Gene Expression Patterns 23-24 (2017) 22–31

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: http://www.elsevier.com/locate/gep

Characterization of arteriovenous identity in the developing neonate mouse retina

Angela M. Crist, Chandler Young, Stryder M. Meadows*

Department of Cell and Molecular Biology, Tulane University, USA

ARTICLE INFO

Article history:
Received 29 June 2016
Received in revised form
14 December 2016
Accepted 31 January 2017
Available online 3 February 2017

Keywords:
Artery
Vein
Differentiation
Retina
Blood vessel
Expression

ABSTRACT

The murine retina has become an ideal model to study blood vessel formation. Blood vessels in the retina undergo various processes, including remodeling and differentiation, to form a stereotypical network that consists of precisely patterned arteries and veins. This model presents a powerful tool for understanding many different aspects of angiogenesis including artery and vein (AV) cell fate acquisition and differentiation. However, characterization of AV differentiation has been largely unexplored in the mouse retinal model. In this study, we describe the expression of previously established AV markers and assess arteriovenous acquisition and identity in the murine neonatal retina. Using *in situ* hybridization and immunofluorescent antibody staining techniques, we analyzed numerous AV differentiation markers such as EphB4-EphrinB2 and members of the Notch pathway. We find that at postnatal day 3 (P3), when blood vessels are beginning to populate the retina, AV identity is not immediately established. However, by P5 expression of many molecular identifiers of arteries and veins become restricted to their respective vessel types. This molecular distinction is more obvious at P7 and remains unchanged through P9. Overall, these studies indicate that, similar to the embryo, acquisition of AV identity occurs in a step-wise process and is largely established by P7 during retina development.

1. Introduction

The formation of the vascular network is a complex multistep process. Numerous molecular and morphological changes are involved in the initial formation of blood vessels (vasculogenesis) and the growth and sprouting of pre-existing vessels (angiogenesis) that are required to establish the cardiovascular system. Adding to the complexity is the overall three-dimensional architecture of this network. For this reason, the murine retina has become an ideal model to study angiogenic vascular development, in part, due to its initial two-dimensional shape. During retinal vascular formation, underlying blood vessels sprout through the optical nerve head at the center and migrate on top of the retina towards the periphery. The result is a highly organized vascular network that can be easily imaged in a flat mount (Fruttiger, 2007). Thus, this simple 2D structure allows researchers to readily observe the many remodeling processes involved in shaping and patterning the vasculature. In addition, this model lends itself to understanding vessel type specification and differentiation given the stereotypical organization and distinctive characteristics of arteries, veins and capillaries.

AV specification and differentiation are critical steps in vascular development, as endothelial cells must acquire and maintain their molecular identity in order to form mature blood vessels. Subsequently, various molecular pathways that drive AV specification and differentiation have been identified throughout the years. Initial observations that EphrinB2 and its receptor EphB4 were differentially expressed in arteries and veins, respectively, were some of the first to indicate that AV identity was genetically predetermined (Wang et al., 1998; Adams et al., 1999). The Notch signaling pathway has been shown to play a predominant role in arterial specification and patterning in the developing vascular network (Duarte et al., 2004; Krebs et al, 2000, 2004; Lawson et al., 2001; Fischer et al., 2004). In fact, arteries are typically identified by Notch signaling components such as the Notch1, 3, 4 receptors, Delta-like ligand 4 (Dll4) and Jagged1 (Jag1) ligands and the basic helix-loop-helix (bHLH) transcription factors Hey1 and Hey2 (Villa et al., 2001; Shutter et al., 2000; Leimeister et al., 1999; Nakagawa et al., 1999; Chong et al., 2011). In addition to Notch signaling components, arterial endothelial cells can be identified via expression of Neuropilin1 (Nrp1) (Herzog et al., 2001; Moyon et al.,

* Corresponding author. Department of Cell and Molecular Biology, Tulane University, 2000 Percival Stern Hall, 6400 Freret St, New Orleans, LA 70115, USA.
E-mail address: smeadows@tulane.edu (S.M. Meadows).

http://dx.doi.org/10.1016/j.gep.2017.01.002
1567-133X/

2001) and Connexin 40 (Cx40) (Chong et al., 2011; Delorme et al., 1997). Venous endothelial cells can also be identified by Neuropilin 2 (Nrp2) (Herzog et al., 2001), Endomucin (Morgan et al., 1999), vascular endothelial growth factor receptor 3 (VegfR3/Flt4) (Chong et al., 2011; Kaipainen et al., 1995) and the Apelin receptor, Apj (Saint-Geniez et al., 2002, 2003).

Conversely, less is understood about acquisition of vein identity, although evidence indicates that inhibition of the Notch pathway during embryonic and neonate development leads to a venous fate (Quillien et al., 2014; Nielsen et al., 2014; You et al., 2005). For instance, the venous expressed transcription factor, chicken ovalbumin upstream promoter-transcription factor (CoupTFII), suppresses arterial differentiation thereby advancing a venous identity (You et al., 2005). Additionally, lineage tracing experiments using a Notch-responsive reporter showed that aortic endothelial cells can down-regulate notch activity and integrate into veins (Quillien et al., 2014). However, recent work in the retina suggests that Notch signaling also actively regulates the patterning of veins and may play a role in venous identity (Ehling et al., 2013).

In many cases, the AV markers used to differentiate and define arteries and veins were initially identified in the embryo, but a number have also been described, to some degree, in the retina, including Apj, Dll4, EphB4, EphrinB2, Flt4, Jag1, Notch1, Notch4, Nrp1, Nrp2, Sox7, Sox17, Sox18 (Saint-Geniez et al., 2002; Hofmann and Luisa Iruela-Arispe, 2007; Tammela et al., 2008; Claxton and Fruttiger, 2004; Corada et al., 2013; Davies et al., 2010; Mahmoud et al., 2010; Zhou et al., 2015; Fantin et al., 2011) and α-smooth muscle actin (αSMA), which identifies smooth muscle cells that predominately surround the neonate arteries (Benjamin et al., 1998). However, to date, acquisition of AV identity has not been comprehensively characterized in the mouse retina.

Due to the wide usage of the retinal model for vascular studies, we aimed to gain insight into how AV cell fates are established as blood vessels migrate from the optic nerve head and mature. In these studies, we categorize the spatiotemporal expression of various AV markers to distinguish when AV identity is established in the vascular network of the murine retina. We find that arteries and veins follow a trend similar to the embryo whereby in early stages, postnatal day 3 (P3), few markers are restricted to a particular blood vessel type. However, at subsequent stages (P5-P7) when arteries and veins are more physically distinct, markers become restricted to their respective vessel type (Fruttiger, 2007; Scott et al., 2010; Hughes and Chang-Ling, 2000; Ishid et al., 2003). Based upon these observations, we conclude that acquisition of AV identity in the mouse retina is underway by P5 and largely defined by P7.

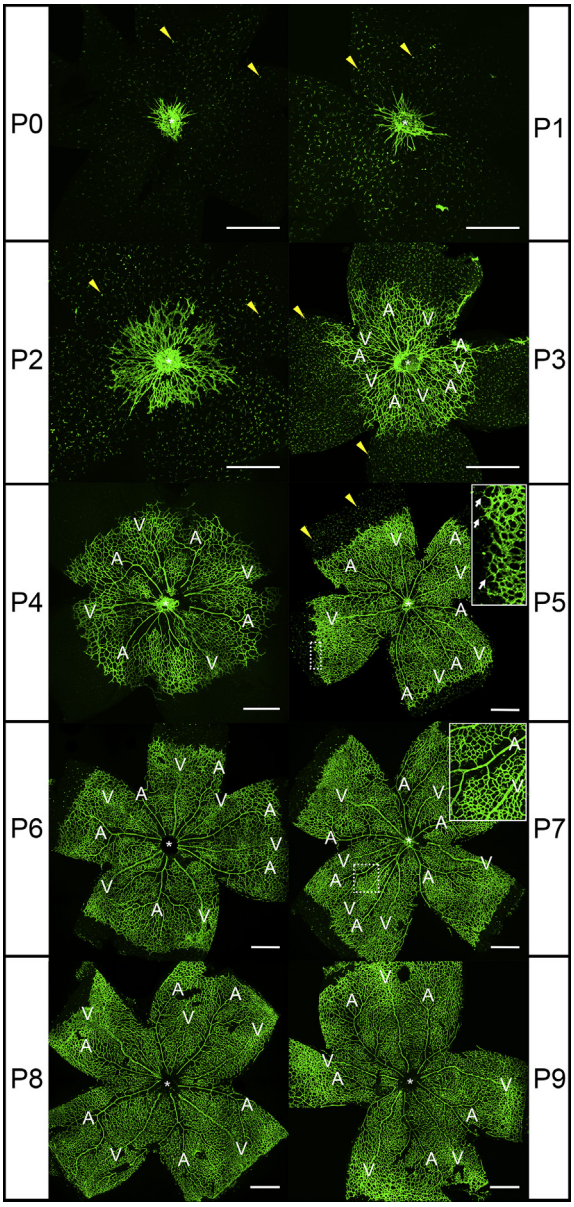

Fig. 1. Formation of the vascular network during mouse neonate retina development. Whole mount confocal images from postnatal (P) day P0-P9 retinas labeled with Isolectin-B4 antibody (green). Isolectin-B4 marks all blood vessels and macrophages (yellow arrowheads). P5 inset shows tip cells (arrows) enlarged from the dotted white box. P7 inset shows a close up of arteries and veins in the dotted white box. Notice a lack of adjacent capillaries next to the artery in comparison to the vein. Artery (A), vein (V) and optic nerve (*) are depicted. Scale bars: 500 μM.

2. Results

2.1. Overview of retinal vascular development

Prior to birth, the mouse retina is completely devoid of a vascular network. However after birth, blood vessels begin to sprout through the centrally located optical nerve and migrate radially to populate the entire retina (Fruttiger, 2007; Stahl et al., 2010). To highlight this process, we labeled P0-P9 mouse retinas with Isolectin-B4, which marks all retinal blood vessels and macrophages (Fig. 1). From postnatal day 1 (P1) to P2, the growing vasculature appears unorganized with no clear distinction between different blood vessel types (Fig. 1). As the network continues to grow from P3 to P9, the vessels become stereotypical in their arrangement and arteries and veins form in alternating patterns with intervening capillary-like vessels connecting them. During this period, tip cells, which are required for growth and extension of the vascular network, can be identified at the leading front extending towards the periphery (Fig. 1, P5 inset). In addition, the blood vessels undergo morphological changes resulting in physical properties that distinguish arteries and veins as shown in Fig. 1, P7 inset (Fruttiger, 2007; Scott et al., 2010; Hughes and Chang-Ling, 2000; Ishid et al., 2003). By P8-P9, the blood vessels have reached the perimeter of the retina, and new vessels sprouting from veins and capillaries near veins begin invading the retina to form additional vascular networks in the deep layer of the retina (Fig. 1) (Fruttiger, 2007; Stahl et al., 2010). After P9 the retinal blood vessels continue to remodel and mature; however for the focus of these studies we have limited our examination to the early neonate retina (P3-P9), at which time arteries and veins develop into