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# Emerging concept in angiogenesis: specification of arterial and venous endothelial cells

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**Abbreviations:** AV, arteriovenous

## Introduction

Therapeutic angiogenesis is a method to induce new blood vessel formation in order to improve health in certain pathological conditions. The primary experimental focus has been to find and test molecules that induce angiogenesis. The main target of such studies has been vascular endothelial cells, as they are the key cell type that constitutes all blood vessels. Targeting vascular endothelial cells is further justified by the fact that capillary angiogenesis is primarily mediated by the assembly of these cells.

Historically, vascular endothelial cells were considered as uniform cell population. However, recent molecular and genetic studies have shown that they are extremely heterogeneous cell population. In particular, significant advance has been made in our understanding of the molecular basis for the difference between arterial and venous endothelial cells.

In this mini-review, I will reflect on the series of breakthrough discoveries that led to our current knowledge of the regulatory mechanisms underlying the molecular distinction between arterial and venous endothelial cells. I will also highlight several key areas of investigation for future endeavor.

## Historical perspectives

Table 1 summarizes the series of breakthrough discoveries leading up to our current understanding of the molecular basis for the difference between arterial and venous endothelial cells. In 1981, Dewey, Davis and Gimbrone demonstrated that shear stress in circulation has regulatory roles in determining the phenotypes of vascular endothelial cells (Dewey *et al*., 1981). The significance of this discovery, however, was not discussed in relation to arteriovenous (AV) distinctions in this original report. However, I believe that it clearly had an impact on the subsequent discoveries, as shear stress is one of the most significant physiological parameters that distinguishes arteries from veins.

In 1998, Anderson's group made a landmark discovery (Wang *et al*., 1998). While studying the function of ephrin/eph family in the nervous system *in vivo*, they realized that ephrinB2 and ephB4 are specific markers for arterial and venous endothelial cells, respectively. Furthermore, they showed that the boundary between the ephrinB2-positive and ephB4-positive vessels coincides with the border between arteries and veins among capillaries. This study, for the first time, experimentally showed that the AV distinction has a genetic basis. Prior to this study, the AV distinction was solely based on anatomical and physiological parameters.

In 1995, Weinstein and Fishman discovered a zebrafish mutant, *gridlock*, that exhibits specific defects in arterial formation (Weinstein *et al*., 1995). This led to the identification of the first genetic regulatory mechanism underlying the specific formation of arteries. This promise of the gene identification was realized by the same group in 2000 (Zhong *et al*., 2000).

Furthermore, in 2001, the Weinstein and Fishman group discovered that the Notch signaling pathway is regulated by the *gridlock* gene (Lawson *et al*., 2001; Zhong *et al*., 2001). It was found that the Notch signaling pathway is activated as endothelial cells adopt a venous phenotype as a part of developmental default processes. However, when this signaling pathway is inhibited by *gridlock* function, endothelial cells assume the arterial fate.

Most recently, in 2003, three groups have independently discovered that VEGF acts as an inducer of the arterial fate of endothelial cells (Lawson *et al*., 2002; Mukouyama *et al*., 2002; Visconti *et al*., 2002). In the case of zebrafish, it has also been found that the *sonic hedgehog* pathway lies upstream to VEGF function in regulating the arterial fate of endothelial cells during embryogenesis (Lawson *et al*., 2002). In mice, it has been shown that angiopoietins modify the function of VEGF in regulating the acquisition of the arterial fate by endothelial cells (Visconti *et al*., 2002). These findings were surprising, as neither VEGF nor angiopoietins had been suspected of discriminating one endothelial type against the other during angiogenesis. More interestingly, it has also been shown that venous-type endothelial cells can be converted to arterial type by VEGF both *in vitro* and *in vivo*, exemplifying the plasticity of vascular endothelial cell phenotypes (Mukouyama *et al*., 2002; Visconti *et al*., 2002).

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**Table 1** Landmark discoveries that have contributed to the molecular basis for the difference between arterial and venous vascular endothelial cells

| *Year* | *Discovery* | *Reference* |
|---|---|---|
| 1981 | Shear-stress regulation of endothelial phenotype | Dewey, Davis, Gimbrone |
| 1997 | Genetic basis of arteriovenous distinction | Wang & Anderson |
| 1995–2000 | Identification of gridlock zebrafish mutant and its mutated gene | Weinstein & Fishman |
| 2001 | Notch signaling regulation of arterial phenotype | Weinstein group<br>Fishman group |
| 2002 | VEGF is an inducer for the arterial phenotype<br>(*It was also shown that angiopoietins modulate this VEGF activity in mice.) | Anderson group<br>Sato group*<br>Weinstein group |

## Future perspectives

### *How do VEGF and angiopoietins regulate the fate of endothelial cell type?*

As described above, it has been shown that VEGF and angiopoietins modulate the fate of endothelial cell type (i.e. arterial vs venous). However, the downstream signaling pathways that are responsible for this action of VEGF and angiopoietins have not been determined. Interestingly, it has recently been reported that the phospholipase C gamma-1 signaling pathway is involved in the specification of arterial-type endothelial cells by VEGF (Lawson *et al*., 2003). It is now important to determine whether this signaling pathway is also modulated by angiopoietins. I am almost certain that there are other pathways that are responsible for the action of VEGF and angiopoietins in regulating the fate of endothelial cell types.

### *How do other angiogenesis-regulating factors work together with the factors that are involved in specifying the endothelial cell type?*

Currently, the list of factors that control angiogenesis is larger than can memorized. During embryonic development and adult angiogenesis, the endothelial cells that become incorporated into newly formed vessels must acquire either the arterial or venous fate. It is now important to understand how each of the angiogenic and antiangiogenic factors coordinate with the factors involved in the specification of arterial and venous endothelial cells.

### *Are there any organ-specific factors that control the specification of arterial and venous endothelial types?*

It has been known that blood vessels in different organs exhibit very different morphology and physiological functions. Blood vessels in each organ must assume such specialized morphology and physiological function during development, in order to support the unique physiological functions of each organ. Recently, the first clue to this organ-specific angiogenesis was uncovered by the identification of endocrine organ-specific VEGF (LeCouter *et al*., 2001). This unique VEGF specifically induces angiogenesis in endocrine organs. I predict that there is a family of factors that control the specification of arterial and venous endothelial types in each organ in a highly specific fashion.

### *What are the factors that control vessel morphology specific to arteries and veins?*

For many years, it has been known that arteries and veins exhibit very specialized anatomical structures. The molecular basis of the formation of these specialized anatomical structures must be clarified.

### *What are the factors that control physiological functions specific to arteries and veins?*

Arteries and veins exhibit very distinct physiological functions in the circulatory system. Endothelial cells that lie at the innermost surface of these vessels play critical roles in many of these functions. It will be important to determine the molecular basis for these specialized physiological functions by arteries and veins.

## Concluding remark

Basic science in the field of angiogenesis has entered into an exciting era. In the last decade, I observed a tremendous leap in our understanding of angiogenesis at the molecular level. I am confident that this trend will continue. I strongly believe that these future advances in basic science will have an important impact on the advancement of clinical medicine, including the field of therapeutic angiogenesis.

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