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Signaling Pathways in the Control of Angiogenesis by Nitric Oxide

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Angiogenesis is a multistep process leading to the formation of new vessels by *sprouting* from pre-existing vessels. In physiological conditions the neovascular growth is fundamental for healing, reproduction and embryonic development. Angiogenesis is dependent upon production of angiogenesis-stimulatory and inhibitory molecules. An imbalance in this regulatory circuit might lead to the development of angiogenesis-mediated disorders, as cancer.

At cardiovascular level angiogenesis is required for healing and tissue reperfusion following ischemia. Under the control of chemical mediators and physical forces, the process involves the exit of endothelial cells from quiescence to promote cell migration, to degrade the extracellular matrix and sustain cell proliferation, ultimately leading to differentiation of vascular buds into functional capillaries. The re-endothelisation process of injured vessels requires endothelial cells to activate similar functions. Gene transfer of angiogenic factors has proved its clinical potential to recover tissue functions in ischemic conditions by favouring endothelial cells growth and migration.

Nitric oxide (NO), produced by the enzymatic conversion of arginine in citrulline through NO synthase (NOS), is the main factor responsible for endothelium-dependent protective effects. At cardiovascular level NO production strictly controls endothelial cell growth and survival, while inhibiting smooth muscle cell contraction and growth, conditions which warrant the physiological vascular responses. When the endothelial cell lining is damaged, the reduction of NO release becomes a major determinant for the occurrence of hypertension, congestive heart failure, disturbed vascular remodelling, atherosclerosis, and ischemia.

NO directs endothelial cells in each step of angiogenesis and its role is twofold. *In vitro* administration of NO donor drugs to sparse coronary endothelium increases their mitotic index, favours cell migration and matrix degradation. *In vivo* NO donors speed up and potentiate neovascular growth, thus demonstrating that NO acts as a true effector of angiogenesis (1,2). On the other hand mediators of angiogenesis, like neuropeptides and bradykinin, signal the angiogenic switch in endothelium by releasing free NO and elevating cGMP levels (2-5). Competent pro-angiogenic activity by the vascular endothelial growth factor (VEGF) is mediated by NO-cGMP signalling of MAPK activation in endothelium (6-8). As a result, impairment of the endogenous NO signalling in endothelium is coupled to inability to mount an angiogenic response to VEGF and vasodilating peptides (2,5,7,9).

In microvascular endothelium NO upregulates the expression of endogenous angiogenic factors like fibroblast growth factor-2 (FGF-2) (4,5). FGF-2 lacks a signalling sequence and is not secreted outside the cell. As a consequence its upregulation results in endogenous accumulation which by a paracrine/intracrine function contributes to improve endothelial cell survival and possibly to inhibit apoptotic events. These experimental observations substantiate the endothelium-protective effect of NO in various clinical settings by the use of different drugs (10,11).

Drugs which restore appropriate NO production by the endothelium have thus the potential to re-address endothelial functions to satisfy metabolic demands, induce angiogenesis, increase the diameter of vessel lumen and prevent stenosis reducing arterial pressure, exert anti-atherogenic and anti-thrombotic effects by promoting intimal re-endothelisation (11). The use of NO donors

and ACE inhibitors, despite their consolidated use in therapy, offers evidence for new mechanistic interpretation of their effect, which can be further exploited.

Elevation of NOS activity in correlation with angiogenesis and tumor growth and aggressiveness has been extensively reported in experimental and human tumors (12). The tumor promoting properties are linked to induction of angiogenesis, downregulation of the immune response and the prevention of leukocyte infiltration. Thus the use of NOS inhibitors or NO scavengers (7,9) seems appropriate to reduce edema, block angiogenesis and facilitate antitumor drug delivery. In order to choose appropriate therapeutic settings it seems crucial to have indications on the expression/activity of specific NOS isoforms inside the tumor mass and the amount of NO produced, as well as its correlation with the angiogenic output of the neoplasia.

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