Review

Growth factor signal transduction defects in the cardiovascular system

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Abstract

Growth factors are important molecules mediating both the development as well as adaptive and pathological changes within the cardiovascular system. Growth factors therefore mediate both beneficial and nonbeneficial effects. The beneficial actions include the improvement of endothelial function, stimulation of vascular repair, the formation of new capillaries (angiogenesis), and the growth of collateral arteries (arteriogenesis). These actions represent the conceptual basis for the therapeutic use of growth factors, based on the idea to therapeutically induce or accelerate a given beneficial process.

The beneficial effects of growth factors have to be separated from effects where growth factors act as mediators of pathological processes such as atherogenesis and plaque destabilization. This review article focuses on the physiological and beneficial effects of growth factors in the vessel wall and describes and explains the dysfunction of these effects under certain conditions now referred to as “growth factor signal transduction defects”.

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1. Vascular signal transduction defects: a novel concept to explain vascular dysfunction and vascular pathology

Vascular diseases are based on the dysfunction of the vascular wall and/or its components. This includes endothelial dysfunction—an early step in the atherogenic process—and the dysfunction of adaptive processes secondary to structural and functional changes within the vasculature, such as angiogenesis and arteriogenesis (the growth of collateral vessels). One possible reason for vascular dysfunction can be the inappropriate function of a growth factor system. To understand the concept of growth factor dysfunction, it is important to briefly review the ordinary function of growth factors, their receptors, and their intracellular downstream machinery; both with regard to their proper biological function, as well as with regard to the underlying molecular mechanisms of action.

2. Basis of the concept: the involvement of growth factors in cardiovascular disease and repair

Peptide growth factors have been shown to be central mediators of cardiovascular function and dysfunction [1]. Growth factors are critically involved in a broad array of actions to control cellular differentiation, cellular growth, and cellular function, which is essentially true for all different cell types within the vessel wall and for all different types of vessels. In postnatal life, growth factors are involved in the control of cellular function and in repair processes (Table 1). In addition, growth factors may be mediating pathological processes including local inflammation and processes of pathological repair. These conditions give rise to several different, clinically relevant vascular disease conditions (Table 2). It is of course important to remember that growth factors as any other biological molecules act in a concentration-dependent fashion, and that inadequately high concentrations may give rise to unwanted pathological responses.
3. Growth factor function

Growth factors and cytokines are grouped to large classes based on the structure and function of their receptors. There are three distinct types of growth factor receptors, namely, (i) receptor tyrosine kinases, (ii) serine–threonine kinases, and (iii) cytokine receptors. The “classical” growth factor receptor model is the receptor tyrosine kinase [2], and this review focuses on “classical” growth factors mediating their signals through this class of receptors. In brief, the receptor is a membrane-anchored molecule with a single transmembrane-spanning domain, an extracellular ligand-binding domain, a juxtamembrane domain, an intracellular tyrosine kinase domain, and a C-terminal tail (see Fig. 1). In the majority of receptor tyrosine kinases, an inserted interkinase domain splits the intracellular tyrosine kinase domain. The receptor is usually activated following ligand binding to the extracellular domain, the dimerization of two receptor molecules, conformational changes, and the activation of the kinase domain. The ATP-binding pocket within the tyrosine kinase domain is the crucial structural element [3], where ATP binds and delivers energy in the form of phosphate groups. Receptor tyrosine kinase activation leads to phosphorylation of tyrosine residues within the intracellular domain of the receptor creating phosphotyrosine residues, which by themselves represent binding sites for signal transduction molecules (with SH-2 domains, phosphotyrosine-binding (PTB) domains, etc.). Activated receptors activate different signal transduction pathways/cascades, finally resulting in the activation of signalling pathways that can induce cell proliferation, cell migration, prevent apoptosis, or induce transcription of specific genes.

4. The concept of growth factor modulation in the cardiovascular system

The concept of growth factor modulation in the cardiovascular system is well-established [1]. In brief, the modification of the expression or activity of one of the vascular growth factors, such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), or transforming growth factor-β (TGF-β), will result in a functional dysbalance within the vessel wall leading to vascular dysfunction. Moreover, such disease-related dysbalances can be counter-acted by therapeutic strategies to either inhibit or stimulate the function of a distinct growth factor, depending on the underlying defect and function. Within the past 7 years, the following advancements have extended and modified this concept: (i) placenta-derived growth factor (PIGF) and other novel ligands have been introduced in vascular biology [4]; (ii) successful ligand–receptor crystallography represents the basis for rational design of specific agonists and antagonists [5]; (iii) empirical research, as well as crystallography, helped to identify specific ATP-binding pockets within the kinase domain, representing the basis for the development of specific antagonists from the tyrphostin (tyrosine phosphorylation inhibitor) class (see review from A. Levitzki in this issue) [6,7]; (iv) several receptor tyrosine kinase inhibitors, such as STI-571 [8], or neutralizing growth factor antibodies, such as avastin (VEGF inhibitor) [9], have been introduced into the clinics and are being successfully used for cancer treatment; (v) finally, novel signalling mechanisms have been described including the molecular transistor between VEGFR-1 and VEGFR-2, which provide further insight into the complexity of endothelial cell regulation [10].

5. VEGF and its relevance for the integrity of vascular function

One functionally relevant vascular growth factor is VEGF [11,12], as it plays a crucial role in vascular development, angiogenesis, collateral growth, and enhancement of collateral perfusion. VEGF therefore exemplifies several aspects highlighted within this review article. A lack of VEGF expression during embryonic development leads to vascular malformations, as VEGF function is necessary for endothelial and vascular development. The lack of only one allele of VEGF-A results in the death of mouse embryos at day 7.5 without generating endothelial cells [13,14]. The same can be observed in mice lacking VEGFR-2 [15], while mice lacking VEGFR-1 do form endothelial cells, but no functional vessels [16]. Additional insight was provided by

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FGF, fibroblast growth factor; TGF-β, transforming growth factor-beta; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; SCF, stem cell factor; CSF, colony-stimulating factor.

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FGF-2, fibroblast growth factor-2; IGF-I, insulin-like growth factor-I; SCF, stem cell factor; GM-CSF, granulocyte-macrophage colony stimulating factor; TGF-α, transforming growth factor-alpha.
mouse models that do not express all isoform of VEGF-A: VEGF 120/120 mice (i.e., mice only expressing the 120-kD isoform of VEGF-A, but lacking the VEGF164 and VEGF188 isoforms) show impaired postnatal myocardial angiogenesis, which is associated with the development of ischemic cardiomyopathy [17]. These mice ultimately die of cardiac failure. More moderate phenotypes have been observed in a rat model of postnatal, pharmacological inhibition of VEGF receptor function—a model for an inducible VEGF receptor signal transduction defect—combined with chronic hypoxia [18]. These rats developed severe pulmonary hypertension based on uncontrolled endothelial cell proliferation. The inhibition of VEGFR-2 prevented the antiapoptotic effect of VEGF-A, leading to abundant endothelial cell death, followed by uncontrolled proliferation within the vessel wall. Other important actions mediated by VEGFR-2 in the endothelium are the production of endothelial nitric oxide synthase (eNOS) [19] and the release of NO [20]. Besides the endothelium, VEGF activation may be critical for monocytes [21]. This is important as monocytes are involved in collateral growth and arteriogenesis [22,23] and as VEGF-induced monocyte migration as well as arteriogenesis are negatively affected in diabetic individuals [24,25] (see below). This correlates well with reduced collateral function in diabetic patients with coronary artery disease [26]. Moreover, an impaired monocyte response may contribute to atherogenesis as these monocytes may be trapped within the plaque and may be unable to migrate out of the plaque again, turning into foam cells.

It is important to mention that there is a balance between pro- and antiangiogenic factors [12]. The (adequate) elevation of proangiogenic factors, such as VEGF, may therefore be the trigger for the stimulation of (normal) angiogenesis. On the other hand, inadequately high concentrations of a growth factor, such as VEGF (e.g., following constitutive overexpression), may lead to a pathological response including the development of disorganized vessels and hemangiomas [27,28].

6. Other growth factors relevant for vascular homeostasis and vascular growth

As pointed out in Table 1, several other peptide growth factors are of critical importance for proper function of and for repair within the vascular system. The fibroblast growth factor family consists of at least 23 different ligands which bind to several different receptors including a total of four receptor-tyrosine kinases (FGFR-1, -2, -3, -4) and several low-affinity coreceptors, such as heparan sulfates and syndecans [29]. FGF-1 and FGF-2 were the first angio-

Fig. 1. Schematic illustration of growth factor activation and receptor tyrosine kinase signalling within a cell (left) and pathological interferences leading to growth factor dysfunction and signal transduction defects (right); ROS: reactive oxygen species.
genic factors that had been identified in the 1980s [30]. FGF-1 and FGF-2 act on both endothelial cells, as well as on smooth muscle cells, and are involved in the stimulation of angiogenesis [1], arteriogenesis [31], and atherogenesis [1]. In addition, FGF-2 has been used in several clinical studies to induce therapeutic angiogenesis in the human heart [32–34] (see article by Annex and Simons, this issue).

Within the vessel wall, PDGF-B plays a crucial role in vascular remodelling, as it acts not only on smooth muscle cells and fibroblasts but is crucially involved in the recruitment of pericytes [35], while PDGF-D is a potent angiogenic molecule [36].

It is well known that vascular remodelling, such as angiogenesis and arteriogenesis, involves an array of several growth factors. Until recently however, it has been uncertain whether a combination of factors will be necessary for a more efficient induction of angiogenesis, or whether a combined action would only speed up vascular growth processes. Recent work from Cao et al. [37] has highlighted that a combination of FGF-2 and PDGF-BB is not only very effective and synergistic in inducing angiogenesis and arteriogenesis, but that the combination of FGF-2 and PDGF-BB is leading to the formation of much more stable vessels that, in contrast to FGF-2- or VEGF-induced capillaries, will not regress over a period of more than 6 months.

In contrast, transforming growth factor-β (TGF-β) is acting differently from the abovementioned growth factors as it is acting via serine–threonine kinase receptors. Besides stimulating angiogenesis, TGF-β is an important mediator of fibrosis. As this review article focuses on receptor tyrosine kinase-mediated signal transduction defects, the molecular basis of TGF-β signalling is not further discussed here but elsewhere in this issue (see article by Lebrin et al.).

### 7. Signal transduction defects as a cause for reduced growth factor responsiveness

Growth factor action is physiologically followed by specific molecular and cellular responses. If growth factor activation is not followed by a proper cellular response or if it does not properly activate downstream signalling cascades, we can call this situation a “signal transduction defect”. Defects can occur on different levels within the signal transduction machinery (Fig. 1): (i) the interaction between ligand and receptor can be inhibited; (ii) the kinase activity or the activation pattern of the receptor can be altered; (iii) docking sites on the intracellular part of the receptor can be modified and can therefore be nonfunctional. In addition, (iv) signal transduction defects can occur downstream of the receptor within the target cell.

There are different causes of signal transduction defects, such as metabolic causes including diabetes mellitus and hyperlipidemia, infection/inflammation, and genetic defects.

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(Table 3). These different causes will be more closely discussed in this review article.

### 8. Metabolic disturbances, infections, and genetic defects can cause signal transduction defects

#### 8.1. Diabetes mellitus and hyperglycemia

It has been shown that diabetes mellitus is associated with an impaired function of growth factors such as VEGF. The reduced growth factor action is paralleled by functional impairment, such as endothelial dysfunction or reduced collateral artery growth [38]. This finding has recently been confirmed and extended by the finding that collateral recruitment is impaired in diabetic patients indicating reduced growth factor action in collateral growth of diabetics [26]. An important cellular component of collateral growth is the functional contribution of monocytes. Monocytes are being activated and migrate into the wall of the growing collateral artery [22,23]. In the case of diabetes mellitus, monocyte migration towards a growth factor, such as VEGF-A, is functionally impaired [24]. It had been shown that overall phosphorylation in monocytes of diabetic individuals stimulated by VEGF appears to be intact, indicating that ligand binding and receptor activation should be functional. On the other hand, monocyte migration towards VEGF is severely impaired, indicating a diabetes-associated signalling defect downstream of VEGFR-1 tyrosine kinase. The biochemical nature of this defect remains to be identified. This defect seems to be rather selective, however, as monocytes from diabetics do migrate towards other strong and independent chemotactic stimuli, such as fMLP, which is signalling via G-protein-coupled receptors. This indicates that the migratory apparatus of these monocytes is still intact.

The role of diabetes mellitus on collateral artery growth has been studied in the mouse model, where an impairment of collateral growth had been demonstrated [39]. These data suggest, however, that the addition of an excess of VEGF ligand can compensate for this defect, and the authors therefore had concluded that the diabetes-related defect of collateralisation in mice is based on the lack of VEGF [39]. Another potential explanation for reduced collateral growth.
in diabetic mice is a bone marrow mononuclear cell dysfunction, which limits the capacity for vascular growth [40]. In both instances, the therapeutic application of either VEGF-A or PIGF improved the impaired response in diabetic animals.

The potential mechanisms leading to the reduced arteriogenic response in patients with diabetes mellitus may be associated with hyperglycaemia, but also other metabolic consequences of diabetes, such as nonenzymatic glycation of proteins or the formation of advanced glycation end products (AGE proteins), may be underlying causes. When testing the effect of isolated hyperglycaemia on the function of VEGF in cultured endothelial cells in vitro, a reduced VEGF response can be observed [41] (own, unpublished data). With this model, we can show a reduced migration of endothelial cells already at slightly elevated glucose levels, such as 14 mmol/L, when exposed to the cells for a period of 16 to 48 h. At higher glucose concentrations, this effect was even more pronounced. In these cells, the activation of phosphatidylinositol 3'-kinase was fully preserved, while the migratory response was highly attenuated. Taken together, hyperglycaemia can lead to an impaired growth factor response on the basis of a signal transduction defect within the endothelial cells. The role of other influencing factors, such as insulin, and the role of protein glycation or other nonenzymatic inhibition of cellular function (e.g., formation of AGE proteins) remain to be investigated in the context of impaired vascular and organ function in diabetes mellitus. It has already been shown in diabetes, however, that reactive nitrogen species (RNS), such as peroxynitrite (ONOO−) [42], can negatively affect receptor tyrosine kinase function (as shown for the EGFR) [43]. Moreover, peroxynitrite can modulate (i.e., positively and negatively affect) the function of a growth factor protein (as shown for FGF-1) [44].

8.2. Hyperlipidemia

There is a long-standing evidence that hyperlipidemia can influence the response to growth factors. Already in 1993, Henry had published the following statement: “One effect of oxidized LDL and its products is to impair transmembrane signalling, a process that might alter responsiveness of endothelial cells to mitogens” [45]. Own, unpublished work supports this view. In case of the endothelium, oxidized LDL induces endothelial dysfunction with a reduced capacity to produce and release nitric oxide. The impaired response to mitogens may be regarded as just another phenotype of endothelial (cell) dysfunction. Likewise, oxidized LDL has been shown to decrease the expression of VEGFR-1 in human-derived macrophages, which results in an impaired response to VEGF [46].

8.3. Infections

Infections can influence the integrity of receptor-mediated signalling and function. As there is no good example with respect to the cardiovascular system yet, findings from the lymphatic system are being discussed here; it has recently been shown that infection of CD8 lymphocytes with the HIV virus isolated from HIV-positive patients showed a defective interleukin-2-dependent STAT5 signalling [47]. This impaired response was a result of an alteration in IL-2 receptor expression and correlated with an impaired activation of IL-2 receptor signalling. Interestingly, antiretroviral treatment resulted in a restoration of this deficit. Whether there are similar examples of infection-related impairment of growth factor responses in the cardiovascular system remains to be demonstrated.

8.4. Genetic defects/genetic alterations of receptor tyrosine kinases

Genetic variations can lead to growth factor dysfunction. It has been shown that molecules involved in growth factor signal transduction are being affected by mutations, mostly point mutations. There are several examples from the cancer field, where mutations in a growth factor receptor are associated with impaired responsiveness of the receptor or receptor system (loss of function) or with enhanced responsiveness (gain of function).

Mutations within receptor tyrosine kinases have been identified and associated with pathological phenotypes. The first example of a genetic defect leading to vascular dysfunction was the discovery of a missense mutation of VEGFR-3 causing autosomal dominant primary human lymphoedema (Milroy’s disease) [48,49]. The polymorphism of the VEGFR-3 gene is based on an inactivating point mutation that leads to a heterozygous histidine–arginine substitution within the kinase domain of the receptor, and prevents autophosphorylation of VEGFR-3. Mice carrying heterozygous inactivating VEGFR3 mutations show swelling of their limbs secondary to a lack of subcutaneous lymphatic vessels. Because of the presence of a remaining wild-type allele, gene therapy using the ligand VEGF-C can stimulate functional receptors encoded by the functional allele and therefore overrule the inhibitory action of the inactive dominant negative VEGFR3 protein, which results in the stimulation of lymphangiogenesis in the mouse model [50]. Therefore, ligand (gene) therapy may be a therapeutic modality for the treatment of diseases associated with mutant receptors.

Outside the vasculature, similar loss of function mutations have been described for other receptor tyrosine kinases. One of them is a mutation within the IGF-I receptor causing intrauterine and postnatal growth retardation [51]. Moreover, activating mutations have recently been described modifying the response to chemotherapy [52]. Patients with non-small-cell lung cancer are usually non-responsive to the tyrosine kinase inhibitor gefitinib, which targets the epidermal growth factor receptor (EGFR). However, non-small-cell lung cancer patients with activating mutations in the EGFR are susceptible to the specific
kinase inhibitor gefitinib. In the future, one may screen for such mutations to identify potential responder patients. Moreover, gain of function mutations may be identified in the cardiovascular system.

9. Conclusions and outlook

Growth factor-related dysfunction has already been shown to be responsible for significant cardiovascular pathology and cardiovascular dysfunction. On the basis of recent data, it may be speculated that growth factor-related cellular and vascular dysfunction might be a thus far underestimated source of pathology and disease. Besides metabolic-related dysfunction, underlying genetic variations could explain thus far unexplained causes of (premature) atherosclerosis and myocardial infarction as a consequence of inappropriate cellular function and repair. The systematic analysis of the genetic basis of such individuals and families may help to understand at least some of the previously unexplained vascular morbidities and mortalities.

Growth factor signal transduction defects may not only be the underlying cause of pathology, but may also account for insufficient therapeutic effects observed in some growth factor therapy approaches (see review by Annex and Simons, this issue). Growth factor therapy (Therapeutic Arteriogenesis) for advanced coronary artery disease had mostly been performed in older patients with severely diseased vessels and under the influence of multiple cardiovascular risk factors. It is well conceivable that the cardiovascular risk factors (such as diabetes mellitus) present in these patients may be responsible for the weak or inconsistent effect of growth factor therapy for therapeutic arteriogenesis.

In the future, we will need to develop tests to predict and estimate the functional impact of growth factor dysfunction and signal transduction defects in individual patients. Moreover, the search for novel treatment strategies of growth factor signal transduction defects will have to follow the systematic analysis of the underlying molecular defects.

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