Catecholamines Regulate Tumor Angiogenesis

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Abstract

Among the regulators of angiogenesis, catecholamine neurotransmitters are of recent interest because of their opposite roles in the regulation of tumor neovascularization. Norepinephrine and epinephrine by acting through specific adrenoceptors increase the synthesis of proangiogenic factors, and thereby, promote tumor growth. In contrast, dopamine acting via its specific D2 receptors inhibits tumor growth by suppressing the actions of vascular permeability factor/vascular endothelial growth factor-A on both tumor endothelial and bone marrow-derived endothelial progenitor cells. These reports identify novel endogenous regulators of tumor angiogenesis and also indicate a new and an inexpensive class of antiangiogenic drugs for the treatment of cancer. [Cancer Res 2009;69(9):3727–30]

Introduction

Angiogenesis is essential for the development of the embryo, tissue repair, and reproductive functions in the adult (1). This process of new blood vessel formation is also critical for the growth and progression of malignant tumors (1). The neovessels in these tumors are mainly formed from the preexisting vessels by proliferation and migration of the adult endothelial cells together with the contribution of bone marrow-derived endothelial progenitor cells (EPC) (refs. 1–3). Although angiogenesis is an intricately balanced phenomenon between the proangiogenic and antiangiogenic factors in normal physiological processes, in pathological conditions like cancer this regulatory balance is lost, thereby leading to the formation of abnormal blood vessels with increased permeability (1). It is now well established that antiangiogenic therapy can retard the growth and progression of malignant tumors (1, 4, 5). Accordingly, there is considerable interest in identifying the antiangiogenic molecules and their mechanism of actions so that newer therapies can be designed to effectively target tumor angiogenesis and growth (1, 4, 5).

Among the several endogenous regulators of angiogenesis, the roles of catecholamines (CA) are important due to their opposite effects on tumor angiogenesis (6–10). Dopamine (DA) by acting through its D2 receptors inhibits angiogenesis by suppressing the action of vascular permeability factor/vascular endothelial growth factor-A (VPF/VEGF) on both adult endothelial cells and EPC (6, 7).

In contrast, norepinephrine (NE) and epinephrine (E) by acting through the β adrenoceptors up-regulate the synthesis of many proangiogenic factors in the malignant tumor cells and induce angiogenesis in the tumor tissues (8–10). The present review briefly discusses the role of CAs on tumor angiogenesis and how they can be utilized for the treatment of cancer.

Catecholamine Neurotransmitters

CAs are a major class of neurotransmitters synthesized from the amino acid tyrosine (11, 12). Besides the brain in the central nervous system, these compounds are endogenously produced in the periphery at the sympathetic nerve endings, in chromaffin tissues like adrenal medulla, in nonneuronal gut cells, and in lymphocytes (11, 12). These neurotransmitters are also considered to be the physiological regulators of flight or fight response during stress and have both excitatory and inhibitory roles (12). NE and E act on their respective target cells through α and β adrenoceptors (12). These receptors are further subdivided into different subtypes; the α1 adrenoceptors act by increasing the intracellular calcium level, whereas the α2 adrenoceptors inhibit intracellular cyclic AMP (cAMP) by down-regulating adenylyl cyclase (12). The β1 and β2 adrenoceptors increase intracellular cAMP by activating adenylyl cyclase (12). However, DA acts on its target cells through its specific receptors, which are broadly classified as D1 and D2 types (12). The D1 class includes the D1 and D5 subtypes, which on activation increase intracellular cAMP (12). In contrast, the D2 class of receptors, which includes D2D, D3, and D4 subtypes, inhibits intracellular cAMP (12).

NE and E Up-regulates Tumor Angiogenesis

Although there are several studies that indicate chronic stress mediates initiation and promotion of different types of experimental and human tumors, these studies suggest that the mechanisms of stress-induced tumor growth are due to major alterations in tumor immunity (13). Interestingly, recent reports have indicated that substantial amounts of NE and E are produced during chronic stress owing to the activation of sympathoadrenal medullary axis, and these CAs act through the β adrenoceptors to directly stimulate the growth of different types of malignant human tumors by up-regulating the synthesis of proangiogenic factors like VPF/VEGF (8–10, 14–16). It is important to mention here that among the several proangiogenic factors, VPF/VEGF is the most critical cytokine required for the induction of tumor angiogenesis, and the action of VPF/VEGF is mediated mainly through its VEGFR-2 receptors present in the tumor endothelial cells (1, 4). There are now several reports indicating that tumor-associated macrophages (TAM) play an important role in promoting tumor angiogenesis by secreting proangiogenic growth factors and matrix metalloproteases (MMP) (refs. 17, 18). It has also been recently shown that NE, by acting on the adrenoceptors present in the TAM,
stimulates production of MMP-9 by these cells and thereby, induces angiogenesis in ovarian cancer (18).

**Signaling Pathways in NE- and E-Mediated Increased Tumor Angiogenesis**

It has been shown that NE stimulates angiogenesis in human malignant ovarian tumors (Hey-A8, SKOV3ip1) grown orthotopically in nude mice by acting through the $\beta_2$ adrenoceptors present in these tumor cells (8). The mechanism of this NE action has been attributed to the increased VPF/VEGF synthesis (8, 9) and over expression of MMP-2 and MMP-9 through the cAMP-protein kinase A (PKA) pathway (8). Thus, the underlying signaling pathway to promote angiogenesis in these malignant ovarian tumors can be summarized as $\beta$ receptor $\rightarrow$ cAMP $\rightarrow$ PKA $\rightarrow$ VEGF. A similar result has been shown in the human pharyngeal carcinoma cell line HONE 1, in which NE by acting through $\beta_2$ adrenoceptors stimulates synthesis of VPF/VEGF and matrix metalloproteinases MMP-2 and MMP-9 (10). Also, NE treatment significantly increases VPF/VEGF synthesis in several human multiple myeloma cell lines (NCI-H929, MM-M1, and FLAM-76) by acting through $\beta_1$ and $\beta_2$ adrenoceptors present in these cells (14). In addition to these signaling pathways, another proangiogenic molecule, IL-6 is also involved in NE- and E-mediated ovarian cancer angiogenesis (15). Recent reports have shown increased synthesis and release of IL-6 in human ovarian tumor cell lines (SKOV3ip1, Hey-A8, and EG) following NE treatment in vitro (15). A significant increase in IL-6 mRNA synthesis and its promoter activity has been observed in these malignant ovarian cells following NE treatment (15). Furthermore, abrogation of this effect by $\beta$ adrenoceptor antagonists confirms that NE regulates the transcription of IL-6 gene through $\beta$ adrenoceptors (15). In depth elucidation of this signaling pathway also indicates Src kinase phosphorylation following NE treatment, which subsequently leads to increased IL-6 mRNA synthesis through enhanced IL-6 promoter activity (15). This NE $\rightarrow$ $\beta$ receptor $\rightarrow$ Src kinase $\rightarrow$ IL-6 pathway in ovarian tumor cells corroborates well with the immunohistochemical analysis of human ovarian cancer tissues demonstrating strong correlation between over expressed Src kinase and the degree of tumor neovascularization (Fig. 1A) (ref. 15). Because activation of Src also induces other proangiogenic molecules like VPF/VEGF and IL-8, these CAs may be responsible for regulating the synthesis of these proangiogenic molecules by ovarian cancer cells (15). However, in a recent study, Landen and colleagues (16) have
shown that NE and E can induce MMPs in ovarian tumor cell lines by activation of STAT3, a transcription factor known to initiate several signaling pathways in cancer via IL-6 independent \( \beta_1/\beta_2 \) adrenoceptor→PKA signaling pathway.

**Dopamine Down-regulates Tumor Angiogenesis**

Recent reports from our laboratory have shown that DA is an endogenous inhibitor of tumor angiogenesis (6, 19). However, unlike NE and E, DA does not influence the expression of proangiogenic molecules like VPF/VEGF in cancer, rather it acts by down-regulating VEGFR-2-mediated signaling pathways in both tumor endothelial cells and EPC through DA D2 receptors present in these cells (6, 7, 19, 20). Accordingly, increased tumor angiogenesis and tumor growth is observed in DA D2 receptor knock out mice (7, 19). In addition, DA treatment also inhibits tumor angiogenesis, growth, and increases the life span of mice transplanted with human stomach, colon, and breast malignant tumors (20, 21). These reports thus confirm that DA inhibits tumor growth by suppressing angiogenesis (6, 7, 19–21).

**Signaling Pathways in DA-Mediated Suppression of Tumor Angiogenesis**

*In vivo* and *in vitro* studies have shown that DA inhibits VPF/VEGF-induced angiogenesis by suppressing VEGFR-2 phosphorylation in tumor endothelial cells (19–21). This DA-mediated suppression of VEGFR-2 phosphorylation, in turn, inhibits MAP kinase and focal adhesion kinase phosphorylation (Fig. 1B) (ref. 21). Furthermore, studies on DA D2 receptor knock out mice also support these findings as increased tumor angiogenesis and growth is associated with significantly increased VEGFR-2 phosphorylation in tumor endothelial cells collected from these knock out animals (19).

In addition, a number of reports have shown the significance of EPC in the formation of tumor neovessels (2, 3). In tumor-bearing hosts, EPC are mobilized from the bone marrow niche by VPF/VEGF through a series of molecular steps like activation of MMP-9, which subsequently promote the mobility of EPC from the bone marrow to the peripheral circulation by release of soluble kit ligand (sKITL) (ref. 3). This is an important step required for the mobilization of EPC into circulation and its subsequent incorporation into the tumor neovessels (3). Our recent investigation has shown a new molecular pathway of this tumor-induced EPC mobilization (7). Although a considerable amount of DA is present in the bone marrow of normal animals, we have shown, for the first time to our knowledge, that bone marrow DA is an important regulator of the mobilization of EPC from the bone marrow during tumor growth (7). It has been shown that the bone marrow DA is significantly decreased in tumor-bearing mice, and this decrease is associated with increased mobilization of EPC from the bone marrow into peripheral circulation (7). The role of endogenous bone marrow DA in the process of EPC mobilization is further supported by increased EPC mobilization in tumor-bearing DA D2 receptor knock out mice (7). In addition, treatment of wild-type tumor-bearing mice with exogenous DA significantly inhibits mobilization of EPC, and DA acts through its D2 receptors present in these progenitors (7). These results confirm the regulatory role of DA in tumor angiogenesis, and the underlying molecular mechanisms have been attributed to DA D2 receptor-mediated inhibition of MMP-9 through down-regulation of ERK-1/ERK-2 signaling pathways in EPC (Fig. 1C) (ref. 7). Lastly, there is also a report indicating decreased tumor angiogenesis and growth in rats with high dopaminergic activity (22).

**Conclusions and Future Directions**

Taken together, the studies outlined above indicate that the CA neurotransmitters are important regulators of tumor angiogenesis (6–10, 13–16, 18–21). NE-mediated activation of \( \beta_2 \) adrenoceptors stimulates tumor angiogenesis (8–10, 13, 14, 16), whereas DA-induced activation of DA D2 receptors inhibits tumor angiogenesis (6, 7, 19–21). It may, therefore, be suggested that these neurotransmitters are possibly acting as an angiogenic switch in the tumor microenvironment. Accordingly, in pathological conditions, in which changes in the expressions of these neurotransmitter receptors occur, the intricate balance between the stimulatory and inhibitory effects of these molecules on angiogenesis may be altered leading to the rapid development of an undetectable tumor mass, which subsequently grows and metastasizes. Accordingly, further research on the specific roles of these neurotransmitters in the development of human malignant tumors is necessary. It is to be noted here that patients taking \( \beta \) blockers have less incidence of cancer (23). Also, recent clinical trials have shown that DA D2 receptor agonists inhibit the actions of VPF/VEGF in ovarian hyperstimulation syndrome patients (24). These clinical reports are very encouraging because both \( \beta \) blockers and DA D2 receptor agonists are in clinical use at present for the treatment of other diseases (23, 24). Therefore, future clinical trials may be undertaken with these inexpensive drugs for the treatment of cancer and other diseases in which angiogenesis play an important pathogenic role (1).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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