN-8-INDUCED ANGIOGENESIS

IL-8 has been shown to be an angiogenic factor (20, 21). Angiogenesis creates a new vascular supply to convey oxygen and nutrients to the involved tissue and to remove the by-products of cellular metabolism, which is necessary for the efficient elimination of invading organisms and wound healing (22). The mechanism by which IL-8 induces angiogenesis is not fully resolved. Some investigators report it is through the direct action of IL-8 on endothelial cells (21, 23), whereas others

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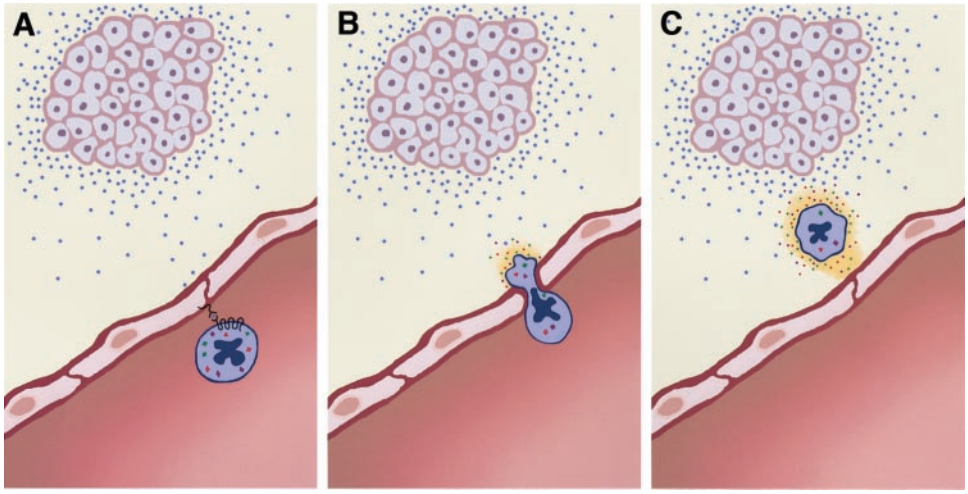

Fig. 1 A schematic representation of a neutrophil responding to IL-8 ectopically released by a tumor with the consequent invasion and remodeling of the ECM. *A*, the neutrophil (represented as a *blue cell*) binds to the IL-8 molecules (*blue dots*) that were released by the tumor and tethered to the vascular endothelial cells. This interaction contributes to the activation of the neutrophil. *B*, the emigration of an "activated" neutrophil from the vascular compartment. During this process, the neutrophil gains access to the ECM (*light yellow area*) and releases vesicles of enzymes (*red*, *green*, and *purple dots*) that initiate ECM remodeling (the *darker yellow*). *C*, the neutrophil, responding to the IL-8 concentration gradient, migrates toward the tumor. Remodeling the ECM during this process thereby establishes an environment more favorable to the progression and metastasis of the tumor cells.

describe it as an indirect mechanism requiring other cell types to work with the endothelial cells (24–26). In response to IL-8, neutrophils release specific proteases and a heparanase that hydrolyze components of the extracellular matrix (ECM); this remodeled ECM is easier for cells to transverse (27–29). During this process, neutrophilic elastase activates latent proteases, which can then cleave and inactivate plasminogen activator inhibitor 1, the natural inhibitor of plasmin (30, 31). This remodeling releases embedded growth factors, such as basic fibroblast growth factor, a potent angiogenic factor that is both a chemoattractant and growth factor for endothelial cells (32, 33). As neutrophils migrate through the newly remodeled ECM, they create channels between the site of extravasation and the involved tissue. This process can promote the migration of endothelial cells during the course of creating a new vascular supply. The channels created by the migratory neutrophils also facilitate the recruitment of other immune cells to the site of the wound, infection, or inflamed tissue. Inflammatory responses are self-limiting under normal physiological conditions (34). Once the infection is cleared or the wound is healed, IL-8 secretion stops, attenuating the influx of neutrophils and the consequential angiogenic and inflammatory responses (35)

INTERLEUKIN-8 AS AN ENABLER OF TUMOR PROGRESSION AND METASTASIS

The normal protective activities of IL-8, when exploited by tumor cells, have the potential to contribute to enhanced tumor growth, progression, and metastasis. Tumor cells that express an undifferentiated phenotype often release large quantities of IL-8 (9, 15, 36). The tumor-associated immune cells release the inflammatory cytokines IL-1β and TNF-α, which appreciably increase the levels of IL-8 released by the tumor cells, thus enhancing their metastatic potential (37, 38). Ectopically released IL-8 could contribute to the progression and metastatic potential of tumor cells via the following mechanisms: (*a*) induction of tumor neovascularization; (*b*) chemoattraction of neutrophils that release enzymes that can enhance tumor cell growth, progression, and metastasis; and (*c*) acting as an autocrine growth and/or chemokinetic factor for the tumor cells.

We hypothesize that the actions of neutrophils as they migrate through the tissue between the point of extravasation and the site of the tumor can contribute to the enhanced metastatic potential of tumor cells producing ectopic IL-8. A schematic representation of the proposed sequence of events initiated by the ectopic release of IL-8 is displayed in Fig. 1. The IL-8 concentration is highest at the site of the tumor, thus establishing a gradient that attracts and retains the neutrophils at the tumor site via direct chemotactic mechanisms. Because neutrophils have a short half-life and a high propensity to undergo lysis, the numbers visualized at the site of a tumor are likely to be a gross underestimate of the numbers that have responded to ectopic IL-8 stimulus. Once the neutrophils have reached the tumor, where the concentration of IL-8 is the highest, they are much more likely to be fully activated and totally degranulate, releasing their remaining stored enzymes and mediators.

NEUTROPHILS IN REMODELING THE EXTRACELLULAR MATRIX

As neutrophils travel toward a source of IL-8, they release a series of enzymes that are instrumental in remodeling the ECM, which favors neovascularization of the involved tissue (20, 28, 39, 40). Similar remodeling by neutrophils responding to IL-8 released by a tumor will create an environment favorable for tumor angiogenesis (41). The matrix metalloproteinases (MMPs) and their inhibitors, the tissue inhibitors of metallopro-

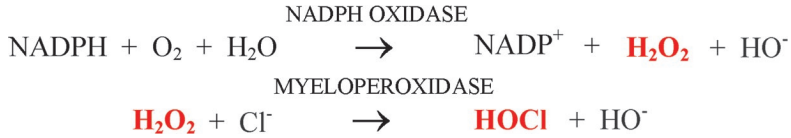

Fig. 2 Production of ROS by neutrophilic oxidases. The oxidative products of NADPH oxidase and myeloperoxidase, H_2O_2 and hypochlorous acid, are shown in *red*.

teinases (TIMPs), are important mediators of ECM remodeling. The MMPs are a family of zinc-dependent extracellular proteases that remodel the ECM by digesting its components. They are usually secreted and often stored as latent or inactive proenzymes. Neutrophils produce and release high levels of MMP-9/gelatinase B, but produce little, if any, MMP-2, which plays an important role in the turnover of various ECM components (42). Neutrophils release a soluble factor in response to IL-8, which activates latent MMP-2 released by other cells (43). In response to IL-8, neutrophils, endothelial cells, and lymphocytes release a specific sulfatase and a heparanase, which are instrumental in releasing embedded growth factors from the ECM (27, 28). This remodeling permits the migration of neutrophils and expedites the recruitment of other immune cells responding to the inflammatory cascade established by the ectopic IL-8 release (27–29). The remodeled matrix offers less resistance to cells leaving the tumor. Enzymes released by the neutrophils within the tumor milieu can activate latent proteases and diminish cell-cell interactions, thereby permitting the dissociation of tumor cells from the main tumor mass (44). The embedded growth factors and chemoattractants released during this remodeling, such as basic fibroblast growth factor, can serve as both a chemoattractant and a growth factor for these tumor cells (45).

Although this review emphasizes the *in vivo* paracrine potential of the IL-8 ectopically released by tumor cells, IL-8 also has been demonstrated to function as an autocrine motility and growth factor for several tumor cell lines. Tumor cells able to respond to ectopically released IL-8 in an autocrine fashion are likely to have an additional growth and progression advantage (46–49).

ROLE OF REACTIVE OXYGEN SPECIES IN ENHANCING TUMOR CELL PROGRESSION

Included among the neutrophilic enzymes used in the production of reactive oxygen species (ROS) are NADPH oxidase and myeloperoxidase (50–52). The reactions catalyzed by these enzymes are illustrated in Fig. 2. The NADPH oxidase reduces O_2 to the superoxide anion, which is converted to H_2O_2. In the presence of chloride ion, hydrogen peroxide (H_2O_2) is converted to hypochlorous acid (HOCl) by the enzyme myeloperoxidase, which is present at high concentrations in neutrophils (53). The oxidative properties of HOCl make it a potent bactericidal agent (52, 54). It is also a potent modifier of several proteins of the ECM. These modifications decrease the adhesion-promoting properties of the ECM (55). A decrease in the cell-matrix interactions increases the probability that tumor cells will leave the primary tumor.

HOCl activates the MMP pro-enzymes by oxidizing specific sulfur-containing amino acids, which alters the enzyme conformation (42, 54, 56, 57). This conformational change either directly activates the MMPs or allows other proteases to activate them, *i.e.*, neutrophil elastase (43, 44). HOCl is a much more potent activator of MMP-2, MMP-7, MMP-8, and MMP-9 than H_2O_2, its oxidative precursor. Furthermore, HOCl inactivates TIMP-1, thereby increasing the proteolytic activity of the MMP-TIMP system used in remodeling the ECM (58–60). Thus, HOCl produced by neutrophils has the potential to enhance the progression and metastatic potential of tumor cells releasing IL-8.

REACTIVE OXYGEN SPECIES AS POTENTIAL MUTAGENS

The ROS appear to have the potential to increase the rate of cellular mutations. Transgenic mouse tumor cell lines were used to determine the contributions of IL-8, neutrophils, and ROS to the rate of mutation of the hypoxanthine phosphoribosyltransferase locus (50, 61, 62). Spontaneous mutations of hypoxanthine phosphoribosyltransferase were observed to be 4-fold higher in cells grown as tumors *in vivo* compared with those grown *in vitro*. Examination of the tumors indicated the predominant host cells associated with these tumors were neutrophils (61). Because human IL-8 is a potent chemoattractant of mouse neutrophils, its gene was transfected into these cells under the regulation of the tetracycline promoter. The presence of tetracycline suppresses the expression of the transfected IL-8 gene. When these cells were grown *in vitro*, the IL-8 released altered neither the growth rate nor the rate of mutation of the hypoxanthine phosphoribosyltransferase gene. However, cells expressing recombinant IL-8 had a higher mutation rate when grown *in vivo*. The increased rate of mutation correlated with both the level of IL-8 present and the number of tumor-associated neutrophils. Treating tumor-bearing animals with tetracycline decreased the amount of IL-8 released and the rate of hypoxanthine phosphoribosyltransferase gene mutation. The correlation between the levels of IL-8 released and the rates of mutations in the hypoxanthine phosphoribosyltransferase gene suggests that an activity associated with the neutrophils contributes to the increased mutation rate. The levels of myeloperoxidase from 45 tumors derived from these transfected cells were compared with the rate of hypoxanthine phosphoribosyltransferase mutations. A correlation between the level of tumor myeloperoxidase and the rate of hypoxanthine phosphoribosyltransferase mutations was found in these tumors ($P < 0.0001$; $r = 0.88$; Ref. 50). When vitamin E, an antioxidant, was orally administered to tumor-bearing mice, the levels of myeloperoxidase activity and the frequency of mutation in the tumor cells were reduced (62–64). This suggests the ROS produced by tumors and their associated neutrophils have the potential to increase the rate of mutagenesis. If this is true, then minimizing the half-life or decreasing the rate of production of such ROS should attenuate the increased mutation rate.

ROS activate nuclear factor (NF)-κB, which is a potent

Table 1 The potential positive and negative roles of IL-8-induced activities

	Protective functions to the host	Properties that enable tumor progression
1 Attract neutrophils	The attracted neutrophils remodel the ECM, thereby allowing them and other inflammatory cells ready access to the involved tissue.	The remodeled ECM more readily allows tumor cells to escape their immediate surroundings. IL-8 can act as an autocrine motility activity for tumor cells.
2 Angiogenesis	Angiogenesis establishes a blood supply to involved tissues, which enhances the rate of tissue repair.	The new vessels supply tumors with a source of nutrients and oxygen to enhance their growth.
3 Enhances the production of ROS by neutrophils	The oxidative species can kill invading microorganisms and activate enzymes for further ECM remodeling.	These species, acting at the site of a tumor, can activate the survival factors, increase the rate of mutations, and activate proteases that can increase the potential for cells to dissociate from the tumor and metastasize.

inhibitor of programmed cell death, or apoptosis (65). Inhibition of the apoptotic cascade will increase the rate of tumor growth and enhance the potential that mutated cells will survive and pass on their genetic changes. NF-κB is normally maintained in the cytoplasm as an inactive heterotrimer. It is activated by the phosphorylation and degradation of the inhibitory subunit, IκB. ROS produced by either neutrophils or tumor cells can activate IκB kinases α and β, which phosphorylate IκB (66, 67). Activated NF-κB is transported into the nucleus, where it acts as transcription factor, promoting the transcription of a series of genes, including IL-8 and several antiapoptotic or survival factors such as Bcl-2 and Bcl-xL (68). The ROS produced by tumor-associated neutrophils enhance the activation of NF-κB and thus increase the potential that the tumor cells will survive, grow, and progress toward a metastatic phenotype. The potential roles for neutrophilic activities used in either host defense or furthering tumor progression are summarized in Table 1.

POTENTIAL THERAPEUTIC TARGETS

Attenuation of either ectopic IL-8 expression or its downstream cascade should diminish the metastatic potential of tumor cells producing IL-8. There are several potential points where pharmacological interventions could be used to attenuate the IL-8 cascade. The first is inhibiting ectopically released IL-8. It has been reported that active NF-κB is an essential transcription factor for the activation of the IL-8 promoter (4). Therefore, the inhibition of the IκB kinases α and β, activators of NF-κB, would be potential targets for inhibiting NF-κB activation and IL-8 expression. *In vitro* studies demonstrate that inhibitors of these kinases inhibit NF-κB activation and the expression of a series of its regulated inflammatory and antiapoptotic genes (69–72). Other likely sites for attenuating the effects of ectopic IL-8 expression lie between the IL-8 receptor and the signals generated by this ligand-receptor interaction. Included in these are the G-protein receptor for IL-8 as well as targets within the responsive elements up to and including the cytoskeleton subunits required for migration or chemotaxis.

Additional points of intervention are the activities released by the neutrophils. The product of NADPH oxidase is H_2O_2, which serves as both an activator of the IκB kinases and a substrate for myeloperoxidase. The suppression of this enzyme is likely to decrease activation of NF-κB. Another potential point of intervention is the enzyme myeloperoxidase, which converts H_2O_2 and the chloride ion to HOCl. Antioxidants such as α-tocopherol or sulfhydryls such as *N*-acetyl cysteine serve as pro-oxidants and free radical traps that can inactivate the ROS including HOCl and thus would attenuate the effect of these oxidants within the tumor microenvironment.

Currently available nutritional supplements and over the counter drugs may also be useful chemopreventive agents to attenuate the progression and metastasis of precancerous lesions or as yet undetected tumors. Nutritional supplements include the antioxidants, in particular vitamin E, which has been shown to lower the concentration of ROS including HOCl. The over the counter drugs that appear most promising as inhibitors of tumor progression and metastasis are the nonsteroidal anti-inflammatory drugs. Many of these are thought to work by inhibiting cyclooxygenase (COX)-1 and/or COX-2, which catalyze the rate-limiting steps in the synthesis of prostaglandins. Another member of this class of compounds that may prove useful is ibuprofen. It has a broad spectrum of activity in that it inhibits both COX-1 and COX-2. Whereas specific inhibitors of either COX-1 or COX-2 were able to inhibit tumor metastasis in mice, a nonspecific COX inhibitor showed better control of tumor growth (73). Ibuprofen not only acts as an inhibitor of the COXs but also acts through COX-independent mechanisms to inhibit the activation of NF-κB and activator protein 1, which serve as positive transcription factors for IL-8 and several of the antiapoptotic factors (74, 75). These compounds may be given as adjuvants to chemotherapeutic regimens or taken as chemopreventive agents. The ability to inhibit the enabling activities at the site of a tumor is likely to mitigate the increased metastatic potential induced by ectopic IL-8 expression, thus increasing both the quality of life and life expectancy of patients.

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