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REVIEW PAPER

History and conceptual developments in vascular biology and angiogenesis research: a personal view

Andreas Bikfalvi[1,2]

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Abstract Vascular biology is an important scientific domain that has gradually penetrated many medical and scientific fields. Scientists are most often focused on present problems in their daily scientific work and lack awareness regarding the evolution of their domain throughout history and of how philosophical issues are related to their research field. In this article, I provide a personal view with an attempt to conceptualize vascular development research that articulates lessons taken from history, philosophy, biology and medicine. I discuss selected aspects related to the history and the philosophy of sciences that can be extracted from the study of vascular development and how conceptual progress in this research field has been made. I will analyze paradigm shifts, cross-fertilization of different fields, technological advances and its impact on angiogenesis and discuss issues related to evolutionary biology, proximity of different molecular systems and scientific methodologies. Finally, I discuss briefly my views where the field is heading in the future.

Keywords Angiogenesis · Vascular biology · History · Conceptual developments

✉ Andreas Bikfalvi
andreas.bikfalvi@u-bordeaux.fr

[1] INSERM U1029 (Angiogenesis and Tumor Microenvironment Laboratory), Allée Geoffroy St Hilaire, 33560 Pessac, France

[2] Université Bordeaux (Angiogenesis and Tumor Microenvironment Laboratory), Allée Geoffroy St Hilaire, 33560 Pessac, France

Introduction

Vascular biology is a vibrant field of investigation in the life sciences. The vasculature plays a leading role in many areas such as atherosclerosis, development, tissue repair, inflammation, cancer and chronic diseases.

In recent years, the field of vascular development has gained the attention of many scientists, and investigations have shed light on how vessel morphogenesis is regulated in development, physiology and pathology [1]. As such, many vascular regulators and their receptors have been identified and their functions in vascular cells investigated. Intracellular factors including transcription factors, microRNAs or long ncRNAs have been characterized and their functions determined [2]. A link with endothelial cell metabolism has been recently established [3]. Therapies either to promote or to inhibit angiogenesis have been developed and have entered the clinic, such as bevacizumab, an inhibitor of vascular endothelial growth factor [4–6], or small chemicals which block the tyrosine kinase domains of receptors at the surface of vascular cells.

There are a number of aspects stemming from the study of vascular development, which are suited to a historical and conceptual analysis and are at present poorly investigated. Such an analysis could show how this scientific domain has developed and what the meaning of the research conducted in this field has for science in general and for the development of scientific ideas or methodologies. I will give in this article my personal view on the subject and focus on selected issues including the analysis of paradigm shift, cross-fertilization of fields, technological advances and impact on angiogenesis, evolutionary considerations, molecular proximity of different systems and methodological considerations.

Changing paradigms

According to Thomas Kuhn, a scientific paradigm is « a universal recognized scientific achievement that, for a time, provides model for solutions of problems for a community of practitioners« [7]. Kuhn makes the distinction between normal science that progresses by accumulation of data and knowledge, and scientific revolutions. Scientific revolutions occur when abnormalities in a research field are encountered which ask to fully reconsider the conceptual framework in which science is conducted at a given time. There is a general tone of scientific relativism in the Kuhnian philosophy but one can easily accept the Kuhnian scheme for scientific « progress » without falling into a relativistic posture. In Kuhn's view a paradigm is the result of a radical transformation of a scientific field which results in new (a) paradigm(s) that are in conflict with the previous paradigms. To my opinion, one can adopt a less restrictive notion of paradigms and broaden its meaning. In this view, paradigms are a sort of frameworks in which the working scientist will do normal problem solving science in order to fill up what is predicated by the paradigm. I would formulate the concept of micro- and macro-paradigms in this respect. Indeed, "revolutions" occur much more frequently on a smaller scale (micro-revolutions) and, in my opinion, they can be considered as micro-paradigms. These micro-revolutions do not affect the whole theoretical edifice of a scientific discipline but shed new light and solidify the whole conceptual structure of a scientific theory.

Regarding our knowledge on vascularization, it has undergone a number of micro- and macro-revolutions leading to new micro- and macro-paradigms. A non-exhaustive list of these micro- and macro-revolutions is given (Table 1). It should be noted that these conceptual leaps have occurred during a period of more than 2000 years!

The discovery of the circulatory system is seen as a macro-revolution that completely changed our way of viewing the organization of living systems. The cell theory applied to the vasculature was another one. Yet another one is the fact that vascularization is dependent on soluble factors produced by normal and pathological tissues, which led to the identification of these factors. Regarding micro-revolutions, I list some of the following: existence of attractive and repulsive factors, the postulate of the specificity of angiogenic factors, the discovery of VEGF, the discovery of lymphangiogenesis factors or the concept of guide cells ("tip"). Based on these scientific revolutions, some of the macro- and micro-paradigms, mostly from an historical perspective as well as the interactions between paradigms specifically related to vascular development are discussed in more detail below (see also Table 1).

Discovery of vascular cells in the capillary wall

The capillary wall was observed for the first time by Theodor Schwann in the tadpole. In 1839 and 1847, Schwann was the first to describe what would later be called the endothelium [8]. He wrote that the capillary vessels in the tail of fully grown tadpoles were surrounded by a thin membrane, clearly visible, which showed no fiber arrangement. The thickness of this "membrane" was not uniform in various areas and was not clearly visible in certain parts. Schwann observed cell nuclei at different locations of the capillary wall that were, for him, either nuclei of cells belonging to the capillary wall or, alternately, adjacent epithelial cells that migrated to the vessel wall. Schwann was rather inclined to the first explanation. If we follow Schwann in his argument, he finds the nuclei in two different places of the capillary wall, inside the wall and near the lumen of the capillary vessel. Schwann seems to consider that these cells were cells of the vessel wall and not invading from the surrounding tissue. Looking at the original drawing by Schwann shown in Fig. 1, we see that the nuclei which he thought to be in the capillary wall are in fact located at two different sites, internal and external. In light of our current knowledge, the cells located in the external position are likely to be a pericyte, and the inner cells in contact with the vascular lumen are what we today call an endothelial cell. However, at the time of his research, he could not definitely decide about the ontology of the vascular cells. The internal layer of cells was later called "endothelium" by His (1831–1904) [9]. The cell theory of the capillary vessel wall was not accepted immediately. But Schwann had supporters. Even Johannes Müller, who was initially hostile toward this idea, afterward changed his mind [10]. However, until the mid-end of the nineteenth century many investigators still believed that there was no real capillary wall and no cellular communication system between the arterial and venous system [11].

Changing ideas about the significance of tumor angiogenesis

Carl Thiersch (1822–1895) was the first to demonstrate the formation of new vessels in the tumor stroma. He showed that new vessels originated from preexisting vessels [12]. Thiersch wrote in a paragraph his enlightening intuition on the importance of the interaction between tumor epithelial cells and blood vessels: « The epithelium is dependent on the vascular stroma in the same way as a plant of the soil in which it has taken root. Like the plant, the epithelium brings with it autonomous development potential and growth and demand nothing else than the contribution of

Table 1 Non-exhaustive list of micro- and macro-paradigms related to vascular biology

	References and year	Micro- or macro-paradigms
1	Ibn Nafis (1242) [73], Colombus (1559) [74], Servetus (1553) [75] Harvey (1628) [76]	Existence of the pulmonary circulation. Nutrition and respiration are not independent in vertebrates but localized in the same circulation
2	**Harvey (1628) [76]**	**The blood circulation in vertebrates is a closed circulation**
3	Harvey (1628) [76]	It is not the diastole that attracts the blood, but the contraction of the heart (systole) that actively propagates the blood through the organism
4	**Schwann (1845, 1847) [8], His (1865) [9]**	**Vessels, in vertebrates, are composed of different layers and exhibit cells that are in direct contact with the blood (endothelial cells)**
5	**Hunter (1794) [77], Goldmann (1907,1908) [13]**	**Vascularization is an active process in tissues**
6	Goldmann (1907,1908) [13]	The vasculature in tumors is part of a host defense mechanism - > **see Girard**
7	**Greenblatt and Shubik (1968) [78], Ehrmann and Knoth (1968) [79]**	**Soluble morphogenic factors are required for vascularization in tissues (tumor-derived factors)**
8	**Folkman (1971) [80]**	**Vascularization is the prime ingredient of the integrated tumor ecosystem and essential for tumor growth**
9	Gimbrone and Folkman (1972) [14]	Blockade of angiogenesis will halt tumor growth
10	Ferrara (1989) [30]	Angiogenic factors have vascular specificity
11	Keshet (1993) [81]	Vascular morphogens act as gradients
12	Folkman (1971) [80], Adams (2001) [82], Eichmann (2004) [83]	There are four types of factors: stimulatory, inhibitory, attractive and repulsive
13	Folkman and Hanahan (1996) [84]	Tumors undergo an angiogenic switch to activate angiogenesis
14	Murray (1932) [85], Dieterlen-Lievre (1975) [86]	The vascular and hematopoietic system has a common origin
15	**Alitalo (1996) [87]**	**The lymphatic circulation is independent of blood vascularization and has its own molecular mechanisms (lymphangiogenesis emerged as subfield)**
15	Betsholtz (1999) [88]	Mechanisms of pericyte recruitment to blood vessels
16	Gerhard and Betsholtz (2003) [51]	Specific cells atop the nascent vessel guide the growth of the vascular tube (TIP cells)
17	Jain (2001, 2003) [19, 20]	Anti-vascular therapy will normalize the vasculature to allow better perfusion which will improve the efficacy of chemotherapy
18	Weinstein (2006) [89], Affolter (2008) [90], Lammert (2009) [91]	Vessel lumen formation is an active process which requires at least one endothelial cell
19	Carmeliet (2004) [92]	Angiogenic factors have extravascular properties
20	Bussolino (2003) [55]; Eichmann (2004) [83]	Vessel guidance is regulated by similar mechanisms as in the nervous system
21	Carmeliet (2013) [93]	Vessel sprouting requires a specific metabolism
22	**Girard (2011) [16]**	**Vessels may exhibit anti-tumor properties via the immune system**
23	Lammert and Melton (2001) [94], Mastumoto and Zaret (2001) [95], Keshet (2011) [41], Rafii (2011) [96]	Vessels have perfusion-independent roles by providing instructive signals to tissues

Macro-paradigms are indicated in bold

substances necessary for its development ». Thiersch had already anticipated this close relationship between the tumor and vessels. However, he had not, in his time, hypothesized an active role ("verlangt nichts weiter") of tumor cells to induce vessel growth, which necessarily implied the existence of a (biochemical) mediator produced by tumor cells.

Ernst Goldmann went one step further in an article published in The Lancet in 1907 and in the Proceedings of the Royal Society of Medicine, where he describes sprouting and budding of capillaries inside tumors [13]. Goldmann saw in tumor angiogenesis a defense mechanism against tumor growth and not a promoting effect on tumor development. To quote Goldmann: "I consider the new formation of blood vessels as a reaction by which the body reacts against the malignant tumor."

This explanation was clearly challenged by Michael Gimbrone and Judah Folkman in 1972, who demonstrated that rabbit tumors only grow in a vascularized tumor environment (which thus has a tumor-promoting effect) and with the discovery of tumor angiogenesis factors [14]. Folkman implicitly stated that tumor cells and the population of capillary endothelial cells within a neoplasm may constitute a highly integrated ecosystem (see below). It is

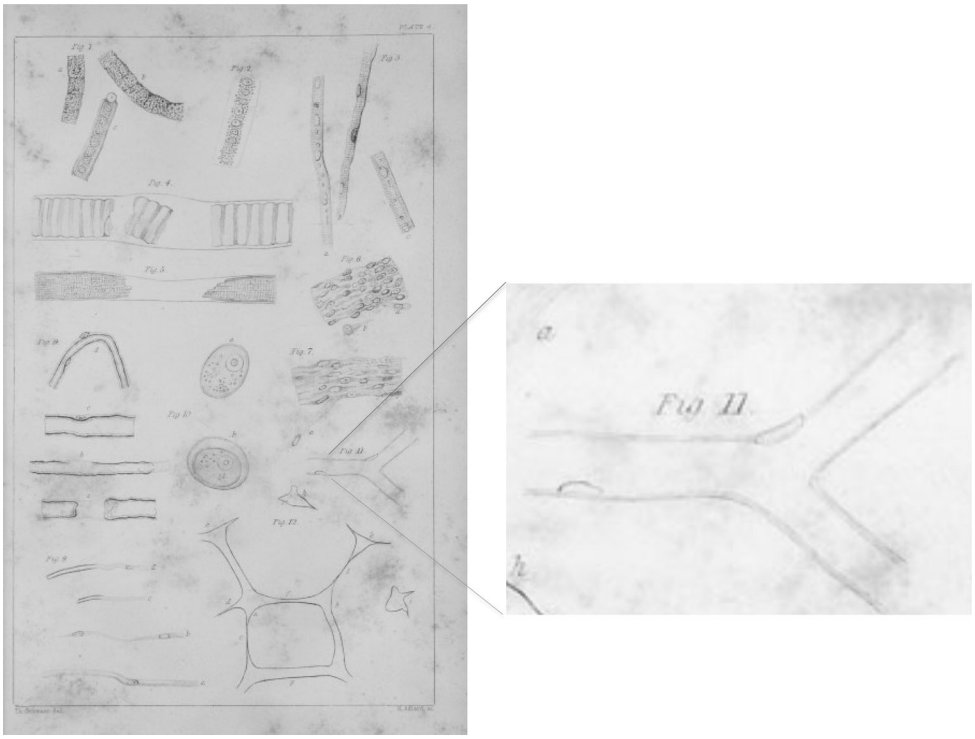

Fig. 1 Original drawing by Schwann depicting two different cell types in the capillary wall. Schwann described these cells as follows [8]: "Very distinct cell-nuclei occur at different spots upon the wall of the capillaries, both of the young and fully developed tadpole. They appear to lie either in the thickness of the wall or on the internal surface of the vessels, on which they often form projections. They admit of a double explanation. They are either nuclei of the primary cells of the capillaries or nuclei of epithelial cells, which invest the capillary vessels. "and he continues later in the text:" that these are the primary cells of the capillaries is, therefore, most probable, although this exclusive argument by no means decides the question"

interesting that (1) the concept of the host defense mechanism precedes the tumor-promoting effect, and that (2) the host defense mechanisms have regained letters of nobility by recent investigation in the field of tumor immunity. Thus, historically speaking the concept of the vascular tumor microenvironment underwent three steps of conceptual modifications: Nourishing (Thiersch) → Host defense (Goldmann) → Tumor promotion (Folkman) → Promotion and defense (present view) ("dialectic" progression) (Fig. 2). It is important to note that Goldmann coined the notion of bodily reaction and host defense that is provided by the vasculature, which seems to be one of the first reports of interdependency between the vasculature and the immune system.

The tumor vasculature—immune interdependency, in the perspective of an anti-tumor response, has only been validated recently by the identification of specialized vessels called high endothelial venules (HEV) in tumors, albeit prior reports indicate that immune cells such as NK cells are required mediators of angiogenesis inhibition by IL-12 and thus provide evidence for NK-cell cytotoxicity to endothelial cells [15, 16]. HEVs are present in some solid tumors such as mammary carcinoma and trigger an anti-tumor immune response by allowing the influx of TH1 cells, cytotoxic effector T cells, and naïve and central memory T cells into the tumor [17]. It would be important to elucidate mechanistically how the number of these vessels can be stimulated to increase the therapeutic efficacy of immunotherapy. Indeed, recently the Bergers laboratory has shown that these vessels can be stimulated by using a combination of immune checkpoint inhibitors and anti-angiogenic therapy [18].

Another important conceptual development was the introduction of the normalization concept in tumor angiogenesis championed by the Jain laboratory [19, 20]. In this concept, anti-angiogenic treatment is destined to kill aberrant vasculature in tumors and to normalize the morphology and functionality of the remaining tumor vasculature. This concept has important consequences for anti-tumor therapy since normalization permits better access of chemotherapy to the tumor due to improved functionality of the vasculature. The normalization concept has been developed for some tumor types such as glioblastoma, but the generalization to all tumors is still a matter of debate and has been viewed critically by some investigators.

Furthermore, the vasculature may provide angiocrine signaling to the tumor and stimulate the proliferation of stem cells including cancer stem cells (see section "Endothelial-derived factors have perfusion-independent effects on organs" for more details).

Angiogenic factors

With regard to the concept of angiogenesis factors, there are two points to discuss, their discovery and their claimed specificity.

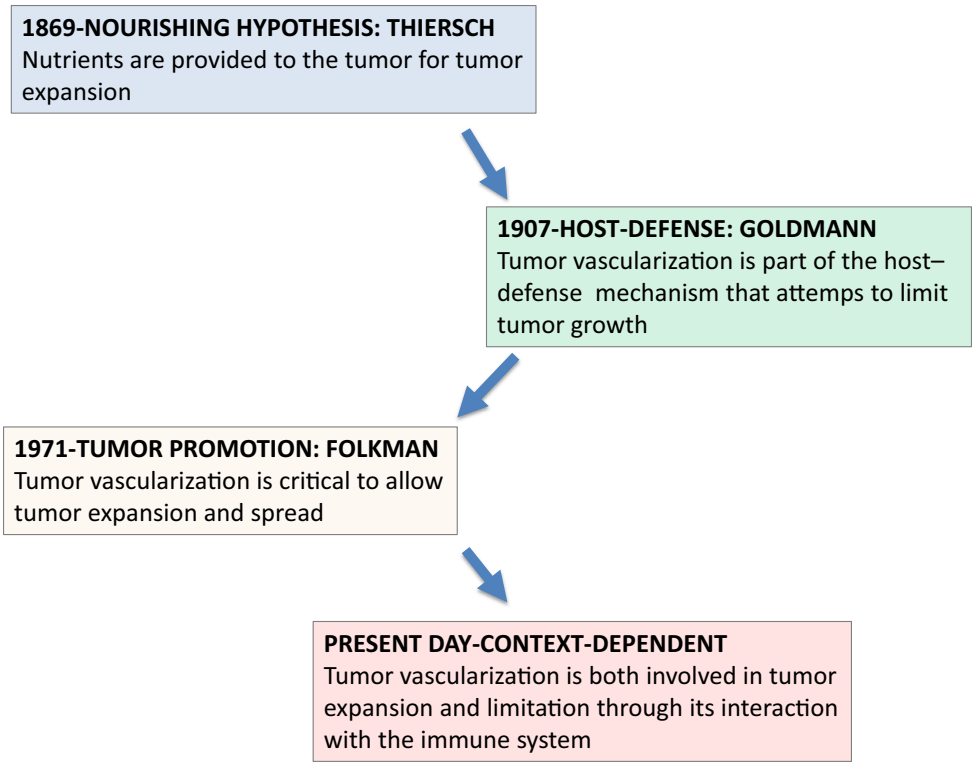

Fig. 2 Evolution of the concept of the vascular TME

Discovery of vascular morphogenesis factors

Until the twentieth century, it was elusive to think about diffusible factors that could control vascular morphogenesis. Vascular morphogenesis was instead viewed more like the unfolding of an internal program within the vasculature itself. Two critical steps had to be taken in the discovery of morphogenic factors: The first was to demonstrate the existence of soluble and diffusible mediators of vascular development. The second was the characterization of the nature of these factors.

Mounting evidence through experiment indisputably proved the existence of a diffusible factor produced by tissues, such as tumor tissue, that could induce vascular growth. Several lines of experiments demonstrated this. Algire had observed changes in blood vessels when fibroblasts were treated with the chemical methylcholanthrene [21]. This suggested that stimulatory factors are produced from cells surrounding blood vessels such as fibroblasts.

In 1948, Michaelson had developed a technique based on the injection of a dye into the arterial system that allowed him to visualize the retinal vasculature [22]. Using this method, he studied the development of retinal vessels and showed that these vessels emerged from the optic nerve to cover the surface and invade the retina. Based on these observations, Michaelson postulated the existence of a factor he called factor X, which induces sprouting of new vessels and that this factor was regulated by hypoxia. In 1951, Campbell observed that the number of capillaries in the retina increased in a low oxygen environment [23]. These observations strongly suggested the presence of factors that stimulate vascular morphogenesis.

The answer to the identity of this factor did not came, strictly speaking, from the vascular biology field, but from tumor biology. Tumors are characterized by abundant angiogenesis, and thus, it is potentially possible to identify this factor from tumor cells or tumor extracts. Pioneering work by Judah Folkman brought the indisputable experimental evidence that the angiogenesis process is crucial for the development of in vivo tumors and postulated that this was due to a diffusible factor he called "Tumor Angiogenesis factor" or TAF, which ultimately led to the identification of these factors [24]. This has led to the discovery of vascular endothelial growth factor from the conditioned medium of follicular stellate cells using heparin-Sepharose chromatography as a critical discovery tool [25] (see section on technology). This discovery was already anticipated by Dvorak and collaborators, who described a factor they called vascular permeability factor (VPF) [26, 27]. The discovery came from the observation that tumors harbor a fibrin meshwork, which could only be explained by an increase in vascular permeability. VPF, which was also purified by heparin-Sepharose chromatography, turned out to be identical to VEGF.

The discovery of soluble TAFs and growth factors raised the question about the quality or properties of

vascular stimulating factors with regard to their multiple functions. These include growth promotion (vascular growth factors), migration (migratory factors), inhibition (negative regulators), guidance (guidance cues) and induction of permeability (permeability factors) [28]. These attributes have been either determined "a priori," can come from experimental evidence or were imported from other scientific domains. "A priori" attributes are such as "if there are positive regulators there must be negative ones." Such a hypothesis may then lead to the search and discovery of these kinds of factors. Another way is experimental evidence where, for instance, a conditioned medium stimulates endothelial cell proliferation or induces vascular permeability, which leads to the identification of such stimulating factors. Yet another possibility is the import of a concept stemming from another field. The attribute "Guidance cue" is such an example. Guidance cue is derived initially from developmental neurobiology and was later introduced into vascular biology [29] (see "Discovery of tip cells and guidance factors").

It is to mention that discoveries made initially within different conceptual frameworks may lead to converging findings as it is the case for VEGF and VPF, which are identical molecules. The discovery of VEGF came from the idea to discover a factor that stimulated specifically the growth of endothelial cells. The discovery of VPF was derived from the idea to discover a factor that regulated vascular permeability.

Specificity of angiogenic factors in question

It was believed at one time that angiogenic factors such as vascular endothelial growth factor (VEGF) were specific for the vasculature and their only role was therefore to stimulate angiogenesis [30]. The thinking was that a pleiotropic factor (i.e., that could interact with other cells outside the vasculature) was unlikely to play a predominant role in the morphogenic process. The concept that specificity equals importance was supported by in vitro studies and genetic experiments in mice where, for instance, VEGF knockout causes a very severe abnormal vascular phenotype [31, 32]. Thus, the wrong belief in the veracity of a concept can even be reinforced by valid experiments and models. It is my view that such kind of reductionist stand has been in the core of vascular biologists at the time and is still for many. Indeed, further studies discovered various other functions outside of the vasculature for vascular regulators [33, 34]. For example, VEGF has roles in the nervous system and the reproductive system. Thus, the quest for a "specific" factor, even if the assumption was proven to be wrong, paradoxically significantly promoted research and allowed the discovery of VEGF and other angiogenic factors. This is an example of a conceptual error that can be, paradoxically, beneficial for research in a particular field of science. To my opinion, if one would had made the initial correct assumption that no vascular-specific factors exist, then already existing alternatives at hand would have satisfied scientist and hindered or slowed down the discovery of major vascular regulators. Fibroblast growth factors (FGFs), which are very potent angioregulatory molecules, were already discovered at that time. However, as the late Werner Risau (personal communication by the author) pointed out at the time of the discovery of VEGF, FGFs were broad-range regulators and had not the "correct" spatial and temporal expression and hypoxia regulation, which of course VEGF possessed. He discussed this issue in a review article entitled "what if anything is an angiogenesis factor" [35] to highlight specifically the problem that scientists had at that time to distinguish the important properties an angiogenesis factor should have from those it should not have. To me, this was also the reason that pushed scientists to look for vascular-specific factors.

Conceptual categories shaping vascular biology and angiogenesis research

When focusing on vascular development, we can already define, in my view, several conceptual categories which include soluble angiogenic factors (module 1), vessel stability and maturation (module 2), sprouting and guidance (module 3), tumor/pathological angiogenesis (module 4) and effects of the vasculature on organ development (module 5).

Module 1 is related to the discovery of soluble angiogenic factors. In this case, a first paradigm is represented by the contention that vascularization is an active process. The following sequence can be envisioned: Vascularization is an active process → soluble factors are required for vascularization → vascular morphogens are specific → vascular morphogens act as gradients.

In module 2, the sequence is the following: Vessels are composed of different cell layers → pericytes are present on capillary vessels → pericytes contribute to barrier function → pericytes stabilize capillary vessels and contribute to the response to angiogenesis factors or inhibitors.

For module 3, the sequence is: Vessels are formed by sprouting → sprouting is dependent on morphogenic gradients → sprouting is dependent on tip cells → the orientation/direction of the vascular sprout is regulated by guidance factors.

For module 4, the sequence is: Tumors are embedded into an integrated ecosystem → tumors are vascularized → vascularization is an active process in tumors with

branches to go either toward a protumor (a) or anti-tumor (b) effect of the vasculature:

(a) Blockade of angiogenesis will halt tumor growth → vascularization is dependent on diffusible factors → blockade of angiogenic factors will halt tumor growth
(b) Vessels interact with immune cells → some tumor vessels have anti-tumor properties by promoting anti-tumor immunity → promoting these vessels will inhibit tumor growth.

For module 5, which is related to organ effects of the vasculature, perfusion-dependent effects precede perfusion-independent effects (perfusion-dependent effects of the vasculature → perfusion-independent effects of the vasculature).

It is important to remember that the progression of each of the conceptual categories is dependent on two factors of variable weight: the preexisting knowledge and the technological development at a given time.

Conceptual categories may also interact with each other (Fig. 3). For example, module 2 (pericyte coverage) and module 4 (tumor/pathological angiogenesis) interact because vascularization in tumors is immature and destabilized with a defect in pericyte coverage. There is interaction between modules 1, 3 and 4 because sprouting is aberrant in tumors and morphogenic gradients are abnormal. Module 5 is a stand-alone module that may only be indirectly connected to module 4 since emerging evidences connect this module to tumor angiogenesis [36]. Thus, conceptual categories that depict different layers of knowledge have strong connections with each other and may contribute to formulation of new hypotheses and paradigms.

Interactions between different scientific fields ("cross-fertilization of fields")

The vasculature, the central ingredient of the integrated ecosystem in tumors

It is surprising that cancer research opened the gate for a molecular explanation of vascularization. Oncology at the time of the pioneering work of Judah Folkman was under the influence of an exclusively tumor cell-driven explanation of cancer. Cancer research was mainly focused on changes taking place inside the tumor cell such as alterations of the cell cycle or the study of oncogenes. The microenvironment was considered as a non-active participant playing no role in development of tumors. However, following the pioneering work of Judah Folkman and other researchers, it could not be ignored that the vascularization played a role in the regulation of tumor development. Folkman further coined the term integrated ecosystem where, in his view, the vasculature had the central role [24]. This concept was instrumental in discovering many factors, receptors and regulatory circuits that not only apply to the tumor context but exhibit a general role in the vasculature. The concept of the ecosystem came from ecology and was introduced by Clapham in 1930, but it was Tansley who fully defined the concept and used it in 1935 in a publication [37]. Tansley devised the concept to draw attention to the importance of transfers of materials between organisms and their environment. Applied to cancer, this would mean that organs and tissues are seen as ecosystems in homoeostasis that tumor cells disrupt. However, this is not the meaning implied by Folkman when he speaks about tumors as integrated ecosystems. In his definition, tumors create

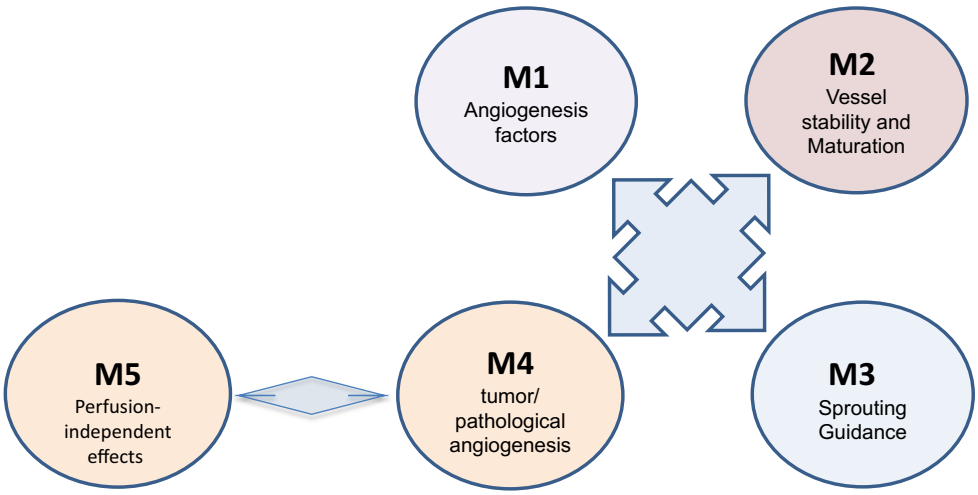

Fig. 3 Five conceptual categories (modules) in vascular development. One can envision four modules with interactions between them. Module 5 seems only indirectly conceptually connected

their own ecosystem by reeducating the host and establishing privileged interactions with components of the microenvironment among which, in Folkman's mind, the vasculature was playing the major role. This means that the tumor ecosystem disturbs the body's own homeostatic ecosystem by redirecting promoting signals to the tumor and by blocking inhibitory input from the body's ecosystem.

Angiogenic factors have extravascular properties and vice versa

The history of VEGF's discovery is a striking example in this context. First, considered as a tumor angiogenesis factor, VEGF conquered developmental biology, cardiology, ophthalmology, neurology and neuroscience to cite only these examples.

A number of molecules and receptors that were largely studied in other fields of the medical and biological sciences have been found to play crucial roles in the vasculature. As such, neuropilins, ephrins or netrins that play an important role in the development of the nervous system were discovered to exhibit important functions in the vasculature [29, 38]. These factors are not only molecules for axonal guidance but are also instrumental for vessel guidance. Indeed, both vascular and nervous systems have an afferent and efferent system (arteries/veins, motor pathways/sensory pathways) and use similar molecular guidance systems. In the vascular system, there is the "tip" cell and in the nervous system the growth cone (see section below). Furthermore, both systems seem interconnected at two levels. Vessels attract peripheral nerves, and nerves on the other hand attract vessels. In the central nervous system, a strong interconnection is found at the level of the blood–brain barrier [39]. Hinman and Davidson proposed the term "Kernel" to designate a molecular regulatory circuit that is similar in different species but that comes in various flavors [40]. It may be not only the result of mutations or gene duplication, but it may be composed of gene products, which have nothing to do with each other on a structural level. Moreover, this "Kernel" can function by transposition of a regulator assembly in another cell and tissue context. What is important in the "Kernel" is that its interrelations of the various molecular components are preserved.

In this context, we could eventually introduce the concept of micro- and macro-Kernel. A micro-Kernel could be the implementation of a specific function (such as the transposition FGF–Notch–Delta in the tracheal system to the VEGF–Notch–Delta in the endothelium), while the macro-Kernel could be a set of nodes, which converge in a common functional purpose.

Endothelial cell-derived factors have perfusion-independent effects on organs

Another example of properties not related to vascular morphogenesis is factors released from endothelial cells for tissue and organ development, which represents a vessel–tissue relationship that is not dependent on the oxygenating/nourishing function of the vasculature. Indeed, inductive signals for tissue morphogenesis derived from the vasculature have been described for organ development such as the dependency of the patterning airway branching on the proximity to the vasculature with an effect on stereotype branching which is dramatically disturbed following vascular ablation [41]. A paradoxical relationship with non-vascular tissue exists such as with the pancreas where, surprisingly, non-nutritional signals from blood vessels act to restrain pancreas growth [42]. VEGF-induced hypervascularization decreases pancreas size. Thus, the vasculature has a positive and negative perfusion-independent action on organ development. There is also a relationship between blood vessel development and Langerhans Islet formation [43, 44]. VEGF seems not to be required for the development of all pancreatic islet capillaries. Furthermore, it has been shown that signals from the endothelium induce pancreatic islet formation and that, in a second step, pancreatic islet cells signal back to the endothelium via VEGF for the maintenance of the islet microcirculation.

Thus, the endothelium provides angiocrine signals that are important for an array of functions including tissue specification, patterning, organ regeneration and maintenance of cellular functions [45]. Indeed, disruption of angiocrine signals from the endothelium impairs stem cell function and organ regeneration. In the brain, these angiocrine signals target neural stem cells and include neurotrophin-3 (NT-3), ephrin B2 and Jagged 1, which maintain them in an undifferentiated state. Other angiocrine factors exhibit stimulatory activities and include BDNF, PEDF, betacellulin placental growth factor-2 (PlGF-2) and VEGF-C, which activates NSCs into transit amplifying cells and neuroblasts. Regenerative processes involving angiocrine signaling are also dependent on other stem cell types such as hematopoietic or spermatogonial stem cells.

Endothelium-derived signaling not only participates in developmental processes but also has a role in pathology. An example is morphogenic factors derived from the endothelium which directly impact disease. An example is hypertension of the pulmonary artery (PAHT) where deficiency of apelin in pulmonary endothelial cells induced an increase in FGF2 via miR-424 and miR-503 release to stimulate smooth muscle cell multiplication and vessel thickness [46]. Elabela/toddler, which functions as a natural APJ receptor agonist, has been shown to be

downregulated in PAHT [47]. Elabela is detected in endothelial cells and may play a similar role as apelin in the EC–SMC interaction. In addition, other signaling molecules for idiopathic PAHT have been described which include the Wnt/planar polarity cell pathway [48]. In addition, it has been shown that endothelial cells are able to signal to cancer stem cells in glioma and that apelin and APJ receptors are implicated (Gavard et al., personal communication). Another EC–glioma interaction has also been described involving EC-specific Pfn-1 phosphorylation, which is associated with tumor aggressiveness in human glioma [49]. Furthermore, FGF4 produced by B cell lymphoma cells activates FGFR1 and upregulates the Notch ligand Jagged 1 on neighboring ECs. EC Jagged 1 feeds back to tumor cells and induces then Notch2–Hey1 [36].

These results illustrate that the nutrient-dependent function of the vasculature has been enriched with an educational role by the vasculature of the surrounding tissue in providing instructive signals.

Discovery of tip cells and guidance factors

The discovery of the tip cells that guide the vasculature stems from neurobiology in analogy to axonal growth. In 1890, Ramon y Cajal described a structure he named the "axonal growth cone" at the extremity of an axon [50]. Axonal growth cones have filopodia and lamellipodia protrusions, which are important for sensing guidance cues. In analogy, this concept has been transposed to blood capillaries in the following way. Sprouting blood vessels are composed of cells, which, like growth cones, lead the vascular tube by sensing environmental cues. These cells have been named "endothelial tip cells" [51].

Both growth cones and tip cells exhibit cellular structures that direct cell movement and are linked to the actin cytoskeleton machinery. The difference between axon growth cones and tip cells resides in the fact that tip cells are cellular structures that are located atop of a growing nascent vessel and thus guide a multicellular unit composed of the tip cell and stalk cells which follow the tip cell. Growth cones, in contrast, represent specific structures at the end of axons. However, both sense the microenvironment for guidance cues to move forward.

Recently, an intriguing observation has been made that two endothelial cells constitute the tip of a nascent blood vessel [52]. Both cells extent filopodia and may also be involved in lumen formation through cord hollowing.

Tip cells are particular cells that exhibit specific morphological, phenotypic and molecular characteristics. Tip cells respond to molecular gradients such as VEGF and VEGFR2 is localized at the filopodia. Furthermore, tip cells and stalk cells are integrated into a molecular circuit that involves VEGFR2, Notch, Delta 4 and Plexin D1 [53]. Indeed, VEGF activates VEGFR2 in tip cells, which in turn increase DLL4 and Plexin D1. DLL4 then interacts then with Notch1 on stalk cells, which decreases VEGFR2 and thus exerts an inhibitory signal. Additional signaling mechanisms and guidance cues have been identified such as endothelial cell-derived sema3A, which exerts a repellent function on tip cell filopodia [54, 55]. Finally, tip cells have specific metabolic regulation, where glycolysis through PFK3B is significantly increased at the level of tip cells in the filopodia [56]. In tumors, PFKFB3 upregulation leads to a more activated endothelium and a dysfunctional vasculature, which can be normalized by PFKFB3 blockade.

More recently, it has been shown through mathematical modeling and live imaging approaches that the rate of tip cell selection determines the length of linear sprout extension [57]. Thus, tip cells not only determine the direction of the nascent sprout but are also critical for sprout extension.

This indicates that the tip cell concept imported into vascular biology from neurobiology in analogy to neuronal growth cones has constituted a fertile conceptual framework, which has led to a significant body of research that has enriched our knowledge of the vascular morphogenic process and may have significance for pathology as well.

Technological advances and impact on vascular biology

One can identify different technological leaps that have accompanied the development of research in the vascular biology and angiogenesis field (Table 2). There are many technological leaps such as, in the early days, injection of specific dyes to visualize the vasculature by John Hunter [58] for the collateral circulation or the visualization of the capillary structure by Theodore Schwann [8]. Another, technology used in the early twentieth century was X-ray imaging that helps to visualize tumor vascularization in humans after bismuth oil administration (Goldman 1907).

One of the most important technological advances, in my opinion, is heparin-Sepharose chromatography [59]. Many morphogens have a strong affinity for heparin, a glycosaminoglycan (GAG) that is found on the membrane of cells. Heparin has been covalently linked to Sepharose beads, and this has allowed the purification of GAG proteins. The interesting feature of this chromatographic method is that it allows the elution of a variety of factors using different ionic strengths. Fibroblast growth factors have a very high heparin-binding capacity, whereas VEGF has much lower affinity for heparin. Ferrara and collaborators used this method to purify VEGF which started by the observation that a mitogenic activity was eluted from

Table 2 Technological leaps in angiogenesis research

Technological leap	References	Consequences
Injection of dyes, etc.	Hunter (1794) [77]	Visualization of in vivo angiogenesis
Microscopy	Schwann (1845,1847) [8], His (1865) [9]	Visualization of vessel structure
X-ray imaging	Goldmann (1907/1908) [13]	In vivo visualization of tumor vessels after injection of bismuth oil
Improvement of biochemical techniques (heparin-Sepharose chromatography)	Singh and Klagsbrun (1984) [59]	Purification of angiogenesis factors
Culture of vascular cells in vitro	Jaffe (1973) [60], Gimbrone (1973) [61], Buzney and Robison (1975) [97], Campbell, Chameley-Campbell (1971,1979) [62, 63]	Study of endothelial cell or vascular smooth muscle/pericyte phenotypes in vitro elucidation of signaling mechanisms
Vascular-specific animal models and gene deletions of vascular genes	Carmeliet (1996) [31], Ferrara (1996) [32]	Validation of an in vivo role of angiogenesis regulators, receptors, etc.
Molecular biology	Ferrara (1989) [30], Ferrara and Williams (1992) [98]	Cloning of angiogenic factors and receptors, microanalysis of tissues at the gene level, etc.
Advanced imaging techniques (intravital imaging, two-photon)	Jain (1987, 1992) [99, 100], Gerhardt (2010) [101]	Visualization of fine morphogenic events (dynamically)
Functional MRI	Ogawa [102]	Measurement of changes in oxygen levels functional parameter of tissue activity
2-DFG-PET	Wolff, Fowler and Kuhl [103]	Measurement of glucose metabolism in tissues

the heparin-Sepharose column at a much lower ionic strength than FGF [25]. This led ultimately to the purification and identification of VEGF. It is noteworthy to mention that Dvorak and collaborators [26] also used heparin-Sepharose chromatography to purify VPF which ultimately turned out to be identical to VEGF. Had this technology not been invented, it would have significantly delayed the discovery of vascular stimulating factors and slowed down the research of the entire field.

Additional important advances have been made by successfully isolating endothelial cells and other vascular cells from various sources and by successfully culturing them in vitro [60–63]. This has allowed the investigation of cell phenotypes and behavior as well as cell signaling. Other not less important discoveries including vascular-specific animal models that allowed specific deletions of vascular genes as well as molecular biology techniques that led to the cloning of vascular morphogens and receptors as well as microanalysis of tissues at the gene level. Furthermore, recent microscopic techniques using two/multiphoton live imaging were instrumental in analyzing precise vascular morphogenic processes dynamically such as tip and stalk cell organization in the nascent vascular tube [64].

Evolutionary considerations and principles

The vasculature comes in different flavors in the animal kingdom. In invertebrates, open and closed vascular systems are encountered, whereas in vertebrates only closed circulatory systems exist. In invertebrates such as drosophila, the oxygenation system is constituted by the tracheal system, which is separated from the vascular system ("dual" mode). A comparison of the vascular system of Drosophila and vertebrates provides valuable insights into how the vasculature is organized. In Drosophila, there are coelomic cavities segmented by the dorsal parts of the mesoderm. The vascular system consists of a central contractile vessel ("heart") that receives hemolymph in the anterior part to be ejected at the posterior part. The wall of the vessel consists of mesothelial cells (also called myoepithelial cells because of their contractile ability) and matrix ("basal"). It should be noted that the matrix is located in the vessel lumen and is therefore exposed to the blood.

In vertebrates, the morpho-functional situation is quite different. Firstly, the system is closed and allows the recirculation of blood through the venous and arterial systems. Secondly, an endothelium covers the internal surface of the vessel and is therefore in contact with the blood. In a pathological situation, the absence of the endothelium (due to damage caused by atherosclerosis, for example) is the initiator of adhesion and activation of platelets. In Drosophila, there is nothing like that. In insects there are, most likely, circulating anticoagulant factors that prevent coagulation and maintain blood fluidity. Thirdly, in vertebrates there is convergence of the functions of nutrition and respiration (oxygen). In Drosophila, the oxygenation system is constituted by the tracheal system, which is separated from the vascular system ("dual"

mode). It should be noted that the hypoxia-inducible system in Drosophila, which involves HIFs, is located in the tracheal system and regulates fibroblast growth factors (FGFs). In vertebrates, the HIF system is located in blood vessels and surrounding tissue and is dependent on VEGFs. Altogether, these morpho-functional differences between invertebrates/insects and vertebrates show that during evolution a "shift" has occurred with regard to this function from the tracheal to the vascular system. As described previously, the term "Kernel" was proposed to designate control modules found in various biological systems, which exhibit different components albeit having the same relationships between each other.

Further insights can be gained from the study of *Botryllus schlosseri*, a marine invertebrate. In *Botryllus*, the endothelium is absent, but *Botryllus* has the ability to form external vascular tubular structures composed of epithelial cells [65]. Surprisingly, these vascular structures express a homologue of VEGF receptor.

This raises the question what the origin of the endothelium at an evolutionary level might be. Theoretically, cell types, hemocytes and epithelial cells could claim this role. Hemocytes are equipped with a homologue of VEGF receptor and are stimulated by VEGF counterparts [65, 66]. This suggests that hemocytes have acquired the ability to become/function as endothelial cells during evolution. This process must be accompanied by acquiring genetic elements of hypoxic regulation. We can infer from these observations that the function of the VEGF system was initially only aimed at controlling the movement of blood cells to the tissues. This function was hijacked during evolution to acquire a new morphogenic and structural role. It was no longer sufficient to convey the blood cells to tissues by an exclusive action on blood cells, but there was also the need to form the channels to achieve this effectively. When put into context, epithelial cells of *Botryllus schlosseri* have gradually lost their dependence on VEGF, the role taken over by endothelial cells probably derived from hemocytes, which already have a dependency on VEGF for inducing cell migration.

The alternative explanation is that the endothelium is derived from mesothelial or myoepithelial cells by transdifferentiation. This is not completely excluded since, as mentioned before, they may express receptors for vascular growth factors. However, this explanation seems unlikely because such functional shift would imply a mesenchymal–endothelial transition. Furthermore, myoepithelial cells have a contractile capacity, which makes them cousins of pericytes and smooth muscle cells.

Do invertebrates have angiogenesis strictly speaking? In vertebrates, angiogenesis is related primarily to the endothelial cells. They are the ones that initiate the cascade of events leading to the formation of the vascular tube. In invertebrates, these cells are absent. However, as we have seen, VEGF receptors are present in some invertebrates and this implies that VEGF is implicated in the formation of tubular structures. As already discussed, *Botryllus* have an internal and external circulatory system where epithelial morphogenesis is controlled by VEGF. However, the regulation of VEGF expression appears to be independent of hypoxia. Munoz and colleagues proposed the term "non-endothelial" angiogenesis for invertebrates [67]. However, more appropriate seems the formation of vessels in invertebrates as "vascular tubulogenesis" to specifically differentiate this phenomenon from "angiogenesis" which is associated with the endothelial organizing principle.

In summary, two important leaps occurred for the vascular tree during evolution: (1) a passage from the organizing principle "epithelium/myoepithelium" to "endothelium" and (2) a recontextualization of regulatory mechanisms with the integration of the hypoxia control system into the endothelium and the surrounding tissue.

Related to this discussion is the concept of vascular mimicry which may represent an ancient form of tube formation. It has been proposed that tumors, in some cases, produce vascular channels devoid of endothelial cells and only lined by tumor cells [68]. This concept was, however, not universally accepted [69].

Scientific methodology in vascular biology

The reductionist approach in the life sciences has produced spectacular results for the knowledge of living systems and led to the development of treatments that have entered routine clinical practice.

Philosophers have extensively thought of how knowledge is acquired during the scientific enterprise and different theoretical frameworks have been formulated, which include inductive and deductive elements in varying proportions. These are inductive inference, deductive inference from hypothesis, the Bayesian approach, error statistical approach and by Inference to the Best Explanation (for details on this topic, see the excellent article by Marcel Weber (http://plato.stanford.edu/entries/biology-experiment/with an extensive bibliography). These principles are of course general to experimental biology, but the reader may find it useful to discuss them in the present context.

In vascular biology, the principal method is inductive inference. Inductive inference obeys the following criteria: (1) simultaneous moment of occurrence of two events (if at any time an event takes place and at another time it does not and has all the conditions in common except one that exists only in the first event, the condition that is different between these two events is the cause or a necessary part of the cause of the observed phenomenon); (2) the two

compared events must be uniform and agent/causal mechanism, and the induced response need only be present in one but not the other situation; (3) other causes that can induce the response should not be present at the time of the experiment. Inductive inference is a key approach in vascular biology and angiogenesis research. It extrapolates from temporal occurrences a causal connection between the molecular alterations and the observed phenotype.

The following examples for inductive inference in vascular biology can be cited: discovery of VEGF and other vascular morphogens, stimulatory or inhibitory effects of factors that modify cellular phenotype or signaling, identification of vascular inhibitory factors, loss-of-function or gain-of-function experiments for vascular morphogens, receptors or signaling molecules, etc.

In biology, there is not, strictly speaking, a similar theoretical framework as in physics, but there is a set of observations/assertions from which hypotheses can be deduced. As such, deductive inference is present in vascular biology, although this is not the "hypothetico-deductivism" which operates in other branches of science. An example of deductive inference is the angiocrine role of the vasculature. This is derived from the hypothesis that, besides the role in tissue oxygenation and nutrition, the vasculature and specifically the endothelium has a supporting role in normal and malignant tissue by producing growth factors, cytokines or other cell-bound or soluble mediators. Another example is guidance (and guidance molecules), a concept which is derived from neurobiology with the formulation of the hypothesis that guidance also represents an important mechanism for directional growth of the nascent vascular tube.

It is noteworthy that inductive and deductive inference is closely linked and may flip back and forth. One can for instance start with a hypothesis and test the hypothesis by deductive inference which may be followed by inductive inference which can lead to new hypothesis that are again experimentally tested and so on.

The Bayesian approach is a statistical approach that assigns a probability to an event related to another condition. The Bayesian approach, back in fashion, found some interest in biology and in particular the analysis of genomic data, which is obviously important in angiogenesis research.

Another approach is the error statistical approach. In this case, there is no probability assigned to hypotheses but the likelihood with which a hypothesis will be tested is evaluated. In this case, the chance is evaluated that a negative hypothesis ("null hypothesis") will pass the test. One can only suspect that this approach was inspired by Karl Popper and his falsification criterion [70]. In this case, a theory seems more solid if it has a significant risk of being falsified but if after evaluation it is not. This has very general meaning and applies to all areas of vascular biology, but we can cite one example. The hypothesis that angiogenesis factors were specific for the vasculature was a hypothesis that had a high chance of being falsified, but it was not for a time being because cell biology and genetics did not invalidate the hypothesis and, on the contrary, reinforced it. Thus, for a time, it could be regarded as a very solid hypothesis. However, the hypothesis was ultimately refuted since more data were produced that demonstrated the contrary.

Inference to the Best Explanation (also called IBE) is an interesting approach for vascular biology. This approach bridges the gap between inductive inference which is exclusively based on temporary occurrences to establish a causal link and mechanistic explanations that involve concepts such as topology, structure or feedback. How is IBE related to angiogenesis research? One example can be given here. By analyzing the structure of the VEGF promoter, the information derived from its structure identifies potential sites that are responsible for the binding of specific transcription factors, the role of which can then be functionally investigated through inductive or deductive inference.

Summary and concluding remarks

We have identified several areas from the vascular development field that can be subjected to a conceptual analysis (Summary Box 1). These include paradigm shifts, cross-fertilization of domains, evolutionary biology issues, technological development and the impact on discovery and knowledge building, and methods for scientific discovery in the vascular biology field. Paradigm shifts, as discussed, take place on a smaller (micro-paradigms) or larger scale (macro-paradigms). It is noteworthy that "false" paradigms can have a positive effect on the discovery path of a given scientific domain such as the assumed specificity of vascular growth factors. One interesting aspect is how vascular development in tumors was viewed during history: first a nourishing tissue, then a host defense mechanism and finally a tumor promoter. Vascular biology has been cross-fertilized by other domains such as cancer biology or neurobiology. Cancer research has allowed the discovery of vascular trophic factors, while neurobiology has introduced concepts such as guidance and tip cells into vascular biology. Thus, concept transposition such as seen for the notion of tip cells and guidance has been/is a valuable device to fuel research in vascular biology and the angiogenesis field. Another important aspect is technology development and methods that are available at a given time, and how they impact on the formulation of concepts and theories. As shown, a simple

Summary Box 1 Conceptual issues

Paradigm shifts occur at a micro- and macroscale in vascular development research

False paradigms may be useful in the discovery path and may lead to progress (i.e., VEGF specificity)

Historically, concepts undergo transformations which may lead to opposing/conflicting views (i.e., the significance of tumor angiogenesis) with possible later resolution

Cross-fertilization from other fields occurs. This occurs at two levels, at a morphological level (i.e., the concept of tip cells and guidance from neurobiology) and at a molecular level (i.e., Netrin as a vascular guidance factor)

Technology development of simple methods may have a major impact on the development of the entire field (i.e., heparin-Sepharose chromatography)

During evolution, the role of the various components of the vessel wall underwent modifications and the organizing principles were changed (i.e., myoepithelium → endothelium)

Micro- and macro-kernels can be defined. A micro-Kernel is related to the implementation of a specific function (i.e., transposition FGF–Notch–Delta in the tracheal system to the VEGF–Notch–Delta in the endothelium), while the macro-Kernel is related to set of nodes, which converge in a common functional purpose

Analysis of scientific methodology still shows a preponderance of inductive inference, even if deductive inference or methods such as Inference to the Best Explanation (IBE) are also in use. Inferences may flip back and forth (i.e., deductive ↔ inductive inference)

Future developments of the angiogenesis field include several aspects:

- Fundamental aspects related to vessels heterogeneity [regional heterogeneity, identification of functionally different vessels such as vessels with anti-tumor activity (HEVs)], construction of larger vessels and interactions with immune cells
- Survival of tumor angiogenesis research at a clinical and translational level depends on the connections that can be made with other field such as tumor immunology

biochemical method such as heparin-Sepharose chromatography has led to the discovery of vascular development factors confirming the anticipated "belief" of the existence of such trophic factors. This has then further spurred not only the vascular biology field but also many other fields such as tumor biology and oncology. Finally, analysis of scientific methodology still shows a preponderance of inductive inference, even if deductive inference or methods such as Inference to the Best Explanation (IBE) are also in use.

An important question is now where the field is heading and what will be the landscape in the next 20 years. I will mention a few examples that, to me, seem important. At present, one important direction that is taken is the study of relation of the vascular system with immunology. The idea of Ernest Goldmann at the beginning of the last century has gained recently attention and, in this respect, recent work by two laboratories stresses the importance of HEVs to trigger anti-tumor immunity [16, 18]. It is important to stimulate HEVs in tumor cells in order to optimize immunotherapy. A step into this direction has been taken by demonstrating the combination of anti-angiogenic and immunotherapy using anti-PDL1 is able to stimulate the number of these vessels in tumors. This effort in this direction must be enhanced.

Another area of investigation is the how larger vessels are construed during development. Where do the endothelial cells that are incorporated into larger vessels come from? Is there retrograde trafficking of endothelial cells from capillaries and smaller vessels?

Important questions are also related to the molecular heterogeneity of vascular cells during development and pathology. Are expression profiles (such as obtained by RNAseq) of endothelial cells in one territory, heterogenous? What is the situation in pathology?

Computation biology has been used by a number of investigators to model vessel formation [71]. However, there is no convincing understanding unless a multilayer approach is yet developed with the integration of morphological and molecular data. This should be tackled in the future and the inclusion of new molecular data as indicated above will help to push into this direction. A step into this direction has been taken by the development of an open library for spatial modeling of vascularized tissues (the microvessel Chaste) [72].

On the translational side, anti-angiogenesis therapy in cancer has deceived clinicians but, at the same time, was very successful in ophthalmology. Will anti-angiogenesis therapy in cancer still be used or abandoned in the near future? Industry is not investing additional efforts in the development of anti-angiogenic compounds in cancer. It is mandatory that new venues are explored, such as mentioned above with immunotherapy, for anti-angiogenesis therapy in cancer to survive.

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