Novartis Foundation Symposium 283

VASCULAR DEVELOPMENT

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Published in 2007 by John Wiley & Sons Ltd,
The Atrium, Southern Gate,
Chichester PO19 8SQ, UK

National 01243 779777
International (+44) 1243 779777
e-mail (for orders and customer service enquiries): cs-books@wiley.co.uk
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Other Wiley Editorial Offices

John Wiley & Sons Inc., 111 River Street, Hoboken, NJ 07030, USA

Jossey-Bass, 989 Market Street, San Francisco, CA 94103-1741, USA

Wiley-VCH Verlag GmbH, Boschstr. 12, D-69469 Weinheim, Germany

John Wiley & Sons Australia Ltd, 33 Park Road, Milton, Queensland 4064, Australia

John Wiley & Sons (Asia) Pte Ltd, 2 Clementi Loop #02-01, Jin Xing Distripark, Singapore 129809

John Wiley & Sons Canada Ltd, 6045 Freemont Blvd, Mississauga, Ontario L5R 4J3, Canada

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Novartis Foundation Symposium 283
x + 250 pages, 46 figures, 3 tables

Anniversary Logo Design: Richard J. Pacifico

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN 978-0-470-03428-6

Typeset in 10½ on 12½ pt Garamond by SNP Best-set Typesetter Ltd., Hong Kong.
Printed and bound in Great Britain by T. J. International Ltd, Padstow, Cornwall.
This book is printed on acid-free paper responsibly manufactured from sustainable forestry, in which at least two trees are planted for each one used for paper production.

Contents

Symposium on Vascular development, held at the Novartis Foundation, London, 13–15 June 2006

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Chair's introduction

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The overall theme of this symposium is vascular development, a subject that has progressed quite rapidly over recent years. It is also a broad subject: often it is described as encompassing just two steps, vasculogenesis and angiogenesis, but it is clear that this division might be somewhat artificial, and moreover, that multiple, complex phenomena are encompassed by these terms. Vasculogenesis involves the differentiation of vascular cells from non-vascular cells (mesodermal or mesenchymal), the assembly of these cells into vessels, and sometimes the incorporation of circulating precursors (bone marrow-derived endothelial progenitor cells) into the vasculature. Angiogenesis is even more complicated, involving a number of different morphogenetic phenomena in the vasculature, such as sprouting of new shoots, circumferential growth, the proliferation and enlargement of vessels, splitting of vessels (intussusception) and remodelling. This last term is often used in the literature as a description of the transition of an immature plexus to a refined hierarchically organized pattern of vasculature. Branch regression is also included in remodelling.

The cellular and molecular processes collected under the simple terms 'angiogenesis', 'vasculogenesis' and 'remodelling' are extremely complicated and we therefore need to diversify our nomenclature in order to define and focus our studies, and know what we are discussing. Just focusing on one of these processes, sprouting in angiogenesis, it becomes immediately clear that even this process is quite complicated. Hypoxia acting via hypoxia-responsive transcriptional machinery leads to the induction of signalling molecules that set off an angiogenic response. This response involves (1) the destabilization and degradation of vascular wall components (the matrix and the dissociation of mural cells that stabilize the vasculature), followed by (2) activation of the endothelial cells, their migration and proliferation to form a sprout, (3) the branching of the sprout and connection of the branches into a communicating and functional vessel network, and finally (4) the stabilization of the newly formed vessels by basement membrane deposition and maturation, and by the recruitment and incorporation of new mural cells.

We are beginning to appreciate that there is functional subspecialization among the vascular cells. This is relatively recent. An example is what occurs at the tip of the vascular sprout, where the leading cell, the tip cell, distinguishes itself morphologically and genetically from the trailing lumen-forming endothelial cells, the stalk cells. There are also mural cells: those associated with the sprouts and subsequent microvessels are referred to as pericytes. These cells are associated with the sprout from the onset of sprouting. Functional subspecialization among the vascular cells, and even within the endothelial compartment, is perhaps something that has been underappreciated so far. It may underlie the unequal cellular responses to the extracellular cues stimulating sprouting, as well as different types of intracellular signalling programmes in the different cell types. The cells are also connected to each other by cell adhesion molecules and junctional complexes; there is likely to be intense cell–cell communication via cell adhesion molecule-associated signalling mechanisms and juxtacrine or paracrine signalling.

One of the emerging themes in vascular development is that the processes that are being uncovered at the growing vessel tips follow the same developmental principles that operate in other organogenesis processes. We have observed attractive signalling, repulsive signalling, cell–cell signalling leading to the establishment of cell boundaries, and we have noticed quite striking similarities to what has been observed for the development of the insect trachea. Remarkably, these processes of tubular sprouting are also similar to a process of subcellular sprout extension, namely neurite extension and guidance.

Vascular development is interesting in its own regard, but one of the major driving forces for scientific progress in this area is the therapeutic promise. There are diseases which could benefit from the enhanced formation of new blood vessels if we learn how to stimulate their formation, such as infarction, fractures, thrombosis and even male baldness. There are other diseases where blood vessel formation is an intrinsic part of the pathological problem. Cancer is the most obvious example, but there is also clinical success with anti-angiogenic treatment in the area of ocular diseases.

What is happening in the therapeutic area? Currently, there is just one validated target for anti-angiogenic therapies, and there are no clinically validated pro-angiogenic therapies. The anti-angiogenic target is vascular endothelial growth factor (VEGF), for which there are currently a few inhibitors in clinical use. Those of us who have been in the angiogenesis field for a while have, however, witnessed a long list of failures in human clinical trials of candidate anti-angiogenic molecules. Endostatin and angiostatin are well known examples, but there are many more that haven't made it into the clinics. It's reasonable to ask why molecules that act potently as anti-angiogenic factors *in vitro* or in animal models, fail in human clinical trials. One of the answers may be that the complexity of vascular development is enormous, and that we are still suffering from a quite profound

lack of insight into the molecular mechanisms of angiogenesis. For example we know very little about the molecular targets of some of the anti-angiogenic drugs that were entered into clinical trials some years ago.

In this meeting we will discuss recent data on the guidance of endothelial cells in angiogenic sprouting and vessel remodelling. We will further discuss cell–cell communication at endothelial cell junctions, polarization of endothelial cells and lumenization of the vessel. Mural cells were in the shadows for a long time but they have recently been highlighted: a couple of papers will look at these. We have a second vascular system to consider—the lymphatics. I am glad that there are representatives of the field of lymphangiogenesis present at this symposium. We are also realizing that the developing vasculature is not just *responding* to surrounding cues, but also delivering developmentally important cues to the organs that it invades (in addition to oxygen and nutrients). Thus, vessels appear to fulfil inductive functions during organogenesis. Reciprocal inductive signalling is a well known principle in epithelial–mesenchymal interactions, and it appears to also take place between blood vessels and surrounding cell types.

Technology is important for studying the vasculature, and experimental genetics has become a major technology for advancing this field. Two participants at this symposium will discuss new developments that push the limits of mouse genetics. Finally, the symposium will have a translational component, as we will discuss vascular drug targets, new and old.

The control of endothelial cell functions by adherens junctions

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Abstract. Cell to cell junctions are important regulators of endothelial responses both in quiescent and angiogenic vessels. Endothelial cells express tight and adherens junctional structures. Although different in their specific molecular composition, these junctional complexes present a relatively similar general arrangement. Both types of junctions are formed by transmembrane adhesive proteins that bind homophilically to identical proteins on an adjacent cell and start a sequence of signalling events. Signal transmission is mediated by interaction with cytoplasmic and transmembrane partners. Adherens junctions are ubiquitous along the vascular tree. In these structures adhesion is mediated by VE-cadherin and its intracellular partners. *In vitro* and *in vivo* data show that VE-cadherin is required for endothelial integrity in quiescent vessels and for the correct organization of new vessels. VE-cadherin regulates endothelial functions through different mechanisms that include: (i) direct activation of signalling molecules such as PI3 kinase and Rac, to sustain survival and organization of the actin cytoskeleton; (ii) regulation of gene transcription, possibly modulating the nuclear level of transcription co-factors such as β-catenin and p120; (iii) formation of complexes with growth factor receptors, such as the type 2 receptor of VEGF (VEGFR2) and modulation of their signalling properties.

2007 Vascular development. Wiley, Chichester (Novartis Foundation Symposium 283) p 4–17

Cell–cell contacts control critical endothelial functions both in quiescent conditions, and in activated situations, such as inflammation and angiogenesis.

Junctional proteins restrain cell migration, inhibit proliferation and apoptosis, and contribute to the maintenance of apical–basal polarity. In general, therefore, junctional signals should counteract angiogenesis and should be inhibited when vessels are induced to proliferate. Indeed, angiogenesis is accompanied by increased vessel permeability (Eliceiri et al 1999) and reduction of endothelial barrier function accompanies most inflammatory situations (Weis et al 2004a).

[1]This paper was presented at the symposium by Elisabetta Dejana, to whom correspondence should be addressed.

A somehow intuitive consequence of these observations is that cell to cell junctions need to be weakened to allow the vessels to grow and be strengthened to maintain the endothelium in a quiescent state. An important aspect to define is the molecular mechanism through which this regulation takes place.

Molecular architecture of endothelial junctions

Various junctional structures have been identified at endothelial cell to cell contacts. Tight junctions, located in the most apical position toward the vessel lumen, exert a strong control on solute permeability and cell trafficking. These structures are present only in endothelia that need to maintain a strict control of permeability, such as brain microcirculation or large vessel endothelia. Adherens junctions on the other hand are ubiquitous along the vascular tree (Liebner et al 2006).

Although the molecular composition of the different types of junctions varies, in general they are formed by both transmembrane and cytoplasmic components. At junctions, dimeric adhesive proteins bind to other identical dimers present on a nearby cell. The result is the lateral clustering of the adhesive molecules at cell to cell contacts, forming a zipper-like structure along the cell periphery (Dejana 2004). The recognition/adhesive information is delivered inside the cell by cytoplasmic and transmembrane partners. Junctions therefore behave as true signalling complexes (Liebner et al 2006).

VE-cadherin and adherens junctions

The major transmembrane component of endothelial adherens junctions is vascular endothelial (VE-) cadherin. This member of the cadherin family of adhesive receptors is expressed specifically in endothelial cells from the time of the earliest endothelial differentiation in the embryo. The extracellular domain of this single-pass transmembrane protein comprises five homologous repeats. The minimal functional unit of VE-cadherin is a homodimer that recognizes as a ligand, in a calcium-dependent way, an identical dimer present on a nearby cell. This starts a process of clustering of VE-cadherin molecules at the apposing cell membranes, which is sustained by both *cis*- and *trans*-interactions between cadherin molecules of the same cell and on the opposing cell, respectively (Gumbiner 2005). From the junction, signals are transmitted with the cooperation of interacting partners such as the intracellular molecules collectively known as catenins: α-, β-, γ-catenin/plakoglobin and p120. β-catenin, plakoglobin and p120 bind strongly to defined sequences of the VE-cadherin cytoplasmic domain (Table 1), while α-catenin binds indirectly through the bridge of β- or γ-catenin. Other cytoplasmic and transmembrane proteins form complexes with VE-cadherin and contribute to the modulation of its adhesive and signalling activities (Table 1).

TABLE 1 VE-cadherin interactors

Transmembrane	*Region of VE-cadherin involved in the binding*	*Constitutive*	*Induced*	*Modulated by cell confluence*	*Functional consequence*
VEGFR2	n.d., β-catenin required	+ (in vivo)	+ (in vitro)	↑	Regulation of VEGFR2 internalization, mitogenic and anti-apoptotic activities (1,2) endothelial barrier-function (3,4)
Dep1	n.d., β-catenin required		+	↑	Down-regulation of VEGFR2, Tyr-phosphorylation, internalization and mitogenic response (1,2)
VE-PTP	membrane-proximal extracellular domain EC5		+	n.d.	Reduced phosphorylation of VEGFR2 in response to VEGF, increased VE-cadherin-mediated barrier integrity, defective vasculogenesis (5,6)
Cytoplasmic					
β-catenin	cytoplasmic domain (aa703–784 in human VE-cadherin)	+		↓	Association with VE-cadherin required to control vessel morphogenesis (7)
γ-catenin	cytoplasmic domain (aa703–784 in human VE-cadherin)	+		↑	Association with VE-cadherin contributes to junction stabilization (8)
p120	cytoplasmic domain (aa621–702 in human VE-cadherin)	+		↓	Involved in the maintenance of endothelial barrier function (9)
p85/PI3K	n.d., β-catenin required	+		↑	Protection from apoptosis activated by VE-cadherin clustering (7)
Tiam	n.d., β-catenin required	+		↑	Regulation of actin and focal contact organization (10)
Csk	phosphotyrosine 685		+	↑	Involved in density-dependent inhibition of cell growth (11)
Src	n.d.	+			Mediates VEGF-induced VE-cadherin phosphorylation *in vivo* and *in vitro* (12)
SHP2	n.d., β-catenin required	+			Down-regulation of catenin's tyrosine phosphorylation. Target of thrombin (13)
Shc	cytoplasmic domain (aa703–784 in human VE-cadherin)	+			Possibly involved in the regulation of response to VEGF (14)

(1) Lampugnani et al 2003, (2) Lampugnani et al 2006, (3) Weis et al 2004a, (4) Weis et al 2004b, (5) Nawroth et al 2002, (6) Baumer et al 2006, (7) Carmeliet et al 1999, (8) Lampugnani et al 1995, (9) Iyer et al 2004, (10) Lampugnani et al 2002, (11) Baumeister et al 2005, (12) Lambeng et al 2005, (13) Ukropec et al 2000, (14) Zanetti et al 2002.

Some of the reported interactions are direct and the molecular domains of VE–cadherin involved have been identified, while others remain to be determined (n.d., not determined). These interactions can be constitutive or induced by cell stimulation or clustering of VE–cadherin at cell to cell contacts as in confluent cultures. VE–cadherin partners may transfer intracellular signals and influence cell behaviour in a way dependent on cell confluency.

VE-cadherin and endothelial functions

Data collected in several *in vitro* and *in vivo* models indicate that VE-cadherin expression and clustering strongly modulate endothelial cell behaviour (Carmeliet et al 1999, Corada et al 1999, 2001, 2002, Liao et al 2002, Weis et al 2004a, Crosby et al 2005). The involvement of VE-cadherin in the organization of new vascular structures is clearly indicated by the effect of homozygous null mutation of the VE-cadherin gene. In the mouse embryo this mutation induces a lethal phenotype at around 9.5 dpc due to major defects in the organization of the vascular tree (Carmeliet et al 1999). Among the various examples that illustrate the requirement of VE-cadherin for the formation of a new endothelial network, we report in Fig. 1 an *ex vivo* model of organ culture. The still avascular allantois of an 8.0 dpc mouse embryo can be explanted and maintained in culture. After about 18 h endothelial cells differentiate and form a vascular network (Fig. 1 a–e) (Drake & Fleming 2000). If the allantois is explanted from embryos with homozygous null mutation of the VE-cadherin gene, endothelial cells differentiate (see PECAM1- and Fli1-positive cells in Fig. 1) (Crosby et al 2005), but they fail to organize in a three-dimensional structure (Fig. 1 f–l). Beyond illustrating the biological role of VE-cadherin, this experimental model is also useful to screen for the anti-angiogenic activity of antibodies to VE-cadherin (Crosby et al 2005).

Conversely, the importance of VE-cadherin in preservation of vascular integrity is underlined by the study of the effects of a blocking monoclonal antibody, BV13, directed to the EC1 region of the extracellular domain of the protein (Corada et al 2002). *In vivo* administration of this antibody strongly increases vascular permeability in several organs of adult mice (Corada et al 1999). Consistently, a monoclonal antibody, BV9, directed to the extracellular domain of human VE-cadherin enhances the permeability of monolayers of human endothelial cells (Corada et al 2001).

While these data support the concept that VE-cadherin is a key element in the control of endothelial integrity, they also imply that targeting VE-cadherin for therapeutic intervention may produce severe systemic effects on quiescent vasculature. However, another monoclonal antibody, E4G10, directed to a different epitope (May et al 2005) was found to target VE-cadherin specifically at angiogenic sites, leaving resting vessels unperturbed (Liao et al 2002). VE-cadherin is in a different conformational state in resting versus growing endothelia and E4G10 recognizes an epitope of VE-cadherin that is accessible only in activated angiogenic vessels, while it is masked in quiescent endothelia.

VE-cadherin signalling pathways

VE-cadherin may modulate endothelial behaviour through different pathways (see Table 1). We have described the association to VE-cadherin of the p85 subunit of

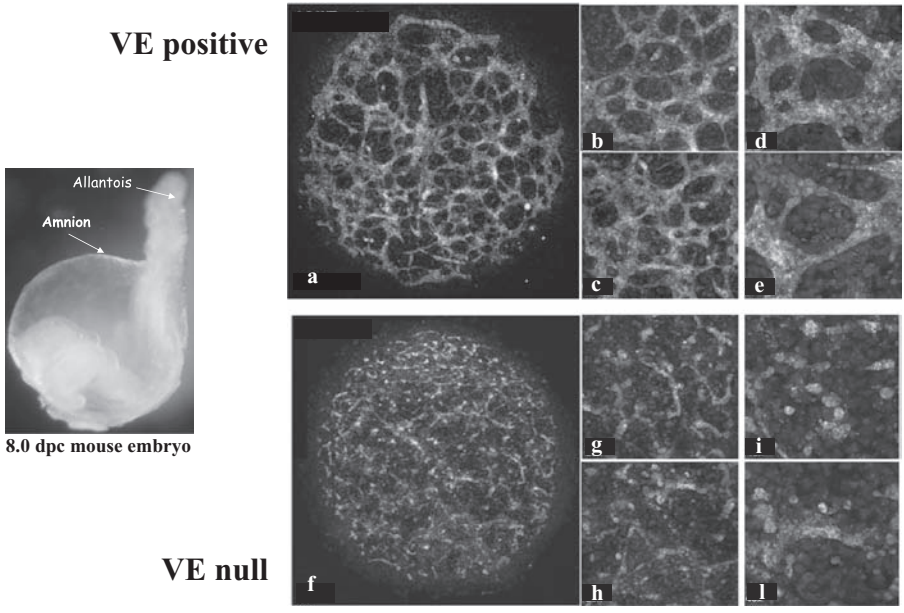

FIG. 1. VE-cadherin null allantois explants fail to undergo normal vascular morphogenesis in culture. Allantoises (small panel on the left) explanted from 8.0 dpc embryos can be maintained in culture for 16–24 hours. In this time frame endothelial cells differentiate, (see PECAM1 [green fluorescence, not visible in this grey scale reproduction, but please see colour figure at *http://www.novartisfound.org.uk/dejana_figure1.jpg*] and Fli1 [red fluorescence] positive cells) and organize in a vascular like network (panel a). The allantois from a homozygous null VE-cadherin mutant embryo contains a comparable number of PECAM1 (green fluorescence) and Fli1 (red fluorescence) positive endothelial cells. However, the cells fail to form stable vascular structures and only form sparse aggregates (f). Small panels on the right (b–e, wild-type, g-l, VE-null) show independent magnifications (b,c and g,h 20×, and d,e and i,l 40×, respectively) of the 10 × magnification fields on the left (a,f).

phosphatidylinositol 3 (PI3) kinase in confluent cells. This results in activation of Akt and enhanced resistance to apoptosis (Carmeliet et al 1999). The C-terminal truncated VE-cadherin, unable to bind β-catenin, does not recruit p85 and does not protect cells from apoptosis. VE-cadherin, again only when clustered in stable junctions, induces recruitment at cell–cell contacts of Tiam, a Rac-specific guanosine exchange factor. The effect is activation of Rac, inhibition of Rho and reorganization of actin cytoskeleton and adhesion plaques (Lampugnani et al 2002). Tyrosine kinases and phosphatases have been reported to form complexes with VE-cadherin. Src kinase is constitutively associated to VE-cadherin and mediates VEGF-induced VE-cadherin phosphorylation (Lambeng et al 2005). This may contribute to the maintenance of VEGF-stimulated angiogenic processes.

C-terminal src kinase, csk, binds via its SH2 domain to the phosphorylated tyrosine 685 of VE-cadherin (Baumeister et al 2005). The functional consequence of such an interaction is inhibition of cell proliferation. Through its most membrane-proximal extracellular domain, VE-cadherin associates with the receptor-type-vascular endothelial protein tyrosine phosphatase (VE-PTP) (Nawroth et al 2002). This reduces tyrosine phosphorylation of VE-cadherin, reduces cell layer permeability, and most importantly, influences a correct vasculogenesis in the embryo (Baumer et al 2006, Nawroth et al 2002). The non-receptor tyrosine phosphatase SHP2 associates with VE-cadherin via β-catenin (Ukropec et al 2000). Thrombin induces phosphorylation of SHP2 and release of SHP2 from the complex. This is accompanied by tyrosine phosphorylation of the VE-cadherin-associated catenins (β-, γ-, p120) and may be part of the mechanism through which thrombin increases endothelial permeability. The transmembrane phosphatase Dep-1 (density enhanced phosphatase 1) is recruited by VE-cadherin and modulates the responses to VEGFR-2 (see below, Lampugnani et al 2003).

It was also found that VE-cadherin regulates gene transcription. This has been observed by comparison of the transcriptome of endothelial cells genetically ablated of VE-cadherin and that of the same cell line gene transduced to express VE-cadherin. 85 and 66 genes have been found up-regulated and down-regulated, respectively, by the presence of VE-cadherin clustered at cell to cell contacts. One reasonable mechanism through which VE-cadherin can exert its control on gene transcription is through sequestration at junctions of transcriptional co-factors, such as β-catenin and p120 (Park et al 2005). This would reduce the nuclear level of these factors and transcriptional regulation of their target genes. While this mechanism may be operating for genes presenting in the promoter regions at least one putative β-catenin/Tcf-Lef-1 binding domain, the precise molecular mechanism of such an effect is still unclear. In addition, the pathways through which VE-cadherin can control genes not under putative β-catenin control remain to be defined.

VE-cadherin can also actively cross-talk with receptors for growth factors. This has been analysed in detail for VEGFR2, a classical modulator of endothelial function (Lampugnani et al 2003). When VE-cadherin is clustered at cell–cell contacts, as is the case in mature quiescent vessels and in confluent cultures, the two proteins can form a complex and, as a consequence, receptor signalling is re-directed from mitogenesis to survival (see below and Fig. 2). The complex between VE-cadherin and VEGFR2 observed *in vivo* is constitutive and transiently disrupted by treatment with VEGF (Weis et al 2004b). While in cell culture VEGF induces the formation of the complex (Zanetti et al 2002). A possible explanation of such discrepancy is the effect of blood flow as a modulator of endothelial functions.

Although the molecular organization of the complex between VE-cadherin and VEGFR2 remains to be defined in detail, we have shown that β-catenin is required

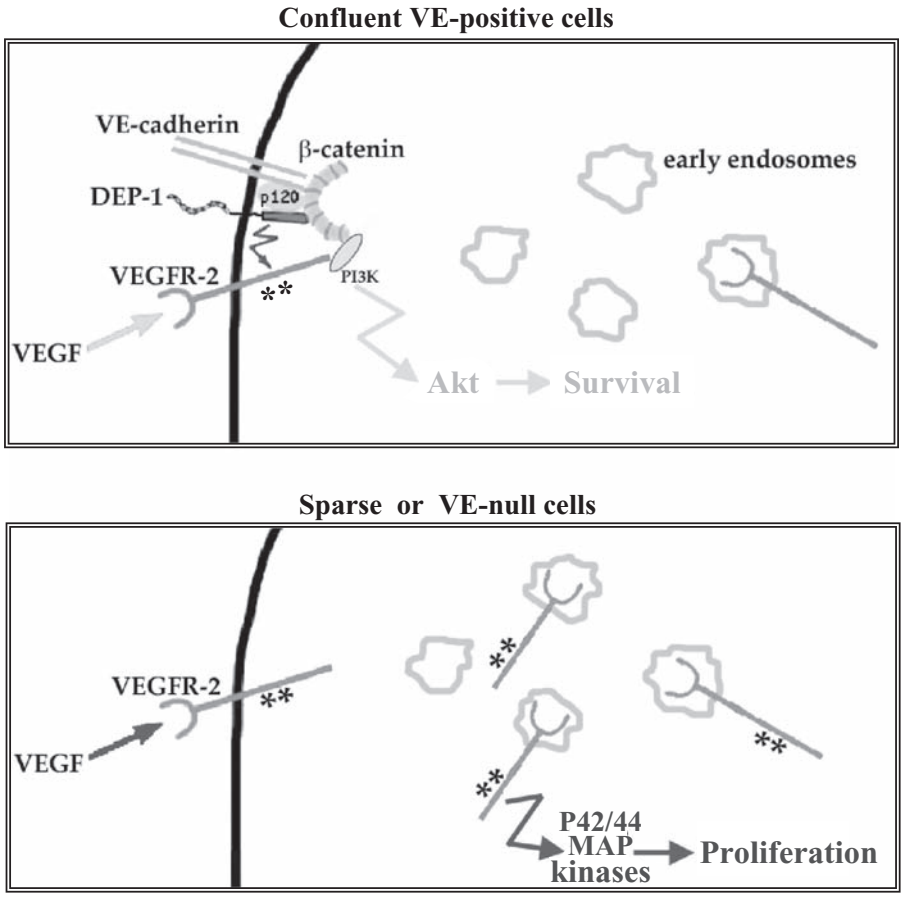

FIG. 2. VE-cadherin clustering at cell to cell contacts directs VEGFR2 signalling. In confluent cells, as in mature quiescent vessels, VE-cadherin clustered at cell–cell contacts can form a complex with VEGFR2. The complex forms in response to VEGF stimulation which induces tyrosine phosphorylation of the receptor (represented as ** in the figure) (Shibuya & Claesson-Welsh 2006). β-catenin is indispensable for the complex to form (Lampugnani et al 2003) and the transmembrane tyrosine-phosphatase Dep1 is recruited in the complex. Association with VE-cadherin reduces VEGFR2 internalization and allows its dephosphorylation by Dep1 (Lampugnani et al 2003). The final outcome is that the internalized receptor is poorly phosphorylated and transfers a weak mitogenic signal from intracellular compartments (Lampugnani et al 2006). In the absence of VE-cadherin, VEGFR2 is highly phosphorylated in response to VEGF (Lampugnani et al 2003), is efficiently internalized and can signal more effectively from intracellular compartments (Lampugnani et al 2006).

for its formation. The biochemical consequence of its interaction with VE-cadherin is that VEGFR2 is less tyrosine-phophorylated following stimulation with VEGF. This results in reduced activation of MAPK and reduced proliferation (Lampugnani et al 2003). Somewhat surprisingly, under the same experimental conditions, the anti-apoptotic response to VEGF is enhanced (Carmeliet et al 1999). The inhibition of tyrosine phosphorylation of VEGFR2 is due to the recruitment of the complex of the transmembrane phosphatase density-enhanced phosphatase 1 (Dep1). This transmembrane phosphatase has been reported to preferentially dephosphorylate particular tyrosine residues of Met receptor (Palka et al 2003) and PDGFβ receptor (Kovalenko et al 2000). If a similar site selectivity also operates for VEGFR2 it may represent the basis of the decreased proliferation and enhanced anti-apoptosis signalled by VEGFR2 in confluent VE-cadherin positive cells (Lampugnani et al 2003). Dep1 could preferentially dephosphorylate the tyrosines involved in recruiting mitogenic mediators (such as PLCγ) (Takahashi et al 2001), leaving intact the phosphotyrosines that sustain the anti-apoptotic signal (Shibuya & Claesson-Welsh 2006).

A further consequence of the formation of a complex between VE-cadherin and VEGFR2 is that the internalization of the activated receptor is inhibited. If VE-cadherin is not expressed or not clustered at cell–cell contacts VEGFR2 can enter intracellular compartments to a greater extent and in a highly phosphorylated form (Lampugnani et al 2006). In this active state, VEGFR2 can activate PLCγ, which can sustain mitogenesis from intracellular compartments. This process is strongly inhibited in VE-cadherin positive cells in which the receptor is retained more effectively at the membrane and, due to Dep1 activity, is actively dephosphorylated. As a consequence, the mitogenic signal from the internalized receptor is much weaker (Lampugnani et al 2006).

The implications of these results are that: (i) signalling from VEGFR2 is under a junctional control; (ii) the formation of a complex between VE-cadherin and VEGFR2 limits its mitogenic signalling; (iii) internalization is a way for the receptor to escape inactivation and signal more effectively and for a longer time; and (iv) any system that modulates the recruitment of VEGFR2 to the complex with VE-cadherin may effectively modulate the activity of this receptor.

Conclusions

It appears that endothelial cell–cell junctions are the association sites of a complex array of transmembrane and cytoplasmic molecules. These molecules functionally interact and the result of such cross-talk is a fine tuning of the endothelial behaviour. Cell–cell junctions represent possible targets of therapeutic intervention since modulation of junction adhesion and signalling may strongly influence endothelial growth, apoptosis and permeability.

Acknowledgements

This work was supported by the Associazione Italiana per la Ricerca sul Cancro, the European Community (QLRT-2001-02059, Integrated Project Contract No LSHG-CT-2004-503573; NoE MAIN 502935; NoE EVGN 503254).

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DISCUSSION

Lammert: Perhaps connecting the sprouts with the cadherins would be an interesting avenue: can we understand how stalk cells proliferate, weakening their junctions, and how this can be explained by VE-cadherin being upstream of VEGF receptor, for example?

Betsholtz: That's a good suggestion. It is an interesting possibility that the junctions are structurally different at the sprout sites.

Dejana: One would expect that confluent cells remain relatively insensitive to VEGF activation. However, it is probably the local concentration of VEGF that makes the difference. There might be a concentration threshold, and cells would start to migrate once this is passed. VEGF can induce phosphorylation of VE-cadherin and reduce the strength of adhesion at junctions. At the right concentration of VEGF, junctions would become weaker and endothelial cells would release themselves from inhibition of growth, and then start to migrate.

Davies: Most of the things you talked about were global responses of a cell to the loss of VE-cadherin expression, such as internalization of receptor and so forth. Do you think everything will be global, or do you have any evidence that part of a cell can develop differently to another part? For example, if one part of a cell has lost expression, it can go into its lamellipodium-forming mode and forge forward, whereas the other side of the cell knows that it is still connected and just needs to pull its neighbours with it. A cell at one position has neighbours on one side but not on the other. One part of the membrane would be responding differently.

Dejana: Let's imagine a wound in the vessel wall. The cells at the front are released from contact inhibition of growth, and would migrate much more effectively. Cells at the migrating front present part of their membrane free, i.e. not in contact with neighbouring cells. VEGF receptors, present in that area, maintain a high response to migration and proliferation. In contrast, receptors at the back where the cells are still in contact with other cells are less responsive. This phenomenon may dictate the direction of movement.

Betsholtz: Jamie Davies, it sounds to me that you are describing the tip cell, which is partially detached at one side and reacts to VEGF with a migratory response. But it still remains attached to the vasculature at the trailing end of the cell.

Davies: It could be the tip cell, or even, if this is true of other cadherins, such as N-cadherin, could be modulated by loss of connection with pericytes.

Gerhardt: We have recently looked at the distribution of VE-cadherin in the growing sprout. In the retinal model, the tip cell seems to have weakened junctions at its contact point with the stalk cell. It also relocates a lot of the VE-cadherin to the sprouting front, to the tips of the filopodia (unpublished results). We know that the response to stimulation of VEGFR-2 also differs between the tip cells and the stalk cell. The question I find interesting in the *in vivo* situation is why the tip cell doesn't respond with proliferation. If you inject VEGF into the retina, almost the entire vascular plexus starts to proliferate. All the cells grow except the tip cells. But these cells retain stable VE-cadherin contacts. If you want to make an analogy to cell culture, I would consider these cells as being confluent. They start to proliferate, not migrate.

Dejana: The concentration of VE-cadherin at junctions doesn't tell the whole story. The most important parameter is the state of phosphorylation of these proteins. When we add histamine or other permeability increasing agents, we don't see cell retraction if we don't use very high concentrations: we just see phosphorylation of VE-cadherin at the junctions. The overall organization of junctions may change depending on the functional state of the cells. Maybe junctions of the cells at the tip allow their migration but not proliferation.

Drake: We have compared tip cells with angioblasts. What we have concluded is the following. Angioblasts do not express VE-cadherin. In the tip cell, VE-cadherin is only expressed at the site where the tip cell and stalk cell share a

common cell-cell junction. Physically, tip cells appear to adhere tightly to the extracellular matrix. I think cell matrix adhesion is what is inhibiting VE-cadherin expression in both angioblasts and tip cells. When one moves back to the stalk cell, which expresses VE-cadherin, the cells are engaged in cell–cell versus cell–matrix interactions.

Dejana: Our view is more concentrated on VE-cadherin. Certainly, interaction with the matrix is important. There are a series of signals from integrin to VE-cadherin and vice versa. VEGFR2, can also interact with integrins, there might be a better interaction of this receptor with integrins while the cells are growing and migrating, while the receptor would interact with cadherins better when the cells are stabilized.

Augustin: What is the relevance of your model in the context of sprouting angiogenesis? I think we would all agree that the tip cells have altered junctional complexes, and that subconfluent cells reflect some of the properties of an activated endothelial cell. Yet, I am not sure whether the two are the same. We have done a lot of transcriptomic profiling of confluent and subconfluent cells. Essentially, we can validate 100% of the genes expressed by subconfluent endothelial cells in the carotid denudation model, whereas we find that only few of the genes expressed by subconfluent cells are actually involved in angiogenesis. Lateral cell migration as it occurs *in vivo* following a carotid denudation is distinctly different in terms of function and phenotype versus the three-dimensional sprouting angiogenesis in a complex matrix, where we see very distinct matrix-dependent gene expression profiles.

Dejana: It might be different, because cell migration is more related to wound repair. In sprouting cells junctions may undergo very subtle modifications. These two models might have something in common but be quite different in nature.

Augustin: You have determined the VE-cadherin-dependent transcriptome of endothelial cells. If you were to validate those genes you presented as candidates, in which model would they validate?

Dejana: The general hypothesis is that VE-cadherin induces stabilization signals. Therefore the genes up- or down-regulated by this molecule should also be up- or down-regulated in confluent, stabilized monolayers.

Owens: I have a question about cell autonomous functions of the cadherins. If you make a chimeric knockout mouse where half of the cells lack the VE-cadherins, do they populate blood vessels or undergo apoptosis?

Dejana: We haven't looked at that, and I'm not aware of anyone else who has. However, VE-cadherin is a way through which cells communicate, if you do not have VE-cadherin you may interrupt communication. So, VE-cadherin null cells may die either because they do not express the molecule (cell autonomous) or because they cannot get in touch with the other endothelial cells.