

The discovery of angiogenic factors: A historical review

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Received 1 September 2001; accepted 1 October 2001

Abstract

Angiogenesis is a biological process by which new capillaries are formed and it occurs in many physiological and pathological conditions. It is controlled by the net balance between molecules that have positive and negative regulatory activity and this concept had led to the notion of the “angiogenic switch,” depending on an increased production of one or more of the positive regulators of angiogenesis. Numerous inducers of angiogenesis have been identified and this review offers a historical account of the relevant literature concerning the discovery of the best-characterized angiogenic factors. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Angiogenesis; Angiogenic factors; Cytokines; History of medicine

1. Introduction

Angiogenesis is a biological process by which new capillaries are formed from preexisting vessels. It is essential in many physiological (embryo development, ovulation, and wound repair) and pathological conditions, such as arthritis, diabetic retinopathy, and tumors.

Angiogenesis is controlled by the net balance between molecules that have positive and negative regulatory activity (Pepper, 1997). This concept had led to the notion of the “angiogenic switch,” depending on an increased production of one or more of the positive regulators of angiogenesis (Hanahan and Folkman, 1996). These can be exported from tumor cells, mobilized from extracellular matrix, or released from inflammatory cells (e.g., macrophages or lymphocytes). The switch clearly involves more than simple upregulation of angiogenic activity and is thought to be the result of a net balance of positive and negative regulators.

Numerous inducers of angiogenesis have been identified, including members of the fibroblast growth factor (FGF) family, vascular permeability factor/vascular endo-

thelial growth factor (VPF/VEGF), angiogenin, transforming growth factor alpha and beta (TGF- α and - β), platelet-derived growth factor (PDGF), platelet-derived endothelial cell growth factor (PDEC GF), tumor necrosis factor alpha (TNF- α), interleukins, chemokines, and angiopoietins (angs).

This paper offers a historical account of the relevant literature concerning the discovery of the best-characterized angiogenic factors.

2. Isolation of the first angiogenic factor

An angiogenic factor was first isolated by Folkman et al. in 1971. The homogenate of a Walker 256 carcinoma—a breast tumour of Sprague–Dawley rats—was fractionated by gel filtration on Sephadex G-100. The fraction that exhibited the strongest angiogenic activity had a molecular weight of about 10,000 Da and consisted of 25% RNA, 10% proteins, and 58% carbohydrates, plus possibly lipid. It was inactivated by digestion with pancreatic ribonuclease, or by heating at 56 °C for 1 h, and was not modified when kept at 4 °C for 3 months, nor when treated with trypsin for more than 3 days. This active fraction was subsequently called “tumor angiogenesis factor” (TAF) (Folkman et al., 1971).

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Both the cytoplasmic and the nuclear fractions of tumor cells stimulated angiogenesis. In the nuclear fraction, this was found to be associated with nonhistonic proteins (Tuan et al., 1973). TAF has since been extracted nondestructively from several tumor cell lines, and several low-molecular-weight angiogenic factors have been isolated, again from the Walker 256 carcinoma: these factors induced a vasoproliferative response *in vivo* when tested on rabbit cornea or chick chorioallantoic membrane (CAM), and *in vitro* on cultured endothelial cells (Mc Auslan and Hoffman, 1979; Weiss et al., 1979; Fenselau et al., 1981).

3. Purification of other angiogenic factors

The 1980s saw the discovery of the first molecules that mediated angiogenesis. A breakthrough in the purification of endothelial cell mitogens came as a result of the observation that an endothelial cell growth factor derived from rat chondrosarcoma had a marked affinity for heparin (Shing et al., 1984). Analysis of the various heparin-binding endothelial cell growth factors by several methods (heparin-affinity column elution profiles, protein sequences, immunological cross-reactivity, and receptor binding) has greatly clarified the relation of these polypeptides to one another.

Heparin affinity chromatography was employed to purify acidic and basic fibroblast growth factor (aFGF and bFGF) (Maciag et al., 1984; Shing et al., 1984) and VEGF (Ferrara and Henzel, 1989; Plouet et al., 1989). These factors have been shown to stimulate endothelial cell growth and migration *in vitro* as well as *in vivo* angiogenesis and have long been considered to be the principal tumor angiogenic factors.

4. FGFs

In 1984, Shing et al. at the Children's Hospital in Boston discovered a tumor-derived factor bound with such a high affinity to heparin that it could be purified 200,000-fold by a single passage over a heparin affinity column. This purified protein had a molecular mass of 14,800 Da and stimulated the proliferation of capillary endothelial cells. These workers later used the chick CAM to show that it stimulated new vessel growth (Shing et al., 1985). bFGF was highly purified from bovine pituitary gland (Bohlem et al. 1984) and bovine brain (Gospodarowicz et al., 1984). Its amino acid sequence was determined by Esch et al. (1985). In 1986, an angiogenesis factor had been isolated from human placenta and human hepatoma cells on the basis of its ability to stimulate protease production in cultured capillary endothelial cells (Moscatelli et al., 1986; Presta et al., 1986). The purified factor also stimulated DNA synthesis and motility in capillary endothelial cells and induced angiogenesis *in vivo*. Amino

acid sequence data revealed that the angiogenesis factor was human bFGF.

The two most extensively investigated proteins of the FGF family are FGF-1 (acidic FGF) and FGF-2 (basic FGF). They were found to be structurally related, having a 53% absolute sequence homology (Esch et al., 1985). They stimulate endothelial cell mitosis and migration *in vitro*, are among the most potent angiogenic proteins *in vivo*, have high affinity for heparin and heparan sulfate, are stored in the extracellular matrix, but lack a signal sequence for secretion. FGF-2 exists in four forms: one low-molecular-weight (18 kDa, FGF-2) and three high-molecular-weight forms (HMWs FGF-2), resulting from alternative initiations of translation. FGF-2 has been isolated from a variety of tissues and cell lines (Bikfalvi et al., 1997).

5. VPF/VEGF

In 1983, Senger et al. described the partial purification of a tumor product that promotes increased VPF in guinea pig skin with a potency some 50,000 times than histamine. VPF selectively permeabilizes venular endothelium to plasma and plasma proteins in a number of vascular beds, including those of skin, subcutaneous tissue, peritoneum, pleura, mesentery, diaphragm, retina, and skeletal muscle. Vascular hyperpermeability becomes evident within a minute or two of VPF injection into normal skin or other tissues and persists for 20 min. The hyperpermeability induced is therefore time-limited, reversible, and is not associated with detectable injury to endothelial cells or other microvascular components (Senger et al., 1983).

In 1989, Ferrara and Henzel and Plouet et al. independently reported the purification (to homogeneity) and sequencing of an endothelial cell-specific mitogen, which they, respectively, called VEGF and vasculotropin. The subsequent molecular cloning of VEGF and VPF (Leung et al., 1989; Kech et al., 1989; Conn et al., 1990) unexpectedly revealed that both activities are embodied in the same molecule. VEGF has been isolated from the conditioned media of a number of cell lines, including bovine pituitary follicular cells (Ferrara and Henzel, 1989; Gospodarowicz et al., 1989), guinea pig tumor (Connolly et al., 1989), NB41 neuroblastoma (Levy et al., 1989), and rat glioma cells (Conn et al., 1990). It elicits a potent angiogenic response when tested in the chick CAM (Leung et al., 1989) or in the rat cornea (Connolly et al., 1989).

VEGF/VPF is expressed by numerous tumor cell lines both *in vitro* and *in vivo*, and receptors for VEGF/VPF occur only on peritumoral capillaries and not on distant endothelial cells (Senger et al., 1995).

Over the past few years, five VEGF-related genes have been identified (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E). There are five characterized VEGF-A isoforms of 121, 145, 165, 189, and 206 amino acids in mammals, generated by alternative splicing of the mRNA

from a single gene comprising eight exons. They display differential interactions with three related receptor tyrosine kinases (VEGFR-1/Flt-1, VEGFR-2/Flk-1, and VEGFR-3/Flt-4). VEGFR-1 and VEGFR-2 are restricted largely to vascular endothelium in their expression, accounting for the specificity of action of this growth factor family. VEGFR-3 is restricted largely to lymphatic endothelium (Kukk et al., 1996). VEGFR-3 may play a role in disorders involving the lymphatic system and angiogenesis and it may be of potential use in drug targeting, in vivo imaging of the lymphatic vessels, and in therapeutic lymphangiogenesis.

Knocking out of a single allele of the VEGF gene mice results in embryonic lethality, suggesting an essential role of VEGF in vascular development (Carmeliet et al., 1996).

Synergistic angiogenesis of VEGF and bFGF has been demonstrated both in vitro and in vivo (Pepper et al., 1992; Goto et al., 1993; Asahara et al., 1995).

6. Angiopoietins

Long after the discovery of VEGF-A, a second family of growth factors specific for the vascular endothelium was identified, with members of this family termed as Angiopoietins (angs) (Davis et al., 1996; Suri et al., 1996; Maisonpierre et al., 1997; Valenzuela et al., 1999). Similar to VEGF, the specificity of the angs for the vascular endothelium results from the restricted distribution of the angs receptors, tie-1 and tie-2, to these cells. Tie-2 has at least four known ligands, ang-1, ang-2, and the yet less characterized ang-3 and ang-4. Ligands for the tie-1 receptor have not been found up to date. Ang-1 and ang-2 are secreted glycoproteins that share 60% amino acid identity and bind with similar affinity to tie-2. Although ang-1 induces autophosphorylation of tie-2, ang-2 is a naturally occurring antagonist that blocks ang-1-induced tie-2 autophosphorylation.

Like VEGF, ang-1 is an endothelial cell-specific growth factor. Ang-1, however, is not a direct endothelial mitogen in vitro. Rather, it induces endothelial cells to recruit pericytes and smooth muscle cells to become incorporated in the vessel wall. Ang-2 blocks the tie-2 receptor and acts to repel pericytes and smooth muscle cells (it is found in tissues like the ovary, uterus, and placenta that undergo transient or periodic growth and vascularization, followed by regression). Moreover, ang-2 seems to be the earliest marker of blood vessels that had perturbed by invading tumor cells (Holash et al., 1999) and is overexpressed in tumor microvasculature of human glioblastoma and hepatocellular carcinoma (Stratmann et al., 1998; Tanaka et al., 1999).

The VEGF/VEGF-Rs pathway seems to control a wide variety of processes during vascular development, from initial assembly of vessels (i.e., vasculogenesis) to the subsequent expansion of vascular network (i.e.,

angiogenesis), while function of the ang/tie-2 pathway seems to be restricted to later stages of vascular development and involved in remodeling and maturation of the vascular network.

7. Therapeutic implications

Considerable benefit can be derived in the clinical setting from manipulating angiogenesis, either positively or negatively. There is a variety of important clinical situations in which it would be desirable to promote angiogenic processes, such as for the induction of collateral vascularization in an ischemic heart or limb (Isner and Asahara, 1999). Conversely, there are pathologic conditions in which preventing angiogenic processes could be useful in the treatment of a growing tumor or a chronic inflammatory process (Folkman, 2001).

Angiogenic growth factors are employed for treatment of patients with ischemic heart or limb diseases and current clinical trials involve members of either the FGF or the VEGF family administered by intravascular infusion (Henry and Abraham, 2000). Treatments resulted in better perfusion of, increased blood flow to, and salvage of ischemic tissues.

Conversely, different strategies have been designed to inhibit VEGF production. Injecting a monoclonal VEGF antibody into nude mice bearing various tumors of human origin significantly suppresses tumor growth and reduces tumor weight of treated animals up 96% (Kim et al., 1993). A humanized antibody against VEGF has recently been designed (Presta et al., 1997) and undergoes testing in cancer patients. Blocking the interaction of VEGF with its receptors has been shown to be another option for antiangiogenic treatment. Blocking of FGF-2 results in inhibition of angiogenesis in vitro and in vivo (Hori et al., 1991) and blocking the intrinsic kinase activity of FGF receptors is a new target for drug development (Panek et al., 1998).

Acknowledgments

This study was supported, in part, by grants of the Associazione Italiana per la Ricerca sul Cancro, Milan; Associazione Italiana per la Lotta al Neuroblastoma, Genoa; and Ministero dell'Università e della Ricerca Scientifica e Tecnologica (local funds), Rome, Italy.

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