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Overcoming Obstacles to Metastasis - Defenses Against Host Defenses: Osteopontin (OPN) as a Shield Against Attack by Cytotoxic Host Cells

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Abstract Osteopontin (OPN) serves both a cell attachment function and a cell signalling function via the $\alpha_v\beta_3$ integrin. In its cell attachment capacity it can promote attachment of both osteoclasts to bone hydroxyapatite and various other cell types to basement membrane/extracellular matrix. In its cell signalling capacity it initiates a signal transduction cascade that includes changes in the intracellular calcium ion levels and the tyrosine phosphorylation status of several proteins including paxillin. Effects on gene expression include suppression of the induction of nitric oxide synthase by inflammatory mediators. OPN can also reduce cell oxidant levels and inhibit the killing of tumor cells by activated macrophages and endothelial cells. We hypothesize that those cancer cells that produce OPN at elevated levels can suppress the oxidative burst, inhibit NO production, and thus protect themselves from killing by specific host cell types. © 1994 Wiley-Liss, Inc.

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For metastasis to occur tumor cells must survive in the hematogenous or lymphatic circulation of the host long enough to reach and establish themselves at a new location. Obstacles to this are both biological (reactive lymphocytes and endothelial cells) and physical (shear forces). It seems reasonable to suggest that anything a prospective metastatic cell can do to overcome these hurdles will work to its advantage. Among the biological hazards to evade is the oxidative burst generated by macrophages and other cell types, which can cause the death of the tumor cell. The oxidative burst consists primarily of reactive oxygen intermediates that are produced by sensitized monocytes or macrophages (for example) upon contact with the tumor cell. As noted below, nitric oxide (NO) can also contribute to the cytotoxic capability of activated host cells to tumor cells. Generation and release of these reactive radicals (e.g., O_2^-, OH, and NO) may result in the death of both the host cell and the tumor cell as the result of the inactivation of critical metabolic pathways. Contributing to the death of the cells are the reactions of the superoxide, hydroxyl, and nitric oxide radicals with enzymes involved in DNA synthesis, intermediary metabolism, and mitochondrial electron transport.

Nitric oxide (at low concentrations) is a potent signaling molecule identified in neurons, endothelial cells, and macrophages [Nathan, 1992; Lowenstein and Snyder, 1992]. It is the product of the action of NO synthase (NOS), which catalyzes a reaction between arginine and oxygen, forming citrulline and NO [Marletta, 1993]. NO stimulates soluble guanylyl cyclase to synthesize cGMP, which modulates the activity of certain ion channels and a variety of enzymes including phosphodiesterases and kinases [Schmidt et al., 1993]. Numerous pathophysiological conditions are associated with abnormal production of NO, including atherosclerosis, ischemia-reperfusion injury, and acute hypertension.

Keller and Kreist [1989] have implicated reactive nitrogen intermediates in the tumoricidal action of marrow-derived rat mononuclear phagocytes and adherent peritoneal cells. Li et al. [1991] observed that nitric oxide produced by cytokine-activated murine lung vascular endothelial cells could also play a major role in the

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lysis of tumor cells. Martin and Edwards [1993] have reported that although reactive oxygen intermediates are responsible for the cytotoxic activity of freshly isolated human blood monocytes, after maturation in vitro to macrophages the cytotoxicity is mediated instead by reactive nitrogen intermediates. Isobe and Nakashima [1993] reported that stimulation of murine macrophages (resident peritoneal macrophages or RAW 264.7 cells) with membranous fragments of tumor cells was sufficient to generate NO; tumor cell cytotoxicity and NO production were both blocked by N-methyl arginine (NMA, an inhibitor of nitric oxide synthase). Yim et al. [1993] have found that infusion of NMA into tumor-bearing mice resulted in increased growth and delayed rejection of the target tumor. It seems clear that NO production by host cells is detrimental to the survival of a tumor cell.

Osteopontin (OPN), a secreted GRGDS-containing acidic phosphoprotein, has been known for some years to be produced at higher levels by many transformed cell lines in comparison to their untransformed counterparts [for reviews, see Senger et al., 1989; Denhardt and Guo, 1993]. Consistent with this is the elevated concentration of OPN in the plasma of patients with a variety of metastatic cancers [Senger et al., 1988]. Good evidence for an important functional role for OPN in malignancy was reported recently by Behrend et al. [1994], who showed that the metastatic ability of a *ras*-transformed NIH3T3 cell line was reduced when OPN synthesis by those cells was specifically inhibited by an antisense RNA. However, the function of OPN in tumor cell biology remains poorly understood, and it has not been evident how elevated OPN expression by cancer cells might contribute to their malignant phenotype.

OPN binds to the vitronectin receptor, the $\alpha_v\beta_3$ integrin, and perhaps to other related receptors [van Dijk et al., 1993]. In some cases, OPN might function in tumor cells as an attachment factor [Chambers et al., 1993]. For example, binding of cells to extracellular OPN might play a role in targeting cells that are metastatic to bone. However, as discussed below, adhesion via a ligand-integrin interaction may be only the first step in a signalling process, raising the possibility that the importance of OPN to tumor cells may be more than simply that of facilitating attachment. In this vein, Cantor and colleagues have characterized OPN (early T-cell activation gene 1 in their terminology) as a cytokine capable of modifying immune cell function (for review, see Patarca et al., 1993].

Integrins are heterodimeric ($\alpha\beta$) cell surface receptors that recognize components of the extracellular matrix and certain soluble extracellular proteins, typically but not necessarily containing an RGD motif. Not only are they important in cell adhesion and migration, they also mediate signal transduction pathways, both from outside the cell to inside via ligand interactions, and from inside the cell to the exterior via modulation of integrin affinities (for review, see Juliano and Haskill, 1993; Williams et al., 1994]. Either the ligand (e.g., fibrinogen or vitronectin, or even RGD-containing peptides at high concentrations) or an antibody to the integrin can initiate conformational changes, promoting receptor clustering and possibly internalization. Since no enzyme activity intrinsic to the integrin has been identified, it is believed that the incoming signal is transmitted via altered interactions with other proteins, particularly those associated with focal adhesions. This integrin-mediated signal can result in altered cell behavior and function, and can communicate information to the cell regarding the external environment. For example, OPN has a chemotactic activity towards macrophages and vascular smooth cells and can promote adhesion of various cell types with the formation of focal adhesion contacts and greater resistance to heat shock [Sauk et al., 1990; Liaw et al., 1994].

Integrin-induced phosphorylation of the focal adhesion kinase pp125FAK, e.g., as a consequence of adhesion of KB cells to fibronectin [Kornberg et al., 1992] or the binding of fibrinogen to αIIbβ3 [Huang et al., 1993], has been reported. Bartfeld et al. [1993] recently described a 190 kDa protein in 3T3 cells that was associated with the $\alpha_v\beta_3$ integrin and became phosphorylated when the cells were stimulated with PDGF; if this affected the conformation of the integrin it could alter the behavior of the receptor and influence the adhesive properties of the cells. Kanner et al. [1993] have reported that activation (by antibodies or ICAM-1) of the β2-integrin LFA-1 (lymphocyte function associated antigen-1) induced calcium mobilization and enhanced tyrosine phosphorylation of phospholipase C-γ1. There are other reports that integrin activation can alter not only intracellular Ca^{++} levels [e.g., Schwartz, 1993; Shankar et al., 1993] but also potassium channels, apparently via a pertussis toxin-sensitive G protein

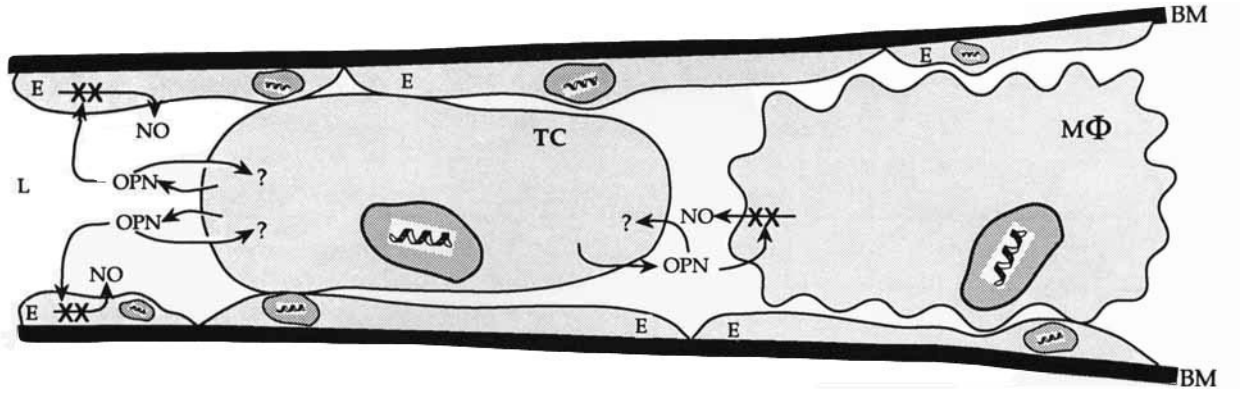

Fig. 1. Stylized representation of interactions among endothelial cells (E), a tumor cell (TC), and a macrophage (MΦ) in the lumen (L) of a small capillary bounded by a basement membrane (BM). The essence of the hypothesis is that the tumor cell *produces OPN in* order to suppress production of NO and possibly reactive oxygen species by macrophages and endothelial cells. The ? indicates the possibility that OPN might act on the TC itself to inhibit potentially cytotoxic induction of NO synthesis by cytokines. The action of OPN any of the cells is considered to be mediated by a signal transduction cascade mediated by an integrin, possibly $\alpha_v\beta_3$. The production of OPN by activated macrophages (not shown) suggests that the picture is actually more complex than illustrated here.

[Arcangeli et al., 1993]. Binding of OPN to osteoclasts via the $\alpha_v\beta_3$ receptor has been reported to alter intracellular Ca^{++} levels [Miyauchi et al., 1991; Zimolo et al., 1994]. OPN signal transduction in these cells could entail activation of phospholipase C [Rolnick et al., 1993].

We have long puzzled over why enhanced expression of this integrin-binding cell adhesion protein might be advantageous to cancer cells. As one possibility, it could protect cells from harmful interactions by occupying binding sites; adhesion of tumor cells to vitronectin receptors on endothelial cells has been shown to be important to endothelial cell killing of tumor cells [Shaughnessy et al., 1991]. However, recent research in our laboratories has given rise to an attractive hypothesis. Hwang et al. [1994] have found that OPN can suppress production of nitric oxide synthase induced by inflammatory mediators in mouse kidney primary proximal tubule cells. This finding has been extended to other NO-producing cells and tissues. Rollo, Feng, Lopez and Denhardt (in preparation) have shown that both NO production and tumor cell killing by the macrophage-like line RAW264.7 can be reduced by OPN. Scott, McCormack, Xuan, and Chambers (in preparation) have found that lipopolysaccharide-induced production of NO by rat thoracic aorta can be blocked with OPN. Together these recent results suggest a novel hypothesis—that tumor cells that produce OPN at high levels are able not only to defend themselves against killing by activated endothelial cells and macrophages/monocytes but also to survive (via an autocrine action of OPN on the tumor cell itself) an otherwise lethal induction of NOS by certain cytokines [Dong et al., 1994]. These points are illustrated in the cartoon in Figure 1. We suggest that OPN provides this protection both by inhibiting NO production and by suppressing the oxidative burst. This would account for the high level of expression of OPN by some metastatic cells and explain its contribution to the malignant phenotype of the metastatic cell. Aspects of this hypothesis that need to be investigated further include determining the effect of OPN on the oxidative burst itself and the extent to which OPN protects the tumor cell from being killed by cytotoxic cells in the animal.

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