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A vascular perspective on neuronal migration

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ABSTRACT

During CNS development and adult neurogenesis, immature neurons travel from the germinal zones towards their final destination using cellular substrates for their migration. Classically, radial glia and neuronal axons have been shown to act as physical scaffolds to support neuroblast locomotion in processes known as gliophilic and neurophilic migration, respectively (Hatten, 1999; Marin and Rubenstein, 2003; Rakic, 2003). In adulthood, long distance neuronal migration occurs in a glial-independent manner since radial glia cells differentiate into astrocytes after birth. A series of studies highlight a novel mode of neuronal migration that uses blood vessels as scaffolds, the so-called vasophilic migration. This migration mode allows neuroblast navigation in physiological and also pathological conditions, such as neuronal precursor migration after ischemic stroke or cerebral invasion of glioma tumor cells. Here we review the current knowledge about how vessels pave the path for migrating neurons and how trophic factors derived by glio-vascular structures guide neuronal migration both during physiological as well as pathological processes.

1. Vasophilic migration of neuroblasts

During adult neurogenesis, subventricular zone (SVZ)-derived neuroblasts migrate tangentially towards the olfactory bulb (OB) where they distribute radially from the center to the periphery until reaching their final destination in the outer layers of the OB (Fig. 1). The migration of neuronal precursors along the vessels was first reported by Bovetti et al. in the OB (Bovetti et al., 2007). Gliophilic migration in the adult CNS is not longer possible since radial glia cells undergo transformation to mature astrocytes at perinatal stages (Cameron and Rakic, 1991). Therefore, Bovetti and colleagues investigated alternative anatomical scaffolds by means of time-lapse confocal microscopy in acute brain slices from young adult mice. They noticed that once neuronal precursors reach the OB, a vast proportion of cells radially migrate associated to the abluminal side of the vasculature allowing the distribution of neuroblasts in this brain region. Analysis using electron microscopy indicated that neuronal precursors in fact associate to the vessels by contacting the extracellular matrix and the astrocytic end-feet wrapping the vessels rather than by direct interaction to the endothelial cells. However, in these studies the molecular players involved in such vessel-associated radial migration of neuroblasts remained to be identified.

Before reaching the olfactory bulb the tangential migration of neuroblasts generated in the SVZ occurs along a highly restricted path called rostral migratory stream (RMS) (Lois and Alvarez-Buylla, 1994). Neuronal precursors in the SVZ are organized in a network consisting of neuroblast chains encapsulated by astrocytic tubular structures that converge to form the RMS (Lois et al., 1996). This cell-aligned translocation is called “chain migration” and it is highly dependent on neurophilic interactions. Initially it was speculated that migrating cells follow a myriad of chemoattractive and chemorepellent clues to generate a unidirectional migration towards the OB (Ng et al., 2005; Paratcha et al., 2006). However, it was difficult to explain how the neuroblasts travel such long-distance and follow the complex shaped curvature of the RMS just being orchestrated by chemical signals. Importantly, Saghatelyan's group demonstrated that blood vessels physically support the neuronal chain migration along the RMS (Snapyan et al., 2009) (Fig. 1). They first observed that vessels are organized parallel to the RMS depicting the route of neuronal precursors while in the core of OB vessels distribute radially also topographically outlining the olfactory precursor pathway as indicated above (Bovetti et al., 2007). High resolution confocal microscopy revealed that vessels are ensheathed by astrocytic processes and neuroblasts envelop these processes along the vessels, thus forming a tripartite complex (Fig. 1). Time-lapse video imaging showed that neuronal precursors migrate along the RMS vessels in a saltatory fashion: cell movement is followed by periods of immobility. The contribution of astrocytes to the RMS assembly is crucial since astrocyte-derived VEGF promotes the appearance and development of the vascular scaffold in the RMS (Bozoyan et al., 2012). Interestingly, astrocytic processes align

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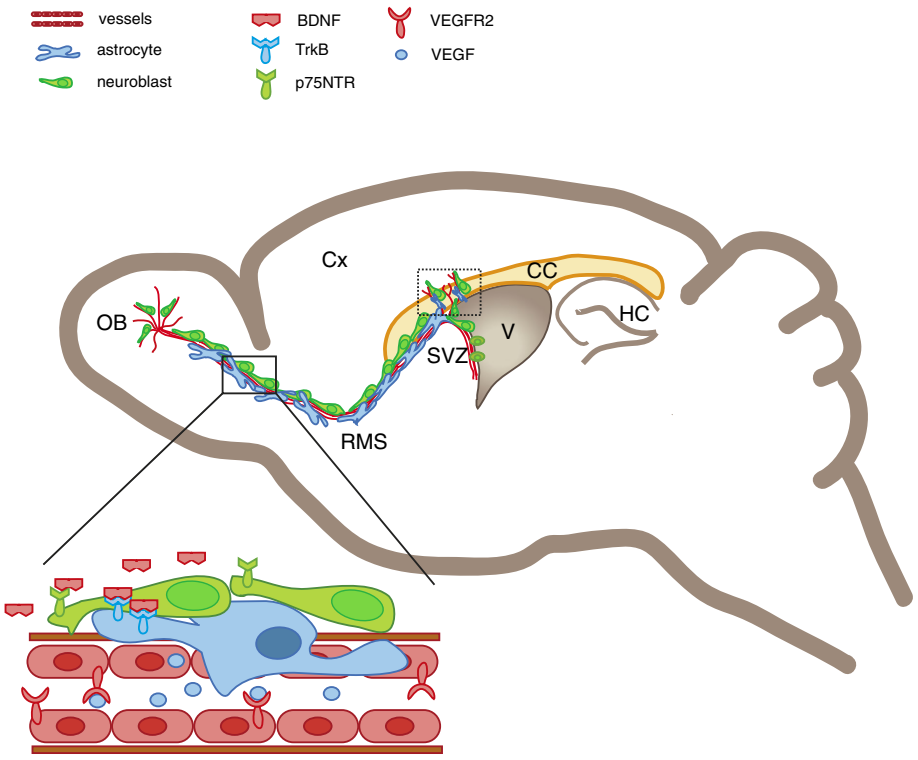

Fig. 1. Vessels pave the path for neuroblast migration. Schematic representation of a sagittal section of the brain depicting the neuronal precursors migratory routes. At perinatal stages, neuroblasts from the SVZ migrate towards the lower layers of the cortex through the corpus callosum along the vasculature (dashed box). In the adult, neuroblasts move tangentially from their germinal niche towards the OB following the RMS route, generated by astrocyte-derived VEGF. This migratory process is supported by tubular structures composed by vessels ensheated by astrocytes that constitute the RMS (detail in solid box). Endothelial cells release BDNF which binds to p75NTR on the migrating neuronal precursors. Furthermore, astrocytes express TrkB, a BDNF high affinity receptor, which regulates the bioavailability of this ligand. When neuroblasts reach the OB they distribute radially towards outer layers also in close association with the vessels. CC = corpus callosum; Cx = cortex; HP = hippocampus; OB = olfactory bulb; RMS = rostral migratory stream; SVZ = subventricular zone; V = ventricule.

in parallel to the vessels of the RMS rather than adopting the common stellate morphology, suggesting that vessel-astrocytic tubular routes support neuronal migration (Whitman et al., 2009). Additionally, Snapyan and colleagues mechanistically defined a molecular pathway that better characterizes the vasophilic migration along the RMS: endothelial cells in the RMS produce brain-derived neurotrophic factor (BDNF) which binds to p75NTR expressed by migrating neuroblasts and promotes their migration (Snapyan et al., 2009). Moreover, astrocytic specific calcium imaging in acute slices indicated that GABA released by neuroblasts induces Ca^{2+}-dependent insertion of TrkB receptors on the plasma membrane of astrocytes. TrkB are high affinity receptors for BDNF, so creating a regulatory system that controls the availability of BDNF. The model proposed by Saghatelyan's group involves vessels promoting neuroblast migration and astrocytes inducing a stationary phase in the motility mode, indicating that the migratory journey towards the OB is a complex regulated process orchestrated by three different cellular components. Supporting this concept, Kaneko et al. demonstrated that migrating neurons dynamically remodel the morphology and organization of astrocytic tubes wrapping the RMS vessels to facilitate directional migration of new neuroblasts in the adult brain towards the OB (Kaneko et al., 2010). It is attractive to speculate that blood vessels could also be actively involved in the dynamic modulation of neuroblast migration towards the OB, however the molecular mechanisms underlying the regulation of vessel–astrocyte guided neuronal migration are still poorly understood. In addition and regardless of the function as migratory scaffold, it has been demonstrated that the RMS has additionally the capacity to generate neuronal precursors independently from the SVZ-restricted neurogenesis (Alonso et al., 2008). Interestingly, it has been recently shown that the newly generated neurons from the RMS are in close association with the vasculature, suggesting that the RMS endothelium has an active role as neurogenic niche besides guiding the neuroblasts towards the OB (Yuan et al., 2015).

Recent evidences indicate that vasophilic migration of neuroblasts might not only be restricted to the adult brain. In neonates, in addition to the tangential neuroblast migration along the RMS, a substantial fraction of neuronal precursors from the SVZ radially migrate through the corpus callosum and incorporate to the lower layers of the cortex (Inta et al., 2008). The group of Hannah Monyer investigated the process of neonatal radial migration and they found that migrating neuroblasts closely associated to blood vessels (Le Magueresse et al., 2012) (Fig. 1). As already described for the process of tangential migration along the RMS in later stages (Bovetti et al., 2007; Snapyan et al., 2009), neuroblasts move along the vessels in a saltatory fashion, combining motility and resting periods, and astrocytic processes tightly wrapping the vessels mediate the physical contact between neuroblasts and the endothelium. The process of radial migration vanishes gradually in rodents 4 weeks after birth, coinciding with a decrease in the vascularization of the corpus callosum and the generation of a glial sheath along the vessels of the RMS (Le Magueresse et al., 2012).

In addition to adult and early postnatal development, the vascular and neuronal systems are in close communication from early embryonic development on. Initially the neural tube (NT) is avascular (James and Mukouyama, 2011) and at mid-gestation (E8.5–E9.5 in mice), VEGF secreted by the NT recruits angioblasts from the mesoderm to form a vascular network around the developing CNS called perineural vascular plexus (PNVP) (Hogan et al., 2004). Vessel sprouts from the PNVP start to invade the NT (at E10.5 in mice) in a highly stereotypical manner. Besides VEGF, Wnt/β-catinin, TGFβ and GPCR124 were identified as regulatory pathways of the CNS vascularization, suggesting that neural-derived cues govern the vascular patterning of the CNS and regulate the establishment of the neurovascular unit (Daneman et al., 2009; Kuhnert et al., 2010; Stenman et al., 2008). Conversely, one could also speculate that this hierarchical vascularization of the CNS may simultaneously support the arrangement of the neuronal populations in the developing

NT beyond providing oxygen and nutrients to the neural tissue. However, this possibility is not yet unveiled. Neural crest cells (NCCs) are a transient multipotent cell population originated from the dorsalmost region of the NT in early development. NCCs extensively migrate throughout the embryo and differentiate into a vast variety of cell lineages, such as neurons and glial cells of the peripheral nervous system, melanocytes, endocrine cells and also skeletal and connective tissue (Huang and Saint-Jeannet, 2004). Interestingly, Saito et al. have recently demonstrated that the dorsal aorta, the first embryonic artery, orchestrate the migration of NCCs and the subsequent lineage segregation into sympathetic ganglia and adrenal medulla *via* vessel-derived bone morphogenetic protein (BMP) signaling (Saito et al., 2012). Thus, this is a relevant first indication of how vessels coordinate the migration and function of neural cells during early development.

In line with a vascular-guided neuronal patterning during embryogenesis, the group of Zoltán Molnár and the group of Arnold R. Kriegstein simultaneously demonstrated that mitotic neuronal precursors are in close proximity to the vessels on the SVZ and the intermediate zone in the embryonic cortex, that neuronal precursors divide adjacent to the vessels and that neurites from differentiated neurons associate to the vessels at the same cortical region (Javaherian and Kriegstein, 2009; Stubbs et al., 2009). In addition to that, Kriegstein's group previously reported that radial glia cells, which are a known scaffold for neuronal migration during corticogenesis (Hatten, 1999; Marin and Rubenstein, 2003; Rakic, 2003), often associate to the vasculature (Noctor et al., 2001). Overall, these observations suggest that blood vessels might be also involved in the proper patterning of neuroblast migration during development of the different layered structures in the brain although the specific contribution of the vasculature remains poorly investigated. Interesting is the fact that we and others have shown that molecular pathways governing vascular morphogenesis (*i.e.* Eph/ephrin, Unc5B, Semaphorin/Neuropilin, Robo/Slit, VEGF/VEGFR2, Notch) have been shown to act directly on neurons and control their migration, suggesting that these pathways could establish a molecular link between vessels and neural cells (Gu et al., 2003; Hashimoto-Torii et al., 2008; Long et al., 2004; Meissirel et al., 2011; Sawamiphak et al., 2010; Seiradake et al., 2014; Senturk et al., 2011).

In conclusion, there are increasing evidences that the endothelium provides a structural scaffold and a chemoattractive route that guide neuroblast migration in physiological conditions. It is crucial to understand the mechanisms underlying the neuronal migration under normal processes in order to decipher the etiology of diseases comprising an abnormal neuronal migration component. Notably, in this respect, the vasculature has an emerging role during pathological conditions that involve neuronal migration in the CNS, such as tissue regeneration after *stroke* or *glioma* invasion. In the following section, we describe the cellular and molecular mechanisms implicated in these two prevalent/lethal pathologies.

2. Neuronal migration in pathological conditions

2.1. Neurovascular microenvironment in ischemic stroke

It is known that tissue suffering from ischemia requires fast establishment of oxygen and energy supply from blood vessels to prevent neuronal cell death. Already in 1994 microscopic studies on clinical samples from patients suffering from ischemic stroke revealed a striking correlation between angiogenic events in the ischemic penumbra region and the recorded survival rate (Krupinski et al., 1994). So far, effective drug treatments for acute ischemic stroke are rare. Remarkably, since the last twenty years, intravenous recombinant tissue plasminogen activator (rtPA) is still the only approved treatment for acute ischemic stroke. Unfortunately, this treatment is only effective if started within 4.5 h from symptoms which leads to many untreated patients due to this narrow time period. This limited pharmacological choice reveals the need to explore the processes of neurovascular repair in order to develop restorative therapies after ischemic stroke (Gomis and Davalos, 2014).

In order to get mechanistic insights rodent animal models were established to mimic a transient or a permanent ischemic attack *via* middle cerebral artery occlusion (MCAO) which is the most frequently damaged artery in stroke patients (Li et al., 2014b; Nih et al., 2012; Thored et al., 2006, 2007). The MCAO method has been widely used to highlight the tight interplay between vascular remodeling and neuronal migration during neurovascular repair after ischemic stroke. Using this model it was shown that ischemia triggers neurogenesis in the SVZ of the brain leading to production of new neurons as a result from increasing numbers of neuronal progenitor cells (NPCs) (Arvidsson et al., 2002; Parent et al., 2002). Live imaging of cultured brain slices after ischemic injury displayed that SVZ-derived NPCs first extended their leading processes to blood vessels and migrated towards them (Kojima et al., 2010; Zhang et al., 2009). In this so-called vasophilic migration mode the neuroblasts closely associated with reactive astrocytes and blood vessels propagating in chains from the SVZ towards the ischemic penumbra (Jin et al., 2003; Kojima et al., 2010; Thored et al., 2006; Yamashita et al., 2006). The majority of the migrating cells exhibit long leading and short trailing processes similar to the observed migration of neuroblasts which travel from the SVZ along the RMS towards the OB (Bovetti et al., 2007; Jin et al., 2003; Snapyan et al., 2009) (see above). In another study, Ohab et al. demonstrated that after stroke clusters of proliferating neuronal cells are localized adjacent to the endothelium proposing the existence of a neurovascular niche. In this neurovascular niche newly-born immature neurons closely associate with the remodeling vasculature, suggesting that neuroblasts preferentially migrate in areas of active vascular remodeling (Ohab et al., 2006).

There are increasing evidences that in physiological conditions microenvironmental trophic factors released by vessels, astrocytes and neuroblasts support the individual cellular compartments at the neurovascular unit. For example, Shen and colleagues have shown that endothelial cells are critical components of the neural stem cell niche by releasing soluble factors that stimulate the self-renewal of neural stem cells, inhibit their differentiation and enhance their neuron production (Shen et al., 2004). In this study the nature of such soluble factors released by endothelial cells remained to be determined. This microenviromental crosstalk between vessels, astrocytes and neuroblasts changes dramatically following ischemic insult. The astrocytes respond to the ischemia and undergo a process called reactive astrogliosis (Sofroniew and Vinters, 2010). Moreover, the ischemic insult causes transient hypoxia that is followed by microvascular growth in the hypoxic penumbra (Marti et al., 2000; Thored et al., 2007). In such ischemic conditions, the penumbra, which is the most hypoxic region, displays a transient change in the expression of growth factors, chemokines and metalloproteinases and their corresponding receptors which in turn stimulates angiogenesis, brain recovery and neuroblast migration (Barkho et al., 2008; Grade et al., 2013; Marti et al., 2000; Ohab et al., 2006; Robin et al., 2006). Cerebral ischemia induces the expression of VEGF in the penumbra, followed by upregulation of VEGFR1 and VEGFR2 along vascular structures that invade the core region of the ischemic tissue (Lennmyr et al., 1998; Marti et al., 2000; Plate et al., 1999). VEGF activates VEGFR2 expressed in ischemic neurons and reactive astrocytes (Kilic et al., 2006). In addition, Angiopoietin-2 expression, together with VEGF, increases after ischemic MCAO in rats (Beck et al., 2000). The upregulation of both factors promotes EC proliferation, suggesting that Angiopoietin-2 is important for the revascularization of the ischemic territory. In line with this finding, the expression of Tie receptors also rises in ECs after focal cerebral ischemia reperfusion in rats (Lin et al., 2000). Furthermore, Kilic and colleagues outlined the protective role of VEGF in the context of focal cerebral ischemia with the help of a transgenic mouse line expressing human VEGF-165 under a neuronal promoter. They demonstrated that neuronal-derived VEGF increases vascular permeability and promotes neuroprotection of the ischemic brain *via* PI3K/Akt (Kilic et al., 2006). Interestingly, VEGF expression is

not only important for the proliferation of endothelial cells within the neurovascular niche. The increase of VEGF and CXCL12 in the ischemic penumbra is necessary to activate MMP expression and promote cell migration (Barkho et al., 2008; Lee et al., 2006; Zhao et al., 2006). MMP9 is mostly secreted by endothelial cells, but in the acute stage of cerebral ischemia it was shown that NPCs differentiate into migratory cells expressing high levels of MMP3 and MMP9 in response to increased VEGF (Barkho et al., 2008). Notably, GFAP expression was co-localizing with MMP9 in structures which resemble microvessel-associated astrocytic end-feet (Zhao et al., 2006). Blocking the expression of MMP3 or MMP9 in NPCs *via* siRNAs interfered with both, the differentiation of NPCs and ischemia-induced cell migration (Barkho et al., 2008).

Several Receptor/Ligand signaling pathways in the neurovascular niche are proposed to regulate neuronal migration in the post-stroke region including Ang1/Tie2, CXCL12/CXCR4, BDNF/p75NTR and TrkB, as well as Epo/EpoR. Ang1 and CXCL12 are induced in the hypoxic peri-infarct region after stroke and regulate stem cell differentiation or migration through their receptors Tie2 and CXCR4 respectively. Stroke-induced Ang1 expression was observed within blood vessels extending from the infarct core to the SVZ along the entire pathway of neuroblast migration and adjacent to cells positive for Tie2 (Ohab et al., 2006). Beneath endothelial cells, the majority of CXCL12 is expressed by reactive astrocytes in the penumbra whereas CXCR4, its receptor, is expressed in NPCs and migrating neuroblasts (Robin et al., 2006). *In vitro* data lead to the postulation that also CXCL12/CXCR4 signaling regulates the directed migration of new neurons to the ischemic area. For example, Thored and colleages have shown that neurosphere cells migrate towards a gradient of CXCL12a in an *in vitro* chemotactic chamber assay. This effect was blocked by the CXCR4 receptor antagonist AMD3100 (Thored et al., 2006). Therefore, these findings suggest that Ang1/Tie2 and CXCL12/CXCR4 signaling pathways might mediate post-stroke neuroblast migration. However, definite genetic proof for this involvement is still missing.

The fact that vascular-derived BDNF is required for the migration of olfactory neurons in the adult brain (Snapyan et al., 2009) suggests that BDNF could also promote injury-induced migration of neuroblasts in the ischemic brain. In agreement with this, endothelial cells in the ischemic striatum secrete BDNF and the recruited neuroblasts express p75NTR, a low affinity receptor for BDNF (Grade et al., 2013). Interestingly, reactive astrocytes located at the injury site and in direct contact with the vessels express TrkB, a high affinity receptor for BDNF and are therefore able to trap extracellular BDNF. Using acute brain slices, Grade and colleagues showed that BDNF released by vessels promotes neuroblast migration in the injured striatum. These studies revealed that injury-induced neuroblast migration shares similarities with migration of NPCs in the RMS regarding its association with vessels, the expression of BDNF and its receptors as well as its role in the initiation of the migratory phase (Grade et al., 2013; Snapyan et al., 2009).

Another molecular player regulating neuroblast migration after stroke is Erythropoietin (Epo), a cytokine known for its important role in hematopoiesis. Epo and its receptor are expressed in the nervous system and are known to be upregulated after hypoxic ischemia (Bernaudin et al., 1999). Interestingly, Tsai and colleagues demonstrated that neuroglial specific EpoR conditional knockout mice displayed impaired neuroblast migration to the peri-infarct cortex. Although these animals preserve the initial post-stroke NPC migration, the number of migrating neuroblasts is significantly reduced after 7 days post-stroke, indicating that subsequent migration towards the infarcted area is disturbed (Tsai et al., 2006).

There have also been some attempts to use stem cell therapy upon ischemic stroke. Li et al. demonstrated that injection of NPCs and embryonic stem cell-derived vascular progenitor cells (VPCs) in a rat stroke model promoted the survival, migration, differentiation and maturation of neuronal and vascular cells derived from the co-transplanted progenitors in contrast to single transplanted control animals. Co-administration of neuronal and vascular progenitor cells triggered the generation of VEGF, BDNF-expressing cells from the grafted NPCs. Interestingly, also the production of VEGF in astroglia was enhanced after co-transplantation. Furthermore, Li and colleagues could show that after co-transplantation cells were able to migrate longer distances compared to single transplantations. Importantly, the co-transplantion of vascular and neuronal stem cells also improved the neurobehavioral deficiencies following stroke, indicating that stem cells based therapies might be beneficial for neurovascular regeneration after ischemic stroke (Li et al., 2014a).

A certain parallelism in the remodeling of the vasculature under hypoxia following ischemic insult in the brain can be drawn with respect to the process occurring during gliomagenesis and glioma progression. In the next chapter we will cover aspects of glioma progression focusing on the role of the vasculature in tumor progression and invasion.

2.2. Glioma invasion is supported by the vasculature

Malignant gliomas are the most aggressive and devastating tumors of the CNS. Glioma invasion is characterized by its diffuse infiltration of the brain tissue which impedes a complete surgical resection and an effective treatment, portending a very poor-prognosis for patients diagnosed for this disease (Cuddapah et al., 2014). Interestingly, glioma cells migrate through the extracellular spaces of the brain often using the vascular scaffold similarly to the vasophilic migration of neuronal progenitors. The perivascular invasion of glioma cells was already reported in 1938 by the neuropathologist Hans Joachim Scherer (Scherer, 1938). He described that gliomas move along defined cerebral structures, such as the blood vessels or the subarachnoid space below the meninges, which are named Scherer's secondary structures. More recent contributions have confirmed Scherer's observations (Farin et al., 2006; Montana and Sontheimer, 2011; Zagzag et al., 2008) and it has been estimated that more than 80% of the glioma tumors develop contiguous to the cerebral vessels (Watkins et al., 2014).

Gliomas are originated from a small fraction of cancer cells with multilineage differentiation and self-renewal capacity called glioblastoma stem cells (GSCs) (Singh et al., 2004). Gene expression studies have demonstrated that GSCs retain high expression similarity with neural stem cells, suggesting that tumors might be originated by mutated neuronal precursors that acquire tumorigenic properties but maintain the self-renewal potential (Phillips et al., 2006). Importantly, Calabrese and colleagues demonstrated that GSCs are preferentially located adjacent to tumor capillaries and that endothelial cells release trophic factors that sustain the stem cell-like state. Co-transplantation of GSCs and endothelial cells in orthotopic tumor xenographs incremented the number of GSCs and accelerated the growth of tumors, highlighting the role of endothelial cells in the tumor microenvironment (Calabrese et al., 2007). The tumor vessels are abnormal, consisting of abundant, leaky and highly disorganized vascular network but, concomitantly, this pathological vascularization generates a hypoxic tumor microenvironment. Notably, GSCs are preferentially located in perivascular or hypoxic areas (Schiffer et al., 2014; Seidel et al., 2010). In these conditions, it has been demonstrated that the hypoxic niche promotes the maintenance of tumor stem cells *via* HIF (Li et al., 2009; Seidel et al., 2010) Therefore, growing tumors benefit from the excessive vasculature not only as enhanced nutrient supply but also as potential cancer stem cell niche to facilitate tumor progression. Interestingly, Wang et al. and Ricci-Vitiani et al. simultaneously reported that GSCs can give rise to endothelial cells in the tumor (Ricci-Vitiani et al., 2010; Wang et al., 2010). These two papers contributed to shed light on the ontogeny of the aberrant vascular bed in glioblastoma tumors and intriguingly illustrate how tumors instruct their niche to develop the malignancy. However, the significance of this basic research finding has recently been questioned by clinical neuropathologists (Rodriguez et al., 2012). In human tumor specimens, most of the tumor vasculature

does not contain cancer genetic mutations (Kulla et al., 2003; Rodriguez et al., 2012) and, therefore, the relevance of the contribution of GSCs to tumor endothelium remains controversial.

Gliomas highly depend on the perivascular niche and progressively colonize the vessels in a dynamic process. The first recording of glioma migratory tracks was accomplished by transplanting fluorescent-labeled C6 rat glioma cells into neonatal rat brains and by analyzing the tumor cell movement in brain slice cultures using time-lapse microscopy (Farin et al., 2006). Notably, tumors invaded the cerebral tissue by moving along the abluminal surface of the vessels but did not infiltrate the vascular lumen. These experiments showed that glioma migratory dynamics resembled those described for neuronal progenitors, which typically extend a prominent leading process attached to the vessels followed by cell motion along the vasculature with fluctuant speed. Later, Winkler and colleagues visualized glioma cell movement *in vivo* by using multiphoton laser scanning microscopy. They were able to track individual glioma cells and demonstrated that gliomas initially grow by cooption of the preexisting microvasculature in the brain and by consequently inducing vascular remodeling to benefit their invasion (Winkler et al., 2009). Previous studies demonstrated that vessel cooption precedes the angiogenic sprouting necessary to support tumor progression (Pezzella et al., 1997). After glioma implantation into the host tissue, tumor cells form cuff-like clusters around preexisting vasculature (Zagzag et al., 2000) without any obvious angiogenic response during the first stages of tumor growth (Holash et al., 1999). Moreover, when glioma cells widespread perivascular through the CNS, new host vessels are coopted by additional satellite tumors. Incipiently, angiopoietin-2 is strongly induced in coopted vessels and subsequently VEGF expression is up-regulated by the remaining tumor cuffs adjacent to coopted vessels, which induces a robust angiogenic response to generate new vessels that support tumor growth (Holash et al., 1999). Therefore, glioma tumors are strongly dependent on the vasculature in two main aspects, as supply for oxygen and nutrients to allow tumor growth and as a scaffold structure to support tumor infiltration.

In order to better track tumor cells *in vivo* the zebrafish has also been explored as a model for cancer (Stoletov et al., 2007, 2010). Ju and colleagues demonstrated that constitutively active smoothened is oncogenic in zebrafish and that co-expression with the human constitutively active Akt leads to glioblastoma-like tumors in the brain, retina and spinal cord (Ju et al., 2009). Recently, they also reported that Shh activation in neural progenitor cells prone the development of optic pathway glioma in the zebrafish (Ju et al., 2014). Beneath the fact that zebrafish can develop almost any kind of tumor it was shown that the zebrafish is suited as xenograft model for multiple human cancer cell lines. Interestingly also for glioma the use of U87 MG (Lal et al., 2012; Yang et al., 2013), U251-RFP (Geiger et al., 2008) and primary glioblastoma multiforme (GBM)-derived cells (Rampazzo et al., 2013) is well documented in the zebrafish. Injection of U87 cells or U251-RFP cells in the yolk sac at 2 days post fertilization (dpf) revealed their capacity to survive, invade and to remodel the vasculature (Geiger et al., 2008; Yang et al., 2013). Geiger and colleagues observed the injected glioblastoma cells *in vivo* and could show that the tumor cells proliferate and recruit the blood vessel from their host (Geiger et al., 2008). Yang and colleagues documented that the human glioma cancer stem cells (GSCs) derived from U87 MG are highly invasive. Interestingly, this invasive capability was associated with enhanced expression of the metalloproteinase MMP9, known for its role during neuronal migration. Using MMP9 inhibitors they could successfully suppress the invasion/spread of GSCs in zebrafish embryos providing an *in vivo* proof for the importance of remodeling of the extracellular matrix for glioblastoma invasion (Yang et al., 2013). U87 MG xenotransplantation into the 4 dpf zebrafish brain by Lal and colleagues highlighted glioblastoma behavior in a functional brain microenvironment: Glioblastoma cells migrated along the blood vessels of the zebrafish embryo up to 450 μm from the site of injection and seemed closely aligned along the abluminal surface of blood vessels, similar to the observations in rodents and humans. Interestingly, this study also showed that the calcium-activated protease calpain-2 is not only critical for glioblastoma cell invasion *in vitro* but also *in vivo*. In zebrafish, Calpain 2 knockdown glioma cells remained in confined clusters in close proximity to the injection site in comparison to the control U87 MG cells.

Different molecular mechanisms have been proposed for the invasive route of glioma cells along the Scherer's structures. On one hand, the perivascular space and the subarachnoid space provide considerably less physical resistance than the parenchyma since they are predominantly fluid-filled compartments. Even so, the extracellular matrix (ECM) that fills the pericellular space needs to be degraded to allow glioma invasion. Glioma cells express several metalloproteinases (including MMP1, MMP2, MMP9 and MMP14) and serine proteases which dig out the route of invasion through proteolysis of the ECM (Mentlein et al., 2012). Furthermore, the remodeling of the perivascular matrix by metalloproteinases also releases growth factors and chemokines entrapped in the ECM which favors tumor progression (reviewed in (Egeblad and Werb, 2002)). On the other hand, several signaling events have been suggested to participate on the establishment of preferential glioma migratory trails. For instance, Zagzag and colleagues showed by immunostaining of human glioblastoma surgical sections that the expression of CXCR4 and CXCL12 highly correlated with the pattern of Scherer secondary structures. They analyzed *in vitro* the CXCL12-mediated chemiotactic migration of glioma cells and proposed that CXCR4-positive glioma cells are being guided by a CXCL12 gradient released by blood vessels (Zagzag et al., 2008). Recently, Cheng and colleagues could demonstrate that CXCR4-positive GSCs are recruited to the perivascular niche and that endothelial cells induce GSC differentiation into pericytes *via* TGFβ1 (Cheng et al., 2013). Notably, these GSC derived pericytes contribute to maintain tumor vessels and in turn support tumor growth. Another important player in tumor cell invasion along the perivascular space is the interaction with the ECM. The cell surface receptors integrins mediate the cellular attachment to the basement membrane surrounding the vessels (Desgrosellier and Cheresh, 2010). Several studies have demonstrated the relevance of integrin binding to ECM proteins during glioma invasion and therefore pharmacological blockers of such interactions are currently used in different clinical trials to halt glioma infiltration (Ohnishi et al., 1997; Ray et al., 2014; Reardon et al., 2008; Serres et al., 2014). In addition, increasing evidences suggest that neurovascular guidance cues, such as Eph/ephrin (Miao et al., 2015; Nakada et al., 2004; Wang et al., 2012), DCC/netrin (Jarjour et al., 2011; Shimizu et al., 2013; Ylivinkka et al., 2013), Robo/slit (Dallol et al., 2003; Mertsch et al., 2008; Yiin et al., 2009), Plexin/semaphorin (Bagci et al., 2009; Li et al., 2012; Sabag et al., 2012; Zhou et al., 2012), are involved in glioma invasion. As an example, EphA2 overexpression was shown to correlate with poor prognosis in glioma patients (Liu et al., 2006). Interestingly, a recent study demonstrates that silencing EphA2 in GSCs reduces the tumorigenecity, stemness and dissemination of intracranially injected tumors in immunodefiencient mice. In agreement with the relevance of the Eph/ephrin axis in tumor infiltration, *ex vivo* GSCs tumor invasion was enhanced in ephrin-A triple knockout brain slices, indicating that ephrin signaling in the tumor microenvironment provides repulsive cues for tumor infiltration (Miao et al., 2015). This has been also proven for other tumors types such as colorectal cancers where downregulation of the repulsive activity of EphB receptors correlates with cancer progresion (Batlle et al., 2005; Cortina et al., 2007).

In order to further investigate the molecular interactions between glioma cells and endothelium, Montana and Sontheimer analyzed patient-derived malignant glioma biopsies and identified bradykinin as an important vascular signal to attract glioma cells towards the cerebral endothelium acting *via* bradykinin 2 receptor (B2R). Moreover, they could impair the affinity of glioma cells for the endothelium by pharmacological inhibition of B2R, thus proposing icatibant (B2R antagonist) as an emerging therapeutic candidate against malignant gliomas (Montana and Sontheimer, 2011). In addition, Sontheimer's group has

recently reported a detailed mechanistic description of glioma invasion (Watkins et al., 2014). Using different glioma mouse models that mimic different clinical disease features, they were able to visualize by two-photon microscopy the interaction of individual glioma cells with the brain vascular network and to correlate changes in vessel size and blood flow to tumor–vessel association. Importantly, this group demonstrated that glioma cells infiltrate the perivascular space and insert their leading processes underneath the astrocytes covering the vessels, causing the displacement of the astrocytic end-feet from the abluminal surface of the vasculature and inducing a local breach of the blood brain barrier (Fig. 2). As the disease progresses, the vasculature gets encased by the tumor and, consequently, the disruption of the astrocytic coupling to the endothelium results in the loss of vascular tone regulated by astrocytes, which correlates with the decreased blood flow observed in glioma patients (Akella et al., 2004). Conversely, glioma cells assume the control of vasoconstriction signals through Ca^{2+}-dependent release of K^{+}. The glioma-mediated reduction in vessel size has been suggested to enable tumor infiltration by enlarging the perivascular space.

Astrocyte end-feet cover 99% of the vasculature in a healthy brain and this vascular–astrocytic interaction is essential to maintain brain homeostasis, blood flow and blood brain barrier (BBB) properties (Iadecola and Nedergaard, 2007). Therefore, given the crucial role of the astrocyte–vessel interface in glioma tumor invasion, it is critically important to molecularly dissect the neuronal–glia–vessel interaction partners in order to design novel therapeutical strategies for this incurable disease.

3. Concluding remarks and further perspectives

Neuronal migration is a coordinated process where neuronal cells do not act individually but in crosstalk with many other cells in the surrounding tissue. The complexity of the brain can be nowadays better characterized by state-of-the-art imaging techniques that allow a more detailed reconstruction of the *in vivo* situation (Grade et al., 2013; Snapyan et al., 2009; Watkins et al., 2014). As summarized in this review, vessels, astrocytes and pericytes ensheathing the vessels play important roles during the process of neuronal migration. Astrocytes react in healthy and pathological situations differently and are able to release important trophic factors such as VEGF which in turn supports the vasculature and neuronal migration (Bozoyan et al., 2012). Pericytes encircle the brain capillaries and are known to regulate a plethora of events including microcirculation, angiogenesis and the blood brain barrier (Armulik et al., 2010). It needs to be further explored whether pericytes might also directly influence the movements of neurons.

Neuronal precursors in the postnatal and adult brain use the interface between vessels and astrocytes to support their displacement. According to the current literature, during embryonic cortical development neuroblasts move either along the radial glia (Noctor et al., 2001; Hatten, 1999; Marin and Rubenstein, 2003; Rakic, 2003) or in a cell autonomous fashion (Franco et al., 2011). However, it is tempting to speculate that similarly the vasculature might be involved in neuroblast migration in during these stages of embryonic development. In agreement with this hypothesis, radial glia cells are aligned to the vasculature (Noctor et al., 2001) and disruption of radial glia anchorage to the meningeal vessels on the pial surface causes defects in neuronal cortical migration (Halfter et al., 2002; Moore et al., 2002). Moreover, meningeal membranes support the tangential migration of hem-derived Cajal–Retzius cells towards the marginal zone during cortex development (Borrell and Marin, 2006), also suggesting that vascular integrity is required for appropriate neuronal migration during brain development.

The neuron–glia–vessel tripartite complex is also important during pathological situations. Unraveling the neuronal interaction with the astrocytes–vessel interface might be of pivotal relevance to understand pathological setting such as glioma invasion or stroke. Current therapeutics has been unsuccessful on halting glioma tumor progression (Paez-Ribes et al., 2009). Instead in the case of cerebral ischemia, experimental models have proven that neurovascular stem cell therapies improve the survival of the grafted cells and the functional neurological recovery (Li et al., 2014a). Therefore, new strategies focused on

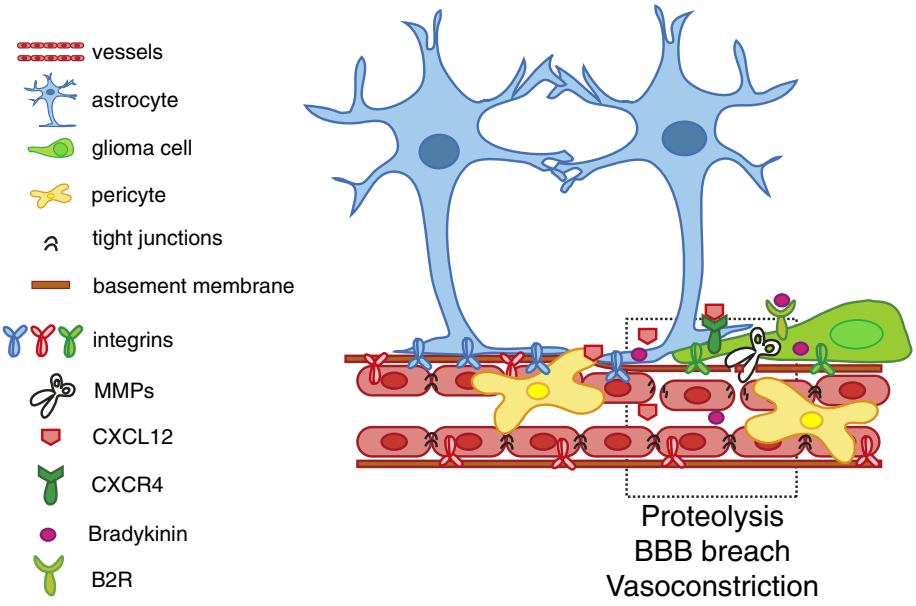

Fig. 2. Glioma tumors preferentially invade the perivascular space. The neurovascular unit includes endothelial cells, mural cells, the basement membrane and the astrocytic end-feet wrapping the basal lamina that surrounds the endothelium. Glioma tumors migrate along the perivascular space recruited by vascular signals, *i.e.* CXCL12 or Bradykinin. The invasion of gliomas to the perivascular space requires a coordinated sequence of cell adhesion and detachment steps mediated by adhesion molecules such as integrins and also the proteolysis of the ECM by MMPs. When glioma cells colonize the vasculature, they displace the astrocytic processes from the vessel surface and degrade the basement membrane. The withdrawal of astrocytic end-feet alters brain functionality in two important aspects: causes a blood brain barrier (BBB) breakdown and disrupts the neurovascular coupling that controls blood flow.

breaching the vascular–neuronal bounding may improve the prognosis of these diseases.

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