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Pericytes are heterogeneous in their origin within the same tissue

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ABSTRACT

Pericytes heterogeneity is based on their morphology, distribution, and markers. It is well known that pericytes from different organs may have distinct embryonic sources. Yamazaki et al. (2017) using several transgenic mouse model reveal by cell-lineage tracing that pericytes are even more heterogeneous than previously appreciated. This study shows that pericytes from within the same tissue may be heterogeneous in their origin. Remarkably, a subpopulation of embryonic dermal pericytes derives from the hematopoietic lineage, an unexpected source. Reconstructing the lineage of pericytes is central to understanding development, and also for the diagnosis and treatment of diseases in which pericytes play important roles.

1. Introduction

Approximately one hundred years ago, Zimmermann (1923) named a population of contractile cells *pericytes* because they were primarily located around blood vessels. The word *pericyte* derives from the Greek *kytos*, a hollow vessel, appropriately describing a cell surrounding a blood vessel. Back then, these cells were identified mainly by their anatomical location and morphology. Pericytes have long projections that encircle the vessel walls which are widely scattered in all tissues (Hirschi and D'Amore, 1996). They surround endothelial cells and communicate with them along the length of the blood vessels by physical contact and paracrine signaling (Diaz-Flores et al., 1991).

Defining a specific molecular marker for pericytes has been challenging. Until recently, light and electron microscopy were the only techniques able to visualize them, thus limiting the knowledge acquired from those studies. In the last years, with the advent of fluorescent and confocal microscopy, technologies combining anatomical location, expression of surface markers, and genetic lineage tracing enabled the discovery of pericytes' varying, sometimes unexpected, roles in health and disease (Birbrair et al., 2015). It is already known that pericytes stabilize blood vessels and participate in vascular development, maturation, remodeling, architecture, and permeability (Enge et al., 2002; Hellstrom et al., 2001; Leveen et al., 1994; Lindahl et al., 1997; Soriano, 1994). Additionally, they regulate blood flow (Pallone and Silldorff, 2001; Pallone et al., 1998, 2003), and, in the central nervous system, collaborate with astrocytes to maintain the functional integrity of the blood brain barrier (Al Ahmad et al., 2011; Armulik et al., 2010; Bell et al., 2010; Cuevas et al., 1984; Daneman et al., 2010; Dohgu et al., 2005; Kamouchi et al., 2011; Krueger and Bechmann, 2010; Nakagawa et al., 2007; Nakamura et al., 2008; Shimizu et al., 2008). Pericytes also may affect immune function by regulating lymphocyte activation and by phagocytic activity (Balabanov et al., 1999, 1996; Bouchard et al., 1997; Castejon, 2011; Fabry et al., 1993; Fisher, 2009; Hasan and Glees, 1990; Jeynes, 1985; Kim et al., 2006; Thomas, 1999; Tu et al., 2011; Verbeek et al., 1995). Interestingly, strong evidence identified pericytes as stem cells capable to form several other cell types (Birbrair et al., 2017a, 2017b, 2014a, 2013a, 2013b, 2013c, 2013d, 2014b, 2015, 2014c; Birbrair and Delbono, 2015; Brighton et al., 1992; Collett et al., 2003; Crisan et al., 2008; Davidoff et al., 2004; Dellavalle et al., 2011, 2007; Diaz-Flores et al., 1992; Doherty et al., 1998; Dore-Duffy et al., 2006; Farrington-Rock et al., 2004; Feng et al., 2011; Olson and Soriano,

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2011; Richardson et al., 1982; Tang et al., 2008).

Pericytes differ in their embryonic origin between tissues (Armulik et al., 2011; Sims, 1991, 2000). Very little is known about the exact identity of pericyte ancestors within developing tissues, and there is evidence for numerous distinct developmental sources (Armulik et al., 2011). Lineage tracing studies indicate that pericytes in the cephalic region and thymus are of neuroectodermal origin (Foster et al., 2008; Muller et al., 2008; Simon et al., 2012; Trost et al., 2013; Zachariah and Cyster, 2010); while in lung, heart, liver and gut, the mesothelium is the main source of perivascular cells (Armulik et al., 2011; Asahina et al., 2011; Cai et al., 2008; Khan et al., 2016; Mellgren et al., 2008; Que et al., 2008; Zhou et al., 2008). In most other organs, pericytes derive from the mesoderm; specifically, the sclerotomal compartment (Armulik et al., 2011; Asahina et al., 2011; Bergwerff et al., 1998; Etchevers et al., 2001; Korn et al., 2002; Que et al., 2008; Wilm et al., 2005; Winkler et al., 2011; Yamanishi et al., 2012).

Understanding the origin and the processes that drive pericyte formation is a central question in developmental biology. Whether all pericytes from the same tissue have the same ancestry remains unknown. Nevertheless, in a recent article in *Cell Reports*, Yamazaki et al. (2017) showed that a pericyte subpopulation within the embryonic skin derives from an unexpected source. The authors used in vivo lineage-tracing technologies to track specifically neural crest-, endothelial-, and hematopoietic-derived cells. These experiments suggested that during development the sources of tissue pericytes are heterogeneous. Strikingly, some of the pericytes in the embryonic skin and brain had hematopoietic origin (Yamazaki et al., 2017). Furthermore, the authors showed defective pericyte development in a mouse model with a known impairment of the myeloid lineage, suggesting that cells from this lineage contribute to pericyte formation in ectodermal organs (Yamazaki et al., 2017). Additionally, this study unravels an important signal (TGFβ) necessary for hematopoietic progenitors to differentiate into pericytes (Yamazaki et al., 2017). This study brings a new possible ancestor for pericytes, and reopens the discussions about pericytes' heterogeneity. These cells are heterogeneous not only in their morphology, distribution, molecular markers and function, but also in their origin even within the same tissue (Fig. 1).

Pericytes have been anatomically defined by their perivascular location in the blood vessel wall in close contact with endothelial cells (Feng et al., 2011; Sa-Pereira et al., 2012). However, not all perivascular cells are pericytes. Besides smooth muscle cells, other cellular types have been described as perivascular: i.e. adventitial cells (Crisan et al., 2012), fibroblasts (Soderblom et al., 2013), and macrophages (Bechmann et al., 2001; Guillemin and Brew, 2004). Classical electron microscopy studies of pericytes reveal their location under the vascular basal lamina (Allsopp and Gamble, 1979), in contrast to other perivascular cells. None of pericyte markers are specific, since they are also expressed by other cell types; and their expression in pericytes is highly dependent on the developmental stages (Armulik et al., 2011). Thus, pericitic markers used in this study could refer to other cell populations. For instance, PDGFRβ is a known marker of other cell types, such as fibroblasts (Soderblom et al., 2013; Spitzer et al., 2012); while NG2 proteoglycan could be expressed in macrophages (Yotsumoto et al., 2015). Additionally, pericytes that do not express NG2 were also recently described (Stark et al., 2013). Recent studies discovered new molecular markers for pericytes, such as Gli1 (Kramann et al., 2015, 2017) and Tbx18 (Guimaraes-Camboa et al., 2017). Whether the perivascular cells derived from hematopoietic progenitors in the embryonic skin are pericytes still needs to be clarified. The combination of pericyte molecular markers with immunolabeling of the basal lamina in genetic lineage tracing models will confirm the nature of those cells.

Surprisingly, Yamazaki et al. (2017) found that perivascular cells were labeled in Vav-Cre/R26R^{EYFP} mice, but not in Tie2-Cre/R26R^{EYFP} mice. It is known that Tie2 gene is expressed by endothelial cells (Maisonpierre et al., 1997; Schnurch and Risau, 1993). However, hematopoietic cells also express Tie2 (Arai et al., 2004; Takakura et al., 1998). Consistent with this, Tie2-Cre mice display Cre recombinase in both endothelial cells and hematopoietic cells, especially in hematopoietic stem cells (HSCs) (Constien et al., 2001; de Lange et al., 2008; Kisanuki et al., 2001; Tang et al., 2010). During development, both endothelium and definitive HSCs which form all hematopoietic cells, arise from a shared precursor, the hemogenic endothelium (Chen et al., 2009; Hirschi, 2012; Medvinsky and Dzierzak, 1996; Nguyen et al., 2014; Rafii et al., 2016). Due to this, it is virtually impossible to avoid some Cre recombinase activity in hematopoietic cells when using endothelial specific promoters with constitutively active Cre recombinase. Similarly, Vav-Cre strains have been shown to target both hematopoietic and endothelial cells (Croker et al., 2004; de Boer et al., 2003; Georgiades et al., 2002). It will be interesting to explore whether the embryonic hematopoietic cells that originate dermal pericytes derive from a different source than the hemogenic endothelium.

Interestingly, a recent study shows that cardiac endothelial cells give rise to ~20% of pericytes in the murine embryonic heart (Chen et al., 2016). Thus, the developmental sources of pericytes are more heterogeneous than previously appreciated. These surprising findings raise the possibility that distinct subsets of pericytes, depending on their developmental origin, could differentially contribute to different pathological conditions.

Additionally, to examine which specific hematopoietic cells form pericytes, CD11b-Cre/TdTomato mice were analyzed (Yamazaki et al., 2017). Nevertheless, pericytes may express CD11b in culture (Balabanov et al., 1996), as well as after stroke (Ozen et al., 2014). Thus, although pericytes in the skin vasculature are labeled in this genetic tracing mouse model (Yamazaki et al., 2017), whether dermal pericytes express CD11b earlier during development or if they derive from non-pericyte CD11b+ cell populations remains to be elucidated.

Although the authors show that dermal myeloid progenitors differentiate into pericytes in culture (Yamazaki et al., 2017), recent studies have shown that cells' behavior in vitro could be completely different from their functionality in vivo (Guimaraes-Camboa et al., 2017; Snippert and Clevers, 2011; van Berlo et al., 2014). Artificial conditions in the dish which characterize cell culture systems may activate differentiation potential that could be not shared by these same endogenous cells in vivo under physiological conditions (Guimaraes-Camboa et al., 2017; Snippert and Clevers, 2011; van Berlo et al., 2014).

Thus, the plasticity observed in vitro might be simply a consequence of the artificial cell culture microenvironment. Based on this, a recent study has challenged the current view about pericytes' capacity to differentiate into other cell types and reopened the discussion about pericytes' plasticity (Birbrair et al., 2017a; Guimaraes-Camboa et al., 2017).

Furthermore, Yamazaki and colleagues used a transgenic mouse model (PU.1 knockout) in which severe impairment of the myeloid lineage was previously reported (McKercher et al., 1996; Scott et al., 1994). In those mice, F4/80+ macrophages were absent from the skin. Although the vascular network covered by endothelial and smooth muscle cells appeared normal, these vessels had a reduction in pericytes (Yamazaki et al., 2017). Interestingly, the reduction in the number of pericytes was approximately 50%, while the proportion of dermal pericytes derived from the hematopoietic lineage seems to correspond to approximately one fourth of all pericytes in the skin. It will be interesting to explore whether the absence of one pericyte subpopulation may influence the development of other pericitic subtypes in the same tissue but of different origin.

2. Perspectives/future directions

Pericytes development and survival are regulated by several signals coming from other cells, i.e. platelet-derived growth factor-β (PDGF-β)