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Review Article

Neuronal clues to vascular guidance

Steven Suchting [a], Roy Bicknell [b], Anne Eichmann [a,*]

[a] Institut National de la Santé et de la Recherche Médicale U36, Collège de France, 11 Place Marcelin Berthelot, 75005 Paris, France
[b] Molecular Angiogenesis Laboratory, Cancer Research UK, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX39DS, UK

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Abstract

The development of the vertebrate vascular system into a highly ordered and stereotyped network requires precise control over the branching and growth of new vessels. Recent research has highlighted the important role of genetic programs in regulating vascular patterning and in particular has established a crucial role for families of molecules previously described in controlling neuronal guidance. Like neurons, new vessels are guided along the correct path by integrating attractive and repulsive cues from the external environment. This is achieved by specialised endothelial cells at the leading tip of vessel sprouts which express receptor proteins that couple extracellular guidance signals with the cytoskeletal changes necessary to alter cell direction. Here, we review the genetic and in vitro evidence implicating four families of ligand–receptor signalling systems common to both neuronal and vessel guidance: the Ephrins and Eph receptors; Semaphorins, Neuropilins and Plexin receptors; Netrin and Unc5 receptors; and Slits and Robo receptors.

Keywords: Vessel guidance; Ephrin; Eph; Semaphorin; Neuropilin; Plexin; Netrin; Unc5; Robo; Slit

Contents

The formation of the vascular system

The vertebrate vascular system is comprised of a highly organised, branching network of arteries, capillaries and veins that penetrates virtually all body tissues, enabling efficient exchange of oxygen and nutrients and removal of waste products. The vasculature forms early in embryo development when endothelial cell precursors called angioblasts differentiate and coalesce into solid cords to form the primary vascular plexus, which eventually gives rise to the dorsal aorta and cardinal veins [1]. New vessels subsequently grow from this primitive network to infiltrate

* Corresponding author. Fax: +33 144271691.
E-mail address: anne.eichmann@college-de-france.fr (A. Eichmann).

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avascular areas of the embryo by either sprouting from parent vessels (sprouting angiogenesis) or by intravascular subdivision (non-sprouting angiogenesis). Oxygen tension is a crucial factor driving this new vessel growth, with regions of hypoxia constituting a strongly attractive signal via the induction of pro-angiogenic molecules such as vascular endothelial growth factor (VEGF). New vessels are subject to intensive remodelling, with many smaller vessels coalescing or expanding to form a hierarchical circuit while excess vessels are pruned away. Haemodynamic forces are critical at this point in shaping the final vascular pattern [2,3]. The maturation of the nascent network is completed through the recruitment of supporting cells (pericytes in medium-sized vessels, smooth muscle cells in larger vessels) and deposition of a new basement membrane.

A striking feature of the vascular system is that its hierarchical branching pattern is highly stereotyped both within and across species, with major and secondary branches forming at precisely designated sites and with organ-specific vascular patterns, e.g. in skin or skeletal muscle, being highly conserved. Such characteristics raise obvious questions as to the controlling factors that guide vascular patterning. Global mechanisms such as haemodynamic forces and oxygen tension, as noted above, have long been recognised as crucial to shaping the vasculature during development. However, in the past decade, substantial evidence has emerged that genetic programs are also fundamental for correct vascular morphogenesis and that families of molecules first described in neuronal patterning also play significant roles in the developing vascular system.

Developmental relationship between nerves and vessels

The vascular and nervous systems share several anatomical and functional parallels. Both systems utilise a complex branching network of nerve cells or blood vessels to penetrate all regions of the body and provide a bidirectional flow of information (sensory and motor neurons; arterial and venous blood flow). The two networks are often patterned similarly in peripheral tissues, with nerve fibres and blood vessels following parallel routes, indicative of shared developmental links between the systems [4]. In terms of behaviour, neuronal and endothelial cell precursors follow similar routes of migration during embryogenesis and respond to similar mesenchymal cues [5]. Genetic studies, meanwhile, have illuminated how both systems regulate each other's development, for example, through the release of neurotrophic factors such as artemin and neurotrophin 3 by vessels [6,7] and pro-angiogenic factors such as VEGF by nerves [8].

The establishment of a precisely wired network requires that correct connections are formed between developing nerves or vessels and is achieved through an ordered series of guidance decisions. Neurons project their axons over very large distances to reach distal targets in a highly ordered and stereotyped manner. Axons must navigate through a complex environment to reach their appropriate targets and do so by continually reassessing their spatial environment to select the correct pathway. This process is dependant upon the growth cone, a sensory structure present at the leading tip of extending axons, which integrates directional information provided by attractive and repulsive guidance cues in the surrounding environment [9]. Recent work has demonstrated that nascent capillary sprouts are likewise guided by specialised endothelial cells called tip cells, which perform an analogous function to growth cones [10,11]. Tip cells, like growth cones, continually extend and retract numerous filopodia to explore their environment and function to define the direction in which the new vascular sprout grows. Whereas tip cells proliferate only minimally, other endothelial cells further down the sprout, called stalk cells, divide to extend the new vessel along the path chosen by the tip cell [11].

The behavioural similarities between the axonal growth cone and endothelial tip cell are complemented by the fact that both systems use a common repertoire of molecular 'sensors', i.e. ligand/receptor signalling systems, which couple the guidance cues detected in the external environment with the cytoskeletal changes necessary to alter cell direction. Four families of classical 'neuronal' guidance cues have so far been identified as regulators of vascular development: the Ephrins, Semaphorins, Slits and Netrins. These guidance cues act to either attract or repel the tip cell, and different cues act over either short or long ranges, depending on whether they are diffusible or cell- or matrix-associated. Furthermore, for a vessel to respond correctly, these cues need to be present in appropriate concentration gradients. In axons, a single cue may be attractive or repulsive depending on the activity of second messengers such as cyclic nucleotides, although this has yet to be confirmed in vessels [12]. A common motif in vessel guidance is for growing vessels to be guided through tissue corridors by combinations of attractive cues made by cells along the corridors and repulsive signals expressed in the surrounding tissues. This is strikingly evident during the formation of intersomitic vessels (ISVs), which sprout from the dorsal aorta into the intersomitic space and grow dorsally between the somites and neural tube, eventually elongating and fusing with vessels from adjacent segments to form the dorsolateral anastomotic vessel (Fig. 1A). Vessel guidance in ISVs is dependant upon genetic programs as these vessels form before perfusion and independent of oxygen signalling. Thus, attractive cues urge vessels forward through the intersomitic space, while repulsive cues prevent them from entering the somite. The behaviour of the vessel sprout is dependant upon the repertoire of receptor proteins and downstream signalling molecules that they express at any given time. Signalling initiated by different cues is integrated and transmitted into changes of the cytoskeleton and, hence, tip cell movement. Members of