VEGF resistance as a molecular basis to explain the angiogenesis paradox in diabetes mellitus

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Abstract

The action of VEGF (vascular endothelial growth factor) is essential to maintain proper endothelial and vascular function. VEGF stimulates virtually all aspects of endothelial function, namely proliferation, migration, permeability and nitric oxide production and release. In addition, the action of VEGF makes the endothelium anti-apoptotic. In turn, the inhibition of VEGF action is associated with endothelial dysfunction. Likewise, endothelial dysfunction can be found in the presence of several cardiovascular risk factors, including diabetes mellitus, hypercholesterolaemia and smoking. As circulating monocytes express functionally active VEGFR-1 (VEGF receptor 1) on their surface, monocytes and the related VEGFR-1-mediated signal transduction cascades have come into focus. The function of monocytes is negatively affected by diabetes mellitus, resulting in monocyte dysfunction. More specifically, a VEGF-related signal transduction defect can be detected in monocytes isolated from diabetic individuals. This reduced monocyte response to VEGF, demonstrated by a reduced chemotactic response, can be regarded as VEGF resistance. It is based on the pre-activation of certain intracellular pathways secondary to the diabetes mellitus-related RAGE (receptor for advanced glycation end-products) activation, ROS (reactive oxygen species) activation and inhibition of PTPs (protein tyrosine phosphatases). This unspecific pre-activation of intracellular pathways represents the molecular basis of VEGF resistance in diabetes mellitus.

VEGF (vascular endothelial growth factor) is a major endothelial growth factor

Peptide growth factors are key molecules that regulate differentiation, growth and activation of vascular cells. VEGF is a key player in endothelial activation and the most important angiogenic growth factor known [1]. Several VEGF genes and isoforms have been identified and characterized. The present review article focuses on the functional effects elucidated by the most common and abundant form, VEGF-A165, abbreviated as VEGF in this article.

VEGF contributes to proper endothelial function and stimulates angiogenesis

The action of VEGF is essential to maintain proper endothelial and vascular function. VEGF stimulates virtually all aspects of endothelial function. This includes the stimulation of proliferation, migration, nitric oxide production and release from endothelial cells, but also the permeability of the endothelial cell layer. In addition, the action of VEGF prevents the endothelium from undergoing apoptosis [2]. The impact of VEGF on endothelial function is illustrated in Figure 1(A).

Pathological conditions are associated with cellular dysfunction

The presence of various so-called cardiovascular risk factors is associated with endothelial dysfunction [3] and correlates with the inhibition of VEGF action. Likewise, endothelial dysfunction can be found in the presence of several cardiovascular risk factors, including diabetes mellitus and hyperglycaemia [4], hypercholesterolaemia [5], hypertension [5] and smoking [6], but also in the presence of more complex situations such as chronic heart failure [7], coronary artery disease [8] and chronic renal failure. These causes and consequences of endothelial dysfunction are highlighted in Figure 1(B).

The monocyte as a model system to study the function and cellular responses of VEGF

In recent years, there has been a strong focus on the functional analysis of the VEGF system because of the important functional impact of VEGF in vascular function. A large number of studies focused on the endothelium in vitro. A novel avenue of research was opened when functionally active VEGFR-1 was found to be expressed on the surface of circulating CD14+ monocytes [9]. More recently, the VEGFR-1-induced signal transduction cascades were described in primary human monocytes [10]. Monocytes have earlier been demonstrated to be negatively affected by diabetes mellitus, as diabetes mellitus is associated
with monocyte dysfunction [11]. More specifically, a VEGF-related signal transduction defect can be detected in monocytes isolated from diabetic individuals.

In fact, on the basis of clinical data in several cohorts with different cardiovascular risk factors including diabetes mellitus, hypercholesterolaemia and smoking, one may regard the monocyte as a living biosensor, as this cell type can indicate cellular dysfunction of any origin [12].

**The molecular basis of VEGF resistance and related monocyte dysfunction**

The monocyte has turned out to be a suitable cellular model to detect impaired VEGF responses, which we now call VEGF resistance [13]. When testing the growth factor response of monocytes, there are three principal types of defective growth factor response: a defect in ligand binding, a defect in signal generation, or a defect in signal transduction (see Figure 3).

Testing the VEGF response of freshly isolated human monocytes from diabetic individuals and characterizing the molecular basis of this diabetes mellitus-related VEGF signal transduction defect has led to the discovery of diabetes mellitus-related VEGF resistance. This reduced monocyte response to VEGF, demonstrated by a reduced chemotactic response, can be regarded as VEGF resistance. It is based on the pre-activation of certain intracellular pathways secondary to diabetes mellitus-related processes [13]. This includes a number of different and partly independent systems (Figure 2A): (i) the activation of RAGE (receptor for advanced glycation end-products) secondary to the hyperglycaemia-related production of AGE (advanced glycation end-products) and the up-regulation of cellular RAGE; (ii) the activation of ROS (reactive oxygen species); and (iii) the inhibition of PTPs (protein tyrosine phosphatases).

Together, all of these current processes result in a rather unspecific activation of intracellular downstream signaling pathways. A specific signal such as VEGFR (VEGF receptor) activation reaches activated mediators and does not result in further activation of the specific VEGFR-related signalling cascades.
VEGF resistance and functional consequences in vascular disease

VEGF resistance as described above is based on the unspecific activation of downstream signalling pathways within the responding cells. It is conceivable that such a pre-activated cell might be resistant to other more specific signals, both disease-related or required during repair processes. Therefore, on the basis of the nature of downstream signalling, such an activation is likely to be unspecific. This means that VEGF resistance is likely to be associated with a resistance to other stimuli, such as other growth factors, as well. As the VEGF response is rather specific for endothelial cells and monocytes, VEGF resistance therefore reflects aspects of endothelial dysfunction and monocyte dysfunction.

VEGF resistance as the previously missing link to explain the angiogenic paradox

The angiogenic paradox describes the fact that diabetes mellitus is associated with both enhanced as well as reduced angiogenesis [14]. More specifically, chronic diabetes mellitus is associated with enhanced angiogenesis in the eye (diabetic retinopathy) as well as in chronic wounds. On the other hand, chronic diabetes mellitus is associated with impaired collateral growth and arteriogenesis [15–17]. So far, there has been no good explanation for the angiogenic paradox in chronic diabetes mellitus. However, the concept of VEGF resistance offers a potential explanation. This is based on three facts: (i) VEGF is a relevant stimulus for both angiogenesis [1] and arteriogenesis [18,19]; (ii) the angiogenic stimulus VEGF is elevated in diabetes mellitus [20]; and (iii) the angiogenic and arteriogenic VEGF response is reduced in diabetes mellitus (VEGF resistance) [13].

The consequences of these three facts are shown in Figure 2(B) and are as follows.

(i) The short-term stimulation of angiogenesis and arteriogenesis in diabetes mellitus is reduced based on a poor response to VEGF, namely VEGF resistance. This is especially true for arteriogenesis, a process that normally takes approx. 2–6 weeks.

(ii) The long-term stimulation of angiogenesis results in a net increase in neovascularization, based on the prolonged stimulation (enhanced stimulus over months and years) despite the poor (but not completely abolished) response to VEGF.

Inducing VEGF resistance as a therapeutic strategy in ophthalmology and oncology (VEGF ablation)

Having unravelled the molecular basis of VEGF resistance in diabetes mellitus, we can ask the question whether the therapeutic induction of VEGF resistance might be an attractive concept to modulate the action of VEGF. Such a therapeutic intervention would result in the modification of endothelial function and angiogenesis.

The therapeutic inhibition of VEGF action has recently been introduced into clinical practice and represents effective therapeutic strategies to inhibit tumour growth in a variety of different tumour types. Furthermore, VEGF inhibition is now used to inhibit angiogenesis in wet AMD (age-related macular degeneration) to preserve vision. These therapeutic approaches of VEGF inhibition may be regarded as therapeutically induced VEGF resistance as VEGF is deprived of its biological action.

There are a number of different molecular approaches to inhibit VEGF action in vitro and in vivo (Figure 3). This includes VEGF antagonism based on neutralizing VEGF antibodies (bevacizumab, Avastin), aptamers that bind to VEGF and block its binding to VEGFRs (pegaptanib, Macugen), the use of soluble VEGFRs or fusion proteins thereof (VEGF Trap-Eye), the use of neutralizing antibodies directed against either VEGFR1 (IMC-18F1) or VEGFR2 (IMC-1121b), or VEGFR-specific tyrosine kinase inhibitors (Sunitinib, Vatalanib, Cediranib, Vandetanib).

It is important to realize that all of the current strategies to therapeutically ablate VEGF activity are different and distinct from disease-related VEGF resistance in diabetes mellitus. An important mechanistic difference is the molecular site of action. Whereas all therapeutic interventions inhibit either VEGF itself or the VEGFR(s), diabetes-related VEGF resistance occurs downstream of VEGFR-1/VEGFR-2. This disease-related VEGF resistance still allows VEGFR activation to occur following ligation, and one cannot exclude that one or several downstream signalling pathways may still be activated and therefore functionally intact. The induction of such a ‘partial VEGF resistance’ may represent an attractive target for future drug development (Figure 3).

The biology of vascular maturation and considerations for the therapeutic use of VEGF and VEGF antagonists

VEGF has a complex role in angiogenesis, and it is not the only angiogenesis factor. Nevertheless, VEGF-A is one of the most potent stimulators of angiogenesis, especially when
it comes to initiating this process. This is also true for tumour angiogenesis, and this is why VEGF inhibition is regarded as a rewarding target for anti-angiogenesis therapy. However, the situation is somewhat more complex in vivo, where VEGF inhibits the [PDGF (platelet-derived growth factor)-driven] maturation of newly formed vessels by inhibiting the function of pericytes and VSMCs (vascular smooth muscle cells), which is achieved by the activation of VEGFR-2/PDGFR-β (PDGF receptor β) dimers in pericytes and VSMCs, which in turn blocks PDGF signalling [21]. This process leads to vessel destabilization. In contrast, the disruption of tumour VEGF leads to increased tumour vessel maturation.

In accordance with these findings, it has been found that the deletion of VEGF in myeloid cells accelerates tumorigenesis. Vessels are less tortuous with increased pericyte coverage (vascular normalization). This deletion of VEGF in myeloid cells was associated with accelerated tumour progression and with less tumour hypoxia, and the susceptibility to chemotherapy was enhanced [22].

A rational consequence for the induction of therapeutic angiogenesis is that angiogenesis should initially be triggered using VEGF (e.g. for 3 days), followed by a combination of PDGF-BB and VEGF-A to allow maturation of the newly formed vessels.

**Summary and conclusions**

The action of VEGF is essential in maintaining endothelial and vascular function. The inhibition of VEGF action is associated with endothelial dysfunction, which is associated with enhanced risk for cardiovascular complications. As circulating monocytes express functionally active VEGFR-1 on their surface, we have studied the related VEGFR-1-mediated signal transduction cascade. Diabetes mellitus is associated with a reduced monocyte response to VEGF, a situation that can be regarded as VEGF resistance. On a molecular level, VEGF resistance in diabetes mellitus is based on the pre-activation of certain intracellular signalling pathways. These novel findings provide the basis to understand the molecular mechanism of VEGF resistance and to design novel strategies for modulating VEGF action in vitro and in vivo. This concept has both diagnostic and therapeutic perspectives.

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**References**


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