Modeling Neural Stem Cell and Glioma Biology

TOBIAS BERGSTRÖM
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Abstract

This thesis is focused on neural stem cell (NSC) and glioma biology. I discuss how NSCs interact with extracellular matrix (ECM) proteins in the stem cell niche, and investigate the consequences of deregulated Platelet-derived growth factor (PDGF) signaling for embryonic NSCs in transgenic mice. Furthermore I present cell cultures of human glioblastoma multiforme (GBM) that models human disease, taking into account the heterogeneity of GBM. Finally, interactions between brain tumors and mast cells are studied using the glioma cultures.

In paper I, the importance of NSC interactions with the ECM in the stem cell niche during development is discussed. Contacts between NSCs and the ECM in the subventricular zone (SVZ) are emerging as important regulatory mechanisms. We show that early postnatal neural stem and progenitor cells (NSPC) attach to collagen I, and that the adhesion is explained by higher expression of collagen receptor integrins compared to adult NSPC. Further, blood vessels in the SVZ express collagen I, indicating a possible functional relationship.

Growth factors, e.g. PDGF, regulate NSC proliferation and differentiation. Aberrant activation of growth factor signaling pathways also plays a role in brain tumor formation. Paper II demonstrates that transgenic mice expressing PDGF-B at high levels in embryonic NSCs displayed mild neurological defects but no hyperplasia or brain tumors. This suggests that a high level of PDGF is not sufficient to induce brain tumors from NSCs without further mutations.

Paper III presents a novel panel of human glioma stem cell (GSC) lines from GBM that display NSC markers in vitro and form secondary orthotopic tumors in vivo. GBM has recently been categorized in molecular subclasses and we demonstrate, for the first time, that these subclasses can be retained in vitro by stem cell culture conditions. We have thus generated models for research and drug development aiming at a focused treatment depending on GBM subtype.

Interactions with the immune system are integral parts of tumorigenesis. Mast cells are found in glioma and in paper IV we demonstrate that the grade-dependent infiltration of mast cells is in part mediated by macrophage migration inhibitory factor and phosphorylation of STAT5.

Keywords: neural stem cell, integrins, glioma, PDGF, mast cell

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


*Equal contribution

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Publications not included in this thesis


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<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ara-C</td>
<td>arabinofuranosyl cytidine</td>
</tr>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>BLBP</td>
<td>brain lipid-binding protein</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>cyclin-dependent kinase inhibitor 2A</td>
</tr>
<tr>
<td>CHI3L1</td>
<td>chitinase 3-like 1</td>
</tr>
<tr>
<td>CXCL12</td>
<td>chemokine (C-X-C motif) ligand 12</td>
</tr>
<tr>
<td>CXCR4</td>
<td>chemokine (C-X-C motif) receptor 4</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>EGF</td>
<td>epidermal growth factor</td>
</tr>
<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
</tr>
<tr>
<td>FGF-2</td>
<td>fibroblast growth factor 2</td>
</tr>
<tr>
<td>GABRA1</td>
<td>gamma-aminobutyric acid A receptor, subunit alpha 1</td>
</tr>
<tr>
<td>GBM</td>
<td>glioblastoma multiforme</td>
</tr>
<tr>
<td>GEMM</td>
<td>genetically engineered mouse model</td>
</tr>
<tr>
<td>GFAP</td>
<td>glial fibrillary acidic protein</td>
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<tr>
<td>GLAST</td>
<td>glutamate and aspartate transporter</td>
</tr>
<tr>
<td>H3F3A</td>
<td>H3 histone, family 3A</td>
</tr>
<tr>
<td>HSPG</td>
<td>heparan sulfate proteoglycan</td>
</tr>
<tr>
<td>IL-1</td>
<td>interleukin 1 complex</td>
</tr>
<tr>
<td>IL-4</td>
<td>interleukin 4</td>
</tr>
<tr>
<td>IL-6</td>
<td>interleukin 6</td>
</tr>
<tr>
<td>IL-8</td>
<td>interleukin 8</td>
</tr>
<tr>
<td>LTB4</td>
<td>leukotriene B4 receptor 1</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen activated protein kinase</td>
</tr>
<tr>
<td>MDM2</td>
<td>mouse double minute 2 homolog</td>
</tr>
<tr>
<td>MET</td>
<td>met proto-oncogene</td>
</tr>
<tr>
<td>MIF</td>
<td>macrophage migration inhibitory factor</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
</tr>
<tr>
<td>NEFL</td>
<td>neurofilament, light polypeptide</td>
</tr>
<tr>
<td>NF1</td>
<td>neurofibromin 1</td>
</tr>
<tr>
<td>NSPC</td>
<td>neural stem/progenitor cells</td>
</tr>
<tr>
<td>PDGF</td>
<td>platelet-derived growth factor</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphatidylinositol-4,5-bisphosphate 3-kinase</td>
</tr>
<tr>
<td>PLCγ</td>
<td>phospholipase Cγ</td>
</tr>
<tr>
<td>PSA-NCAM</td>
<td>polysialylated neural cell adhesion molecule</td>
</tr>
<tr>
<td>PTEN</td>
<td>phosphatase and tensin homolog</td>
</tr>
</tbody>
</table>
RB retinoblastoma protein
RGD Arg-Gly-Asp, integrin binding motif
RTK receptor tyrosine kinase
SCF stem cell factor
SDF-1 stromal cell-derived factor 1
SH2 Src homology 2
SLC12A5 solute carrier family 12, member 5
STAT5 signal transducer and activator of transcription 5
SVZ subventricular zone
SYT1 synaptotagmin 1
TCGA The cancer genome atlas
TGF-β transforming growth factor β
TNF-α tumor necrosis factor α
TP53 tumor suppressor protein 53
VEGF vascular endothelial growth factor
WHO World Health Organization
Neural stem cells during CNS development

The central nervous system originates from a thickened layer of epithelial cells called the neural plate. The thickening of the epithelium and the formation of the neural plate mark the start of the part of organogenesis known as neurulation. The neural plate folds into a hollow structure called the neural tube, and subsequently the spinal cord and the three primary brain vesicles (prosencephalon, mesencephalon and rhombencephalon) are formed. Later, the three primary vesicles transform into five secondary vesicles (telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon) from which the different parts of the brain emerge (Gilbert and Singer, 2006).

During the development of the central nervous system, neural stem cells shift identity more than once. Neurulation starts around embryonic day (E) 8.5 in the mouse and at this time neural stem cells are the neuroepithelial cells making up the neural plate and later forming the neural tube. At this time, the neuroepithelial cells undergo symmetric division and proliferate to expand the pool of neural stem cells (Copp et al., 2003). When neurogenesis begins around E10 in the mouse (García-Moreno et al., 2007), neuroepithelial cells differentiate to radial glial cells and lose some of their epithelial characteristics in the process, notably tight junctions (Aaku-Saraste et al., 1996) and basal-apical polarity of cell membrane proteins (Huttner and Brand, 1997; Kosodo et al., 2004). In addition they gain astroglial features such as glycogen granules (Gadisseux and Evrard, 1985), GLAST (Shibata et al., 1997) and BLBP expression (Feng et al., 1994). The common astrocyte marker GFAP is expressed in radial glial cells in primates (Choi, 1981; Levitt and Rakic, 1980) but not in rodents (Bignami and Dahl, 1974; Schnitzer et al., 1981).

Radial glial cells extend processes from the ventricular to the pial surface of the neural tube providing a scaffold for newly born neurons to migrate along (Fig. 1). For quite some time this was believed to be the main function of radial glial cells. This is perhaps not surprising, as it has been shown that 80-90% of neuronal precursors migrate along glial fibers (Hatten, 1999). Another prevailing idea was that neurons and glial cells had separate origins considering that neurogenesis and gliogenesis are consecutive events during development. Later, this idea was proven wrong, exemplified by a study by
Noctor and others (2008) demonstrating that a radial glial cell that first gives rise to neurons later transforms into an astrocyte. This is an example of neurogenic to gliogenic transition in the same cell. Most of the radial glial cells differentiate to astrocytes after neurogenesis (Culican et al., 1990; Edwards et al., 1990; Voigt, 1989), making them candidates for glial progenitors. However, the identity of the neuronal precursor was at that time unknown. Some ten years later, studies emerged demonstrating that radial glial cells did indeed produce neurons and thus established the identity of radial glial cells as neural stem cells responsible for almost all of the neurons, astrocytes and oligodendrocytes that make up the brain (Anthony et al., 2004; Malatesta and Hartfuss, 2000; Noctor et al., 2001; 2008).

It is worth noting that radial glial cells by no means are a homogeneous population of cells. This is evident when one considers the different set of transcription factors they express and the diverse set of progeny they produce (reviewed in detail by Kriegstein and Alvarez-Buylla (2009)). In order to achieve diversity, radial glial cells seem to be organized in radial units with different progenitors giving rise to distinct progeny (Rakic, 1988; 1995). The radial unit hypothesis does not fully explain the diversity seen in the neocortex where most of the progeny stems from the radial glial cells of a single germinal zone. Here it seems that distinct types of cells are born in a timed sequence (Fig. 1A-C). This sequence is believed to arise either through different precursors programmed to produce their progeny at defined times or through one progenitor that changes its potency during development (reviewed by Franco and Muller (2013)).

![Figure 1. Different stages of neural stem cell development. (A) Neuroepithelial cells undergo self-renewal. (B) Early radial glia give rise to neurons. (C) Late radial glia produce astrocyte and oligodendrocyte precursors. (nIPC, aIPC and oIPC denote neuronal, astrocytic and oligodendrogial intermediate precursor respectively)](image-url)
Adult neural stem cells

In some species, radial glial cells persist into adulthood where they act as neural stem cells along almost the entire ventricular zone (i.e. in songbirds (Nottebohm, 2004)). In mammals, most of the radial glial cells differentiate to astrocytes (Culican et al., 1990; Edwards et al., 1990; Voigt, 1989) but some persist into adulthood and become neurogenic astrocytes located in the subventricular zone of the lateral ventricles (Merkle et al., 2004). Also neurogenic astrocytes in the hippocampal formation have an embryonic origin (Li et al., 2013).

Several decades ago, traces of neurogenesis in the adult hippocampus were shown (Bayer et al., 1982; Bayer, 1985; Kaplan and Hinds, 1977). These early studies did not convince the scientific community of the presence of adult neural stem cells, mainly due to technical limitations. However, when Reynolds and Weiss (1992) demonstrated that neural progenitors isolated from the striatum had the potential to differentiate into neurons and astrocytes in vitro, the first evidence of the presence of adult neural stem cells was presented. Subsequent studies refined the localization to the subventricular zone of the lateral ventricles (Lois and Alvarez-Buylla, 1993) and neurons born in the subventricular zone were later found to migrate to the olfactory bulb (Lois and Alvarez-Buylla, 1994). A few years later the neural stem cell in the subventricular zone was identified as a “type B” astrocyte (Doetsch et al., 1999a). In parallel, the subgranular zone of the hippocampus was investigated. Now, with refined methods, it was possible to show that also the hippocampal formation harbors multipotent neural stem cells (Gage et al., 1995; Palmer et al., 1997; Suh et al., 2007). Neurogenesis in the hippocampus is important for learning and memory, and has also been shown to increase in conditions such as stroke and epilepsy (reviewed in (Zhao et al., 2008)).

There seems to be a difference regarding the in vivo potential of neural stem cells in the hippocampus and the SVZ. Whereas SVZ neural stem cells differentiate into olfactory bulb neurons (Doetsch et al., 1999a) and oligodendrocytes (Hack et al., 2005; Menn et al., 2006), neural stem cells in the hippocampus instead differentiate into neurons and astrocytes (Suh et al., 2007). The findings described so far concern adult neurogenesis in rodents, where the most detailed studies have been made. Corresponding features are described in the human brain, but are not elucidated to the same extent as in rodents (reviewed in Ihrie et al. (2011)).

Postnatal neural stem cells in the SVZ

The SVZ undergoes quite dramatic changes during the first two weeks of postnatal (P) life. The abundant radial glia of the embryonic ventral zone are
gradually changing and eventually transforming into neural stem cells of the adult SVZ. At P0, around 95% of the ventral zone cells are radial glial cells. These numbers decrease to 60% at P7 and by P15, all radial glial cells have either differentiated into more mature cells or transformed into adult neural stem cells (Tramontin et al., 2003).

Neural stem cells in the SVZ of the adult mouse brain are GFAP positive astrocytes. These cells, called type B cells, reside in the subependymal layer of the lateral ventricle wall. During adult neurogenesis type B cells differentiate into transient amplifying precursors, or type C cells, that proliferate more actively leading to an increase in numbers. A majority of C cells differentiate further into migrating immature neuroblasts called type A cells. Type B astrocytes are a heterogeneous cell population and produce different types of neurons depending on their developmental origin (Kelsch et al., 2007; Kohwi et al., 2007; Merkle et al., 2007). The A cells migrate along the rostral migratory stream to the olfactory bulb where they mature to granular and periglomerular neurons (Doetsch et al., 1999a). Some type C cells instead differentiate into oligodendrocyte precursors; the decision between these two fates is governed by the transcription factors Pax6 and Olig2 (Hack et al., 2005). SVZ astrocytes also respond to myelin damage by increasing the production of oligodendrocyte progenitors, which migrate to the site of damage and mature into myelinating oligodendrocytes (Menn et al., 2006; Nait-Oumesmar et al., 1999).

**Neural stem cell niches**

A stem cell niche is an environment where stem cells are kept in an undifferentiated and multipotent state. When the stem cell undergoes asymmetric division and produces a daughter cell, the stem cell is kept in the niche while the more differentiated progenitor migrates away to its final destination. Originally proposed in the seventies by Schofield (1978) the niche is both a physical and functional location where humoral and paracrine factors, the extracellular matrix, non-protein properties such as the oxidative state and Ca$^{2+}$-levels and niche cells all contribute to the specialized microenvironment (Ferraro et al., 2010).

In the subventricular zone, type B astrocytes can be divided into two subtypes based on their location. Type B1 astrocytes have a single ciliated apical ending in the ventricle wall and these apical endings of the B1 cell are surrounded by multiciliated ependymal cells in a pinwheel structure (Doetsch et al., 1997; Mirzadeh et al., 2008). Type B2 astrocytes on the other hand are situated in the border between the SVZ and the striatum, and do not contact the ventricle (Doetsch et al., 1997; Mirzadeh et al., 2008). SVZ ependymal cells are also divided in E1 and E2 subtypes based in part on the number of cilia they have (Mirzadeh et al., 2008). Ependymal cells, also of
radial glial cell origin (Spassky, 2005), have been suggested to be the neural stem cells of the SVZ (Johansson et al., 1999a). This is not unlikely as ependymal cells share many features with immature neural cells, such as expression of Sox2, Nestin, CD133, Msh1 and Msh2 (Doetsch et al., 1997; Ellis et al., 2004; Ferri et al., 2004; Sakakibara et al., 2002; Weigmann et al., 1997). The location of type B1 astrocytes, intermingled within the ependymal cell layer, further complicated the identification of the neural stem cell. Later, ependymal cells were shown to respond to injury by producing neuroblasts and astrocytes, although they remain non-dividing during normal conditions (Carlén et al., 2009).

Interactions with the vasculature are important for the control of proliferation, self-renewal and differentiation in a variety of stem cell niches. Examples range from the hematopoietic stem cell niche (Kiel et al., 2005) and the bulge of the hair follicle in the skin (Fuchs et al., 2004), via the higher vocal center in adult songbirds (Louissaint et al., 2002) to the subgranular zone in the hippocampal formation (Palmer et al., 2000). The SVZ is no exception, where most of the dividing cells are close to a blood vessel (Shen et al., 2008; Tavazoie et al., 2008). The basal endfeet of a majority of the type B1 cells attach to a blood vessel (Mirzadeh et al., 2008) and the contact with the SVZ vasculature occur in places where blood vessels lack astrocyte end-feet and pericyte coverage. Considering that 98% of type B1 cells also make apical contact with the ventricle (Mirzadeh et al., 2008), almost all of the neural stem cells in the SVZ are subject to signals from both the blood and cerebrospinal fluid. (Fig. 2) The vasculature is planar alongside the SVZ.
with a different organization compared to the cortex where blood vessels are branched frequently and in a more random fashion (Tavazoie et al., 2008). The difference in vasculature is not only anatomical, the rate of blood flow in the SVZ vasculature is significantly lower compared to flow rates in the striatum. Although lower blood flow rates would indicate that the SVZ is a hypoxic environment, only the ependymal cell layer and a subset of neurons show evidence of hypoxia (Culver et al., 2013). Interestingly, blood flow rates have been shown to influence the transcriptional signatures of endothelial cells (Ohura et al., 2003; Tzima et al., 2005), an important part of the neural stem cell niche shown to influence self-renewal (Shen, 2004).

The extracellular matrix is a part of the adult neural stem cell niche

The ECM regulates stem cell behavior in the SVZ niche, providing structural support, growth factor gradients and location-based instructive cues. An extensive basal lamina is found in the SVZ, made up of laminins (Kazanis et al., 2010), tenascin-C (de Chevigny et al., 2006; Garcia et al., 2004; Kazanis et al., 2007; Peretto et al., 2005), heparan sulfate proteoglycans (Fuxe et al., 1994; Kerever et al., 2007), chondroitin sulfate proteoglycans (Akita et al., 2008; Thomas et al., 1996), nidogen and collagens (Kerever et al., 2007; Mercier et al., 2002). Small finger-like structures of basal lamina, called fractones, extend from blood vessels and come into contact with neural stem cells in the SVZ (Kerever et al., 2007; Mercier et al., 2002). Fractones contain laminins and HSPGs among other ECM proteins. HSPGs have been shown to sequester FGF-2 in the neural stem cell niche, illustrating how the ECM can modulate proliferative signals (Kerever et al., 2007). The heterogeneous expression of laminin isoforms in the SVZ hints at the complexity of SVZ signaling, where neural stem cells are exposed to different laminin heterotrimers from the vasculature, ependymal cells and fractones (Kazanis et al., 2010).

Collagens – structure and classification

Collagens are the most abundant proteins in animals. They are composed of a triple helix of polypeptide chains that are made up of repeats of Gly-X-Y where X is often a proline and Y often a hydroxyproline. Because of the tightly-packed triple helix, every third amino acid is required to be glycine. Hydroxyproline provides stability to the triple helix. Fibrillar and network-forming collagens belong to classic categories with collagen I, II and III exemplifying fibrillar collagens and collagen IV being a network-forming collagen. In addition, three more categories of collagens are described;
FACITs (fibril-associated collagens with interrupted triple helices), MACITs (membrane-associated collagens with interrupted triple helices) and MULTIPLEXINS (multiple triple-helix domains and interruptions). 46 different polypeptide chains make up at least 29 different collagen triple helices. Homotrimers do exist, but the most commonly found triple helices are heterotrimers. For example, collagen I consists of a heterotrimer composed of col1a1 and col1a2 polypeptide chains in a [col1a1]2 [col1a2]1 conformation (Shoulders and Raines, 2009).

Collagens in the central nervous system

Collagens in the central nervous system (CNS) have been described as mainly belonging to connective tissues (e.g. in the meninges), basement membranes between the nervous and vascular systems and the sensory end organs. In addition, collagens play a role in the developing nervous system in processes such as axon guidance and synaptogenesis. Fibrillar collagens I and II are found both in the leptomeninges (pia mater and arachnoidea) and in the dura mater. The basement membranes harbor collagens IV, XV and XVIII (Shoulders and Raines, 2009). Collagens are not abundant in the parenchyma, but there are a few exceptions. In the hippocampus, COLXVI mRNA has been found, and hippocampal neurons express this collagen in culture (Hubert et al., 2008). In the SVZ, parenchymal collagen I not explicitly associated with fractones was found (Kerever et al., 2007; Mercier et al., 2002). Many collagen isoforms have been implicated in diseases of the nervous system, such as tumors of the CNS (glioma, medulloblastoma), tumors of the PNS (Schwannoma, neurofibroma) and Alzheimers’ disease (Hubert et al., 2008).

ECM receptors within the SVZ

Given the abundance of laminins in the SVZ, it is not surprising that laminin receptors make up a large part of the ECM receptors found there. The main laminin receptors are integrins, but dystroglycan and syndecans also bind laminin and are found both during neural development (Lathia et al., 2007) and in the adult SVZ (Kazanis et al., 2010). In addition, other cell adhesion molecules are described, both during development and in the adult neural stem cell niche. For example E-cadherin has been proposed to regulate neural stem cell self-renewal in the SVZ (Karpowicz et al., 2009). It is also expressed transiently during development where it is important for the segmentation of the brain (Matsunami and Takeichi, 1995; Shimamura and Takeichi, 1992).

One of the properties of the stem cell niche is to keep neural stem cells in a specific location, often achieved via interactions with the ECM. When the cell is in a specific location it can be subjected to signaling by receptors and
signaling molecules that operate over short distances such as gap junctions (Cheng et al., 2004) and Notch signaling (Campos et al., 2006; Hitoshi et al., 2002; Louvi and Artavanis-Tsakonas, 2006). When the cell divides and one of the daughter cells move away from the niche, the short-range signaling is terminated. PSA-NCAM is another cell adhesion molecule that together with Doublecortin is used as a marker for migrating neuroblasts in the rostral migratory stream (Brown et al., 2003; Seki and Arai, 1993).

Integrins

Integrins are one of the most frequently described cell adhesion receptors in the context of neural stem cell regulation. They are heterodimeric cell surface receptors with affinities to a variety of ECM molecules and cell surface receptors. There are 24 αβ heterodimers built up by 18 α and 8 β subunits that can be divided in four groups; RGD receptors (binds to the RGD sequence found in e.g. fibronectin), collagen receptors, laminin receptors and leukocyte-specific receptors (Barczyk et al., 2010). In addition to various ECM molecules, integrins have been shown to bind to several cell adhesion molecules, such as E-cadherin and PECAM-1 (reviewed in (Humphries et al., 2006)).

Integrins are abundant proteins and every nucleated cell expresses integrins on its surface (Barczyk et al., 2010). The first discoveries regarding integrin function described integrins as receptors linking the ECM to the cytoskeleton and their name describe the observation that they maintain the ECM-cytoskeletal integrity (reviewed in (Hynes, 2004)). Further investigations have revealed that in addition to this, integrins mediate signaling, both from the extracellular space to the cytosol, “outside-in” signaling, and vice versa, “inside-out” signaling. An example of outside-in signaling is the phenomena known as anchorage dependence. It has been known for a long time that almost all cells require binding to a substrate in order for the cell to respond to e.g. proliferative RTK signaling and to proceed through the cell cycle (Assoian, 1997), a requirement lost in many types of cancer cells (Desgroisellier and Cheresh, 2010).

Upon integrin binding, a complex of proteins is formed that links the ECM on the outside to the cytoskeleton. All integrins bind to the actin fibers in the cytosol, except for α6β4 which binds to the intermediate filaments. (Hynes, 2002). The protein complex, called a focal adhesion, attracts adaptor proteins such as talin, vinculin and paxillin, and regulatory proteins, e.g. focal adhesion kinase and Src (Deakin and Turner, 2008). Focal adhesions mediate cellular responses such as migration, survival, proliferation and cytoskeletal reorganization (Hynes, 2002).

Folded down, the integrin heterotrimer cannot bind ECM ligands, which is almost a prerequisite for leukocytes circulating in the blood stream. The intracellular events leading to activation of the integrin, i.e. conformational
changes leading to increased affinity for ECM ligands, are termed inside-out signaling. Talin is an example of a protein known for mediating such effects (Shattil et al., 2010).

**Integrins on embryonic and adult neural stem cells**

As already mentioned, neural stem cells are exposed to different laminin heterotrimers (Kazanis et al., 2010; Lathia et al., 2007), both during development and in the SVZ. Neural stem cells express a plethora of integrins, among those the integrin laminin receptors. There are four main integrin laminin receptors, integrins α3β1, α6β1, α6β3 and α7β1. Of these four, integrin α6β1 is the best studied in neural stem cells. It has been demonstrated that integrin α6β1 is expressed on dividing cells in the SVZ, and that this expression decreases with increased distance to blood vessels (Shen et al., 2008). SDF-1/CXCR4 pathway signaling has been demonstrated to upregulate integrin α6β1 and EGFR on activated B1 and C cells (Kokovay et al., 2010).

Integrin α6 is also important for migration, and the different response to SDF-1 signaling on integrin α6 expression in type A, B1 (higher expression) and C cells (lower expression) provides a model for the exit from the ependymal to the vascular niche and further on to the rostral migratory stream. Since SDF-1 induces high levels of integrin α6 on activated B1 and C cells, they attach readily to the laminin-expressing blood vessels where they divide; the lower levels of integrin α6 on A cells instead facilitate the exit from the SVZ. Though the amount is lower, there is still some integrin α6 expressed on A cells which allow them to migrate along the rostral migratory stream (Kokovay et al., 2010).

It seems that a difference in ECM signaling is achieved by activating the β1 integrin on type B1 cells in the SVZ. When the anti-mitotic drug Ara-C is infused into the SVZ, type C and type A cells are depleted and type B1 cells become activated in order to replenish the SVZ (Doetsch et al., 1999b). Upon Ara-C infusion, β1 integrin expression occurred on type B1 cells at the time of mitotic activation (Kazanis et al., 2010). *In vitro* and *in vivo* studies on embryonic neural stem cells show the importance of β1 integrins for migration (α3β1, α5β1, α6β1, αvβ1) and proliferation (αvβ1, α5β1) (Anton et al., 1999; Jacques et al., 1998; Marchetti et al., 2010). Another study reveals the importance of α5β1 for migration of striatal precursors (Tate et al., 2004) indicating that there are distinct integrin expression patterns in different parts of the developing brain. Another integrin subunit involved in migration is β8, which together with αv forms an RGD receptor capable of binding fibronectin. Integrin β8 is essential for neuroblast migration in the rostral migratory stream, and consequently β8−/− mice have smaller olfactory bulbs (Mobley and Mecarty, 2011). Another important function of β8 integrins is to regulate neurovascular homeostatis as demonstrated by an increase in
intracerebral blood vessels with perivascular astrogliosis in integrin β8-/- mice (Mobley et al., 2009).

Four integrins function as collagen receptors, α1β1, α2β1, α10β1 and α11β1, with different affinities for fibrillar and basement membrane collagens. α2β1 and α11β1 prefer fibrillar collagens, such as collagen I, while α1β1 and α10β1 prefer basement membranes, e.g. collagen IV. α2β1 is the only collagen receptor thus far that has been shown to bind fibrillar collagen I with high affinity (Jokinen et al., 2004). In the same study, α1β1 was shown to prefer monomeric collagen I over fibrillar collagen I (Jokinen et al., 2004)
Platelet-derived growth factor

Receptors and ligands
The platelet-derived growth factor (PDGF) family consists of four ligands that bind two receptor subtypes. Classical ligands PDGF-A and -B have been studied since the seventies (Kohler and Lipton, 1974; Ross et al., 1974; Westermark and Wasteson, 1976) while PDGF-C and -D were more recently discovered (LaRochelle et al., 2001; Li et al., 2000). PDGF ligands bind two receptor tyrosine kinases, the PDGF α- and β-receptor. The receptors dimerize upon ligand binding and are known to form homo- and heterodimers. The classical ligands PDGF-A and -B form disulfide bridged homodimers -AA and -BB, and the heterodimer -AB. The more recent PDGF-C and PDGF-D ligands differ from -A and -B in that they require proteolitical cleavage to be activated and only form homodimers. In vitro, PDGF homo- and heterodimer ligands bind the three receptor variants with different specificity. PDGF-AA, -BB, -CC and -AB bind the PDGF αα receptor, -BB, -CC, -DD and -AB bind the αβ receptor while only -BB and -DD have been shown to bind the ββ receptor. However, all of these interactions have not been demonstrated to occur in vivo (Andrae et al., 2008).

PDGF receptor signaling – a brief summary
Upon ligand binding, PDGF receptors dimerize and autophosphorylate in trans. The initial phosphorylations in the kinase domains of the receptors lead to an increase in kinase efficiency (Fantl et al., 1989; Kazlauskas and Cooper, 1989). The following tyrosine residue phosphorylations serve as SH2 docking sites that enable binding of a variety of kinases, phosphatases and adaptor proteins that carry the SH2 domain (Pawson, 1997). The most important signaling pathways are PI3-kinase/Akt, PLC-γ, Shp-2 and Ras/MAPK (Heldin et al., 1998). Phosphatidylinositol 3’ (PI3)-kinase binds PDGF receptors with their SH2 domain and activates Akt/PKB that in turn has anti-apoptotic effects (Dudek et al., 1997; Kauffmann-Zeh et al., 1997). PI3-kinase also activates the Rho family of GTPases, known for their involvement in actin rearrangements (Hawkins et al., 1995). Phospholipase C-γ activation leads to increased levels of Ca²⁺ and diacylglycerol, which leads to activation of protein kinase C (Berridge, 1993). In some cells, this leads to
cell growth and increased motility (Kamat and Carpenter, 1997). Shp-2 is a phosphatase that among other substrates binds and dephosphorylates the PDGF receptors, serving as a negative-feedback loop (Heldin and Westermark, 1999). Finally, the adaptor protein Grb2 forms a complex with Sos, which is a guanine nucleotide exchange factor for Ras, activating Ras by converting it from Ras-GDP to Ras-GTP (Rozakis-Adcock et al., 1992). Active Ras-GTP in turn activates the mitogen activated protein kinase (MAPK) cascade Raf/Mek/Erk. MAPK signaling is involved in cell growth, migration and differentiation (Heldin et al., 1998).

PDGF during development

PDGF ligands and receptors are widely expressed during development and are involved in the formation of kidney, lung, testis, placenta and skin among other organs (Andrae et al., 2008). The embryonic lethality of both α- and β-receptor knock-out mice illustrate the importance of PDGF signaling during development (Kaminski et al., 2001; Soriano, 1997). During development of the brain, PDGF ligands and receptors are found on a variety of neurons (Reddy and Pleasure, 1992; Yeh et al., 1991). PDGF α-receptors are integral in oligodendrocyte development and can be found on oligodendrocyte progenitors in the developing brain (Pringle et al., 1992), while the β-receptor is expressed on neurons (Smits et al., 1991). Conditional knock-outs of the β-receptor has no effect on viability of the transgenic mice, but neurons lacking the β-receptor seem to be more susceptible to stress-induced apoptosis (Ishii et al., 2006).

PDGF and neural stem cells

PDGF is an important regulator of neural stem cells during development and PDGF ligands and receptors are expressed in the embryonic as well as the adult CNS. The first reports of PDGF in the CNS demonstrated a role for proliferation and differentiation of oligodendrocyte progenitor cells (Heldin et al., 1981; Noble et al., 1988; Raff et al., 1988). Later studies showed that PDGF exerts neurotrophic effects (Smits et al., 1991), promoted neuronal differentiation (Johe et al., 1996; Williams et al., 1997) and has a role in neuroprotection (Pietz et al., 1996). Furthermore, PDGF retains partly differentiated neural progenitor cells in an immature state of rapid proliferation but cannot replace FGF-2 or EGF as a stem cell mitogen. (Enarsson et al., 2002; Erlandsson et al., 2001; 2006). This is evident when examining the transcriptional profile after PDGF-treatment, which is an intermediate between that of neural stem cells and their differentiated progeny (Demoulin et al., 2006). Endogenous PDGF was found to stimulate progenitor prolifera-
tion and its inhibition caused differentiation, which shows that autocrine signaling occurs by this growth factor (Erlandsson et al., 2006). Embryonic NSPC from rodents express the PDGF α-receptor in vitro and during differentiation in culture the α-receptor expression level is maintained while the β-receptor expression is increased (Erlandsson et al., 2001). The α-receptor has also been found in the SVZ of adult mice, but the evidence is not conclusive as to which cell type, or cell types that expresses the receptor (Chojnacki et al., 2011; Jackson et al., 2006).

**PDGF and tumor formation**

Simian sarcoma virus was found to induce gliomas when administered intracerebrally to newborn marmosets (Deinhardt and Klein, 1980). A few years later it was discovered that the sequence of v-sis (the oncogene from the simian sarcoma virus) was highly similar in amino acid sequence to PDGF-B (Doolittle et al., 1983; Waterfield et al., 1983). Since then, the importance of PDGF signaling in brain tumor formation has been illustrated in several studies (Fleming et al., 1992; Hermanson et al., 1992; 1996; Kumabe et al., 1992).

Several rodent models have been set up to characterize glioma formation using PDGF as an oncogene. There have been essentially two approaches, retroviral insertion or transgenic overexpression. Retroviral overexpression of PDGF has succeeded in producing tumors in embryonic, newborn, and adult mice (Appolloni et al., 2009; Assanah et al., 2006; Dai et al., 2001; Lindberg et al., 2009; Uhrbom et al., 1998). If the PDGF overexpression is combined with additional mutations (such as in tumor suppressor knock-out transgenic mice), the resulting tumors have higher grade, shorter latency and higher incidence (Hambardzumyan et al., 2009). Transgenic over-expression of PDGF has resulted in hypercellularity of oligodendrocyte precursors upon PDGF overexpression in neurons (Calver et al., 1998) or oligodendrocytes (Forsberg-Nilsson et al., 2003). However, despite the increase in cell number neither study showed tumor development. Similarly, overexpression of PDGF in embryonic neural stem cells did not cause hyperplasia or tumors (Niklasson et al., 2010). For tumors to develop from transgenic overexpression, it seems that additional mutations are required (Hede et al., 2009). The difference in tumor formation between retroviral and transgenic overexpression of PDGF highlights the importance of insertional mutagenesis in this process (Johansson et al., 2004).
Glioma

Glioblastoma is the most common primary malignant brain tumor afflicting 3 out of 100,000 individuals each year (Dolecek et al., 2012). Complete resection is almost impossible to obtain due to the invasive nature of the tumor, thus the tumor recurs in nearly every patient (Lowenstein and Castro, 2012). The standard care of treatment for glioblastoma is surgery followed by radiation and chemotherapy. Radiation therapy with concomitant administration of the alkylating agent temozolomide followed by adjuvant temozolomide has proven beneficial with a 2.5-month increase in median survival. Even so, the median overall survival is still poor at ~15 months (Stupp et al., 2005).

Clinical features, WHO classification

Tumors of the central nervous system are classified and graded according to guidelines from the World Health Organization (WHO). The classification is based on histological resemblance to normal cells and does not necessarily reflect the cellular origin of the tumor. Tumors are graded on a “malignancy scale” from grade I to IV with grade IV being the most malignant. Grade I tumors usually has a low proliferative potential and can often be treated by surgical resection. Tumors with grade II on the other hand are infiltrative and often recur despite low rates of proliferation, some types also progress into higher-grade tumors. Lesions with grade III have histological features of malignancy such as nuclear atypia and mitotic activity. Finally, grade IV tumors are neoplasms that are cytologically malignant, mitotically active and prone to necrosis. A rapid disease evolution and fatal outcome accompanies grade IV tumors, in the central nervous system exemplified by glioblastomas, many sarcomas and most embryonic neoplasms (Louis et al., 2007).

The most common primary neuroepithelial tumors are gliomas where the main types are astrocytomas, oligodendrogliomas and ependymomas (Dolecek et al., 2012). Astrocytomas occur with various grade of malignancy, starting with the low-grade tumors pilocytic astrocytoma (grade I) and subependymal giant cell astrocytoma (grade I). The spectrum of diffuse infiltrative astrocytomas starts with pleomorphic xanthoastrocytoma (grade II) and diffuse astrocytoma (grade II). Grade II astrocytomas are defined as neoplasms with only cytological atypia. Tumors that also features mitotic activi-
ty and anaplasia (cellular dedifferentiation) are denoted anaplastic astrocytomas (grade III). Neoplasms with further addition of microvascular proliferation and/or necrosis are diagnosed as glioblastoma (grade IV).

WHO grades are used as a tool to predict outcome and response to treatment. Clinical findings such as age, tumor location, radiological features, extent of surgical resection, proliferation index and genetic alterations also contribute to the overall estimate of diagnosis. However, the predictive power of tumor grade is manifested in differences in survival. Patients with grade II tumors typically survive more than 5 years while overall survival for patients with grade III tumors is 2-3 years. When it comes to grade IV tumors the prognosis depend on whether there are effective therapy regimens at hand for the actual tumor (Louis et al., 2007). While glioblastoma patients survive only ~15 months (Stupp et al., 2005), patients with grade IV medulloblastoma have a 5-year survival of 60-70%. The increase in survival among medulloblastoma patients is attributed to recent advantages in surgery, anesthesia, neuroimaging, peri-operative care and combination regimens with both radiation therapy and chemotherapy.

Mutations and perturbed signaling pathways in Glioma

There is a distinction between primary and secondary GBM. Secondary GBM progress from a lower grade diffuse astrocytoma or anaplastic astrocytoma whereas primary GBM present at diagnosis as fully developed tumors without evidence of previous lesions. This distinction is reflected in the mutations and perturbed signaling pathways important for GBM disease progression. In short, primary GBM is characterized by EGFR amplification and PTEN mutations, while mutations in TP53 are genetic alterations leading to secondary GBM. Loss of heterozygosity of chromosome 10q is frequent in both primary and secondary GBM. (Ohgaki and Kleihues, 2007). EGFR amplification occurs in 40% of primary GBM and of these 70% also harbor rearrangements of the gene (Ekstrand et al., 1991; 1992; Malden et al., 1988; Sugawa et al., 1990; Yamazaki et al., 1988). An increase in PDGF ligand and receptor expression has been demonstrated in astrocytic tumors (Nistér et al., 1988), but gene amplification is only seen in a subset of GBM (Hermanson et al., 1992). The tumor suppressor PTEN is seen mutated in 20% of anaplastic astrocytomas and 25% of primary GBMs (Davies et al., 1999; Ohgaki and Kleihues, 2007; Watanabe et al., 1998). Another important tumor suppressor is TP53, which is mutated in 65% of secondary GBM (Ohgaki and Kleihues, 2007; Watanabe et al., 1997; 1996). The rate of TP53 mutation in primary GBM is significantly lower at around 30% (Ohgaki et al., 2004; Watanabe et al., 1996). Loss of the tumor suppressor P14ARF occurs in 76% of GBM. P14ARF bind MDM2 thereby inhibiting TP53 degradation (Nakamura et al., 2001). Finally, loss of heterozygosity of chromo-
some 10q is frequent in GBM with no difference between primary and secondary GBM (Balesaria et al., 1999; Ichimura et al., 1998; Ohgaki and Kleihues, 2007).

In conclusion, glioblastomas display frequent genetic alterations in RTK/RAS/P13K pathways, \(TP53\) signaling and \(RB\) signaling. As many as 74% of GBMs harbor mutations in all three of these core pathways. This is in concordance with the notion that tumors progress by evading senescence and apoptosis, increasing proliferative signaling and avoiding cell cycle arrest (Cancer Genome Atlas Research Network, 2008).

**Glioblastoma molecular subclasses**

In glioblastoma, molecular subgroups have been described. Based on genetic and epigenetic profiles these molecular subclasses can to some extent be used to predict prognosis and response to therapy (Brennan et al., 2009; Phillips et al., 2006; Sturm et al., 2012; Verhaak et al., 2010). GBM is a highly heterogeneous tumor, and subtyping of GBM patients has emerged as a novel way of classifying tumors with the aim of finding stratified treatment modalities.

According to the study based on the largest set of samples, GBM can be divided into the Proneural, Neural, Classical and Mesenchymal subtypes, referred to as the TCGA classification (Verhaak et al., 2010). A focal amplification of the 4q12 locus harboring \(PDGFRA\) together with high levels of \(PDGFRA\) gene expression define Proneural gliomas, a subclass that also harbors an enrichment of \(IDH1\) point mutations. The Neural subtype has an increased expression of neuronal markers such as \(NEFL\), \(GABRA1\), \(SYT1\), and \(SLC12A5\). Classical glioblastomas display \(EGFR\) alterations in 97% of cases together with a distinct lack of \(TP53\) mutations. Chromosome 7 amplification together with loss of chromosome 10 was frequent in Classical glioblastomas, which also feature focal loss of 9p21.3 targeting the \(CDKN2A\) locus. Deletions of the \(NFI\) gene and increased expression of genes in the tumor necrosis factor super family pathway are features of the Mesenchymal subtype. Also high expression of genes in the \(NF-kB\) pathway and expression of mesenchymal markers such as \(CHI3L1\) and \(MET\) characterizes Mesenchymal glioblastomas.

The more recent classification by Sturm et al. (2012) also takes methylation patterns into account and includes pediatric glioblastomas. This has lead to a refined TCGA categorization by confirming the Classical (called RTKII) and Mesenchymal subtypes, while dividing the Proneural subtype into RTKI/\(PDGFRA\)-amplified tumors and \(IDH\)-mutated tumors. In addition, distinct mutations in the \(H3F3A\) gene coding for a histone protein corresponds to epigenetic profiles with global methylation patterns.
Glioma models

Attempts to model glioma began in the 1940s and 1950s with transplantation of human brain tumors into rodents (Greene, 1952; Greene and Arnold, 1945). In the mid 1960s the first chemically induced rat brain tumor model emerged (Druckrey et al., 1965). At the same time the athymic nude mouse was discovered which increased the reliability of transplantation (Flanagan, 1966). In parallel, adherent glioma cell lines were established from human tumors (Pontén and Macintyre, 1968; Westermark et al., 1973). In the 1990s the advent of genetically engineered mouse models (GEMMs) (Danks et al., 1995; Holland et al., 1998) opened up opportunities to manipulate genes involved in molecular pathways important for tumor initiation and propagation. The most recent additions to the “modeling toolbox” include xenograft models based on GBM cultured using stem cell methods (Lee et al., 2006). (For a historical overview see Huszthy et al. (2012))

Chemically induced models

N-nitroso compounds such as methyl nitrosourea and ethyl nitrosourea can be used to induce glioma-like lesions in rats (Schmidek et al., 1971). Cell lines have been created by cloning tumors induced with N-nitroso compounds, such as the C6, 9L and CNS-1 (Barth and Kaur, 2009). Worth noting is that the C6 line was induced in an outbred Wistar rat, making it allogeneic with all available inbred strains. This is evident by the strong humoral immune response developed when introducing intracranial and subcutaneous C6 tumors simultaneously, leading to a survival rate of 100% compared to 11% with a single intracranial injection (Parsa et al., 2000).

N-nitroso compounds have been less proficient in producing tumors in wildtype mice, but in a p53 knockout transgenic model gliomas and medulloblastomas are formed upon transplacental injections of ethyl nitrosourea (Oda et al., 1997).

Adherent serum-derived human glioma cell lines

In addition to the U-series (Pontén and Macintyre, 1968; Westermark et al., 1973) of permanent GBM cell lines established in Uppsala, the D- (Bigner et al., 1981), LN- (Studer et al., 1985) and SF-series (Rutka et al., 1987) have been established at Duke, Lausanne and University of California San Francisco respectively. These lines have been utilized extensively in glioma research both for in vitro studies and xenograft transplantation.

When serum-derived adherent lines are xenografted the resulting tumors does not fully reflect the histological appearance of the human disease. For instance, there is limited single cell infiltration into healthy tissue and ne-
croses and microvascular proliferation are often absent (Lee et al., 2006; Mahesparan et al., 2003).

When serially propagating tumor material in nude mice, another set of challenges becomes apparent. When compared to the original biopsy, only one out of seven tumors retained their chromosomal profile (Bigner et al., 1989). Furthermore, array comparative genomic hybridization and whole genome sequencing revealed that serum-derived cell lines displayed genomic profiles distinct from those typically found in GBM primary tumors (Clark et al., 2010; Ernst et al., 2009). Finally, a report demonstrated that gene expression profiles of serum-derived adherent GBM lines were not representative of primary human GBM (Li et al., 2008).

Better results regarding these matters have been reported when using biopsy spheroid cultures, which are made up of minced pieces of tumor grown in serum-containing medium on soft agar (Bjerkvig et al., 1990). When transplanted, the resulting tumors display single cell infiltration. Additional GBM traits e.g. microvascular proliferation and necrosis occur after serial xenografting (Sakariassen et al., 2006; Wang et al., 2009a). Lastly, genomic profiles of biopsy spheroid cultures seem more representative of the parental gliomas (De Witt Hamer et al., 2008).

Spheroid cultures retain parts of the microenvironment of the parent tumor such as resident macrophages, vessels and extracellular matrix components. This could be an explanation for the seemingly better behavior of spheroid cultures compared to adherent serum-derived lines regarding histopathological traits and genomic profiles (Huszthy et al., 2012).

Glioma cells cultured with neural stem cell methods

By using protocols used to culture adult neural stem cells from rodents (Reynolds and Weiss, 1992) and humans (Johansson et al., 1999b), researchers have been able to culture glioma cells that harbor neural stem cell traits (Hemmati et al., 2003; Ignatova et al., 2002; Singh et al., 2003). In short, cells are grown as free-floating aggregates called neurospheres in serum-free medium supplemented with insulin, EGF and FGF-2. When xenografted, these cells retain features of their parent tumor (Galli et al., 2004) such as an infiltrative behavior and the presence of necrosis and microvascular proliferation. In addition, the molecular profile is stable over time and is closer to the parent tumor (Chen et al., 2010; Günther et al., 2008; Lee et al., 2006). In another report, glioma cells cultured with neural stem cell methods gave rise to xenografts with a prominent invasive behavior (Schulte et al., 2011), an important feature when modeling the disease.

An adaptation to the neurosphere technique has been reported where cells are cultured adherent on a laminin substrate (Pollard et al., 2009a). This facilitates high-throughput screening, clonal culture and also circumvents some of the issues with neurosphere culture such as inadvertent fusion of
spheres, differentiation and lack of access to nutrients within the sphere (Reynolds and Rietze, 2005; Singec et al., 2006; Suslov, 2002). Whether sphere culture or adherent culture is preferred is surrounded by some controversy (Pollard et al., 2009b; Reynolds and Vescovi, 2009).

Genetically engineered mouse models
There are two main objections against using xenografts to model glioma. The first issue is that the mice used as hosts lack a proper immune response. Tumorigenesis to some extent involves evading immune surveillance as will be mentioned in more detail below. Thus, the selection mechanisms of cancer cells during tumor development as a result of immune system effects are not represented in xenograft models. The other issue is that a large number of tumor cells are usually used to initiate tumors which is different from the general conception that tumorigenesis consists of a series of events that starts with the transformation of a single cell (Hambardzumyan et al., 2011).

The advent of advanced genetic tools and an increased knowledge of the genetic aberrations involved in brain tumorigenesis lead to the creation of several GEMMs focusing on different aspects of glioma biology. The beauty of GEMMs is the possibility to dissect molecular pathways and study the influence of single mutated genes on tumor initiation and propagation. The first models introduced overexpressed viral oncogenes (Brinster et al., 1984). If germline mutations are introduced that inevitably turn normal cells into tumor cells, the resulting mouse would most certainly be embryonic lethal. Therefore these models rely on subsequent mutations to occur for the initiation of tumors and can be considered models for cancer predisposition. The development of technologies that gives temporal and spatial control of gene expression allow for postnatal tumor initiation. GEMMs are also useful in the context of tumor/stroma-interactions since the mice are endowed with (depending on the mutation induced) a fully functional immune system.

Several models have been reported with mutations or overexpression in pathways important for glioma such as Ras, Akt, Rb, PDGF and EGFR (Guha, 1998; Henson et al., 1994; Holland et al., 2000; Ueki et al., 1996). (For an overview see Huszthy et al. (2012).) Below is an overview focused on modeling different molecular subtypes of glioma. The different molecular subclasses can be narrowed down to circle around mutations in PDGF, EGF and NF1 pathways. These pathways are linked since PDGFR and EGFR both activate Ras/Mapk pathways and NF1 is a GTPase-activating protein for Ras. In short, the three different groups of mutations all seem to induce altered Ras activation.

PDGF models
In addition to transgenic models for PDGF-driven tumors, two models for the study on PDGF effects on tumorigenesis make use of retroviral transfer
of PDGF-containing vectors. The first model uses a construct with a recombinant Moloney murine leukemia virus containing PDGF-B to infect dividing cells (Uhrbom et al., 1998; 2000). The second model uses RCAS/tv-a technology (Fisher et al., 1999) to induce PDGF expression in different cell types depending on the gene controlling tv-a expression. Three tv-a mouse lines are used for this purpose, the first two express tv-a under the NES and GFAP promoters respectively (Holland and Varmus, 1998; Holland et al., 1998) and has been used to generate PDGF-B driven tumors (Dai et al., 2001). The third tv-a line express tv-a under the control of CNP, a marker for immature oligodendrocytes, and was used to demonstrate for the first time that gliomas can develop in a committed glial progenitor (Lindberg et al., 2009). PDGF overexpression together with loss of tumor suppressors is reported to give tumors of higher malignancy (Tchougounova et al., 2007). As described earlier in this thesis, overexpression of PDGF ligands in the CNS is not sufficient to induce tumors (Calver et al., 1998; Forsberg-Nilsson et al., 2003).

NF1 models

NF1 is a tumor suppressor and is mutated in patients with Neurofibromatosis type I. Mutated NF1 is a genetic predisposition factor for glioma since the prevalence of glioma in persons with NF1 mutations is higher than in the general population (Gutmann et al., 2003; 2002). When an Nf1+/- mutation is introduced in a mouse already harboring a Trp53+/- mutation, glioma arises (Reilly et al., 2000). This model is hampered by incomplete and variable penetration, but was used to delineate the influence of mouse strain genetic background on tumor incidence (Reilly, 2004). The next generation of NF1 mice uses a GFAP-driven cre/lox (Kilby et al., 1993; Macleod and Jacks, 1999) system to introduce different combinations of null and null conditional alleles of Nf1 and Trp53 tumor suppressors. These mice develop tumors with 100% penetrance and demonstrate that it is sufficient to mutate two tumor suppressors to induce malignant gliomas (Zhu et al., 2005). Intriguingly, the order of Nf1 and Trp53 mutations is important for the development of tumors. Gliomas were induced only when loss of Trp53 preceded or coincided with loss of Nf1 (Wang et al., 2009b; Zhu et al., 2005).

EGFR models

Overexpression and amplification of EGFR is a common genetical aberration in high-grade glioma (Ekstrand et al., 1991). The first glioma model with a variant of EGFR introduced a transforming variant of EGFR (v-erbB (Burgess, 2008)) under the control of the S100b promoter, which induced low-grade oligodenrogliomas in 20% of founder mice. In addition, the penetrance and malignancy increased considerably when the loss of Trp53 or Cdkn2a was introduced (Weiss et al., 2003). Given the S100b-v-erbB induced tumors it is unexpected that another transforming variant of EGFR
(EGFRvIII (Gan et al., 2013)) was unable to drive gliomagenesis under the control of the GFAP promoter. This indicates that the tumor inducing capacity of EGFR variants is dependent on the cellular environment. When EGFRvIII was co-expressed with an active form of Ras, oligodendrogliomas occurred (Ding et al., 2003). Co-dependence on tumor suppressor loss was demonstrated in a cre/lox model where either Cdkn2a or Pten loss is required for tumors to develop upon EGFRvIII expression in adult animals (Zhu et al., 2009). Taken together, this evidence indicates that high levels of EGFR in itself is not an oncogenic event (Hambardzumyan et al., 2011).

Cancer stem cells

The concept of cancer stem cells was originally hypothesized in the field of hematopoietic cancers (Bonnet and Dick, 1997; Lapidot et al., 1994). The term cancer stem cell comes from the observation that only a subset of tumor cells has the ability to form new tumors. These cancer stem cells share many traits with normal stem cells, and often it is the signaling pathway responsible for normal stem cell self-renewal that lead to tumorigenesis when dysregulated. Whether this means that it is the stem cell that is the target of neoplastic transformation or another more differentiated cells is not clearly demonstrated (Fig. 3). In general, stem cells have the ability to self-renew and differentiate to produce tissue-specific mature cells. The ability to undergo self-renewal means that stem cells continously divide in tissues over long periods of time, thus increasing the likelihood of accumulating mutations that cause neoplasia (Reya et al., 2001).

![Diagram of neural stem cell, neural progenitor, and mature cell](image)

Figure 3. Cells of the neural lineage are possible candidates for brain tumor initiating cells.
Cancer stem cells has the ability to self-renew, to give rise to new tumors and to produce all of the different cells found in the tumor. Experiments on acute myeloid leukemia (AML) indicate that cancer stem cells are rare cells and that only a few cells has the ability to seed new tumors (Bonnet and Dick, 1997; Hope et al., 2004). A more recent study raises the concern that the low estimate of cancer stem cell number in fact is dependent on the immunocompetence of the host used for grafting of the AML cancer stem cell (Kelly et al., 2007). This reasoning is also valid for melanoma as demonstrated by Quintana et al. (2008).

Demonstrating the presence of cancer stem cells in solid tumors has been considerably harder, in part owing to the lack of defined markers to prospectively sort for stem cells. In addition, in vivo models of for instance lung, colon and bladder cancers are technically challenging, which further complicates analysis (Ailles and Weissman, 2007). Breast cancer was the first solid tumor that were reported to harbor cancer stem cells (Al-Hajj et al., 2003), later more examples have been demonstrated such as pancreatic, colon, and prostate cancer (Ailles and Weissman, 2007).

In the field of brain tumors, there have been a few studies demonstrating culture of tumor cells with neural stem cell culture methods but without prospectively sorting cells (Galli et al., 2004; Hemmati et al., 2003; Ignatova et al., 2002). The first study to prospectively sort cells used the glycoprotein CD133 as a glioma stem cell marker (Singh et al., 2003). Later studies has questioned the use of CD133 as a marker for glioma stem cells, since cells negative for CD133 were reported to give rise to new tumors (Beier et al., 2007; Chen et al., 2010; Wang et al., 2008). Other markers such as integrin α6, SSEA-1 and A2B5 have been used to prospectively sort for tumorigenic cells (Lathia et al., 2010; Ogden et al., 2008; Son et al., 2009). Perhaps the elusive identity of glioma stem cells relates to the importance of interactions with the microenvironment for tumor-initiating capacity (Prestegarden and Enger, 2010). It is tempting to assume that the glioma stem cell model infers that the cell of origin is in fact a neural progenitor. However, this cannot be taken for granted. Several parallel lines of evidence points to either differentiated astrocytes, neural progenitors or neural stem cells (Fig. 3) as the cell of origin of glioma (Jiang and Uhrbom, 2012; Stiles and Rowitch, 2008).
Glioma and the immune system

Immune responses in the CNS

The brain elicits a distinct immune response not seen in other organs, largely due to the different properties of the CNS compared to other tissues. A series of studies initiated almost a century ago pointed to a lack of immune response to antigens from grafts to the brain parenchyma. A few decades later the term “immune privileged” was coined by Billingham and Boswell (1953), to describe a lack of immune response. This has later been shown to be true for various antigens presented to the brain parenchyma (reviewed by Galea et al. (2007)). The blood-brain barrier (BBB) and a relative lack of lymphatic drainage are key features of the CNS important for the distinct immune response in the brain. In essence, the BBB is composed of specialized endothelial cells with tight junctions and adherens junctions (Hawkins and Davis, 2005; Hermann and Elali, 2012) that provide a physical barrier separating the CNS from the circulation. It is also a selective exchange barrier allowing for the entrance of nutrients and peptides needed for proper neuronal function (Lampron et al., 2013), as well as of leukocyte recruitment mainly upon disease and injury (Ransohoff et al., 2003).

The brain has no proper lymphatic drainage into dedicated lymph vessels. Instead, the cerebrospinal fluid exits the brain through the thin bone structure called the cribriform plate and ends up in lymphatics in the nasal mucosa (Cserr and Knopf, 1992). As much as 50% of injected albumin into the caudate nucleus was recovered from cervical lymph indicating that there is a significant drainage of CSF (Boulton et al., 1999). Dendritic cells, the professional antigen presenting cells, are not found in the healthy brain parenchyma but are present in the choroid plexus and the meninges (Matyszak and Perry, 1996; McMenamin, 1999). No priming of naive T lymphocytes occur in the brain, further indicating the lack of resident dendritic cells (Mendez-Fernandez et al., 2005). Upon inflammation, dendritic cells are found in the brain parenchyma (Matyszak and Perry, 1996) but it is uncertain whether they migrate out of the parenchyma to prime T or B lymphocytes in the cervical lymph nodes or if they have functions within the inflamed CNS (Galea et al., 2007).

The innate immune system in the CNS is largely made up of microglia, although recruitment of granulocytes and monocytes occur upon damages such as infections and chronic diseases like multiple sclerosis (Wilson et al.,
Microglia are resident macrophages in the CNS, estimated to make up 5-20% of all glial cells (Benveniste, 1997; Lawson et al., 1992; 1990). Primitive myeloid progenitors invade the developing CNS already before E8 in the mouse and contribute to the majority of the pool of adult microglia (Ginhoux et al., 2010). Resting microglia are found “roaming” the brain parenchyma and examine the microenvironment through pinocytosis and by interacting with neurons (Nimmerjahn et al., 2005). When activated, microglia show increased proliferation, migration, phagocytic activity and release of cytokines and reactive oxygen species (Carbonell et al., 2005; Eliason et al., 2002; Liu et al., 1998; Zhang et al., 2001).

The immune response in glioma

Patients with glioma have reduced immune function evident by impaired lymphocyte activation (Brooks et al., 1972; Dix et al., 1999; Elliott et al., 1984), increased expression of FasL (Badie et al., 2001), and altered cytokine secretion from microglia (Kostianovsky et al., 2008). Even though almost one third of the invading cells in gliomas express microglia markers (Badie et al., 2000), tumors continue to grow aggressively suggesting that the anti-tumor activity of microglia is low. Not only resident microglia infiltrate glioma, several studies report that macrophages originated from peripheral bone-marrow derived mononuclear cells associate with tumors. However, the exact lineage origin for these macrophages has been notoriously hard to decipher (reviewed by Charles et al., 2012 and Kennedy et al., 2013). To further increase the complexity, tumor-associated macrophages are polarized; different subtypes are described based on activating signals, receptor expression, cytokine production and behavior (reviewed by Kennedy et al., 2013).

Microglia present in and around tumors seem to promote tumor growth. In part by the previously mentioned immunosuppression but also by the secretion of matrix metalloproteinases (MMPs) (reviewed by Charles et al., 2012). In addition, normal features of microglia such as phagocytosis and antigen presentation are suppressed in the context of glioma (Flügel et al., 1999; Hao et al., 2002). Not only microglia but also T and B lymphocytes, NK cells, mast cells and dendritic cells infiltrate brain tumors (Polajeva et al., 2011; Tran Thang et al., 2010; Yasuda et al., 1983).

Mast cells

Mast cells are myeloid derived sentinel cells of the immune system with a dual nature; depending on the context their actions are beneficial or detrimental (Leslie, 2007). One prominent cellular feature is the secretory gran-
ules that contain several mediators e.g. proteases, cytokines, histamine and proteoglycans (Metcalfe et al., 1997). Found in regions where the body meets the environment, e.g. in airways and in the skin, their pathological role in anaphylaxis and asthma is well documented. In these archetypical allergic reactions mast cells release the contents of their granules upon IgE-receptor stimulation, causing swelling of the mucous membranes and itching skin (Galli et al., 2008b). In addition to their role in allergy mast cells have proven essential as a first line defence against bacterial infections, demonstrated in e.g. a model of induced peritonitis (Echtenacher et al., 1996; Malaviya et al., 1996).

In rodents, mast cells were first categorized as mucosal-type or connective-tissue-type based on their dye-binding properties (Enerbäck, 1966b; 1966a). Human mast cells are categorized based on their protease content; tryptase-only, chymase-only and both tryptase- and chymase-containing types have been described (Moon et al., 2009). Mast cells originate in the bone marrow and circulate the blood stream as immature precursors (Kitamura et al., 1977; Rodewald et al., 1996). The maturation and subsequent granulation occurs in peripheral tissues (Galli et al., 2008a; Moon et al., 2009). Different peripheral tissues offer different microenvironments, so it is perhaps not surprising that mast cell heterogeneity between and within different locations has been described (Moon et al., 2009). Rodent mast cells even seem to have the ability to trans-differentiate between mucosal and connective-tissue type mast cells (Kitamura, 1989). In the brain, mast cells are found in perivascular locations in the parenchyma and meninges where they are thought to promote BBB damage, brain edema and prolonged extravasation in the case of cerebral ischemia (Lindsberg et al., 2010). In the healthy rodent, mast cells are found in the hippocampal formation (Nelissen et al., 2013).

Mast cells and tumors

Mast cells have a dual nature, in addition to their essential function in responding to cell injury and defending bacterial infections they can also play detrimental roles in the context of tumors, for example by inducing tumor angiogenesis and dampening the immune response (reviewed by Maltby et al., (2009)). With such an abundance of secreted factors, it is no suprise that the effects of mast cells are numerous and diverse. For instance, mast cells secrete MMPs that disrupt the ECM potentially increasing tumor invasiveness. VEGF, FGF-2 and IL-8 are examples of pro-angiogenic factors that induces neovascularization. Another detrimental effect is the induction of tumor growth seen from secretion of FGF-2, VEGF, PDGF, SCF and IL-8. At the same time, secretion of tryptase, chymase, IL-1, IL-4, IL-6, TNF-α, TGF-β, INF-α, LTB4 and chondroitin sulfate exert beneficial effects such as
tumor inhibition, tumor apoptosis, inhibition of metastasis and inflammation (Ribatti and Crivellato, 2009).

Mast cells have been implicated in a variety of tumors, some of which are mentioned here. Mast cells have been shown to be essential for polyp development in hereditary colon cancer (Gounaris et al., 2007). In a pancreatic β-cell tumor model, mast cell recruitment is essential for tumor expansion and blocking mast cell degranulation lead to hypoxia and apoptosis of the tumor (Soucek et al., 2007). In a model of squamous carcinoma, the presence of mast cells coincides with an increase in angiogenesis and MMP-9 activation (Coussens et al., 1999). Finally, mast cell infiltration is correlated to poor prognosis in Hodgkin’s lymphoma, where higher number of mast cells is coupled to nodular sclerosis histology and worse relapse-free survival (Molin et al., 2002).

**Mast cells in glioma**

In the first study to demonstrate mast cell accumulation in glioma Põlajeva et al. (2011) reported that higher number of infiltrating mast cells correlates to higher tumor grade. Furthermore the study shows that mast cells express high levels of CXCR4 and respond to CXCL12 secreted by glioma cells by infiltrating the tumor. Whether mast cells play a beneficial or detrimental role in glioma remains to be determined.
Present investigation

Aims

- **Paper I** – to elucidate the adhesion properties of mouse NSPC at different developmental stages.
- **Paper II** – to characterize the effect of PDGF-B overexpression in neural stem cells during mouse development.
- **Paper III** – to analyze if molecular subclasses of glioma can be modelled in vitro by using stem cell culture methods.
- **Paper IV** – to investigate mechanisms for grade-dependent mast cell recruitment to glioma.

Results and discussion

**Paper I – Temporally Regulated Collagen/Integrin Interactions Confer Adhesive Properties to Early Postnatal Neural Stem Cells**

The extracellular matrix is an important part of the neural stem cell niche in the SVZ. Keeping cells in specific positions is an important tool in regulating stem cell behavior. The laboratory has previously described that early postnatal neural stem/progenitor cells (NSPC) from the SVZ adhered to a hydrogel composed of collagen and hyaluronan, and that this culture paradigm favored neuronal differentiation (Brännvall et al., 2007). In the present study, this adhesive behavior was further analyzed. NSPC from embryonic, early postnatal and adult mice were seeded on collagen I, but only early postnatal cells were capable of adhering to the substrate. The adherent cells stained positive for the focal adhesion component paxillin, which suggests that the adhesion was mediated by integrins. Integrins α1β1, α2β1, α10β1, α11β1 bind collagen with varying affinities for collagen I. Indeed, blocking antibodies directed to integrin β1 could inhibit the adhesion. Furthermore, early postnatal NSPC express higher mRNA amounts of integrin collagen receptor subunits α1, α2 and α11 than the other ages investigated, which may explain the distinct adhesive behavior observed. Next, we turned our attention to the subventricular zone, and in particular the presence of collagen I. We found collagen I expressed on blood vessels in both the adult and early postnatal SVZ, indicating that interactions through integrin collagen recep-
tors may constitute a possible regulatory mechanism for NSPC in the SVZ. Integrin α2β1 has also been reported to bind laminin subunits α2 (Colognato, 1997) and α5 (Doi et al., 2002), both of which are highly expressed on blood vessels in the SVZ (Kazanis et al., 2010). Laminin α5 together with laminin subunits β1 and γ1 (also found on blood vessels in the SVZ (Kazanis et al., 2010)) form the laminin trimer LM-511 that has been reported to support embryonic stem cell self-renewal in vitro (Domogatskaya et al., 2008). This provides a rationale for continuous evaluation of integrin α2 expression and function in the context of neural stem cell regulation.

The hydrogel used in the previous study (Brännvall et al., 2007) contained both hyaluronic acid and collagen and provided a three-dimensional (3D) environment for cells to grow in. A 3D matrix composed of two ECM molecules offers many parameters to explain the difference seen in adhesion and differentiation potential. In an attempt to analyze the different components important for the observed cellular responses, the present study has so far been focused on integrin-mediated adhesion to collagen. We concluded that differences in integrin expression were the likely reason for the observed differences in adhesion. We aim to further study CD44/hyaluronan interactions since CD44 is known to modulate focal adhesions (Cohen et al., 2006) and a variety of cellular responses including differentiation (Ponta et al., 2003).

Another parameter to be considered is the stiffness of the hydrogel, which is considerably softer than regular culture plastics. It has been shown that maturation of neurons is enhanced on a soft gel coated with fibronectin (Teixeira et al., 2009). Preliminary results indicate that P6 NSPC seem to differentiate to neurons to a greater extent on top of a collagen gel compared to collagen-coated culture plastics. However, this setup does not separate collagen concentration from matrix stiffness as potential causes for the observed behavior. At present, a method to study matrix stiffness has been set up using polyacrylamide, to create matrices of various rigidities (Tse and Engler, 2010). On these substrates, differentiation will be evaluated on matrices with varying stiffnesses but with the same concentration of collagen. This will shed light on the process of mechanosensing, which is believed to be mainly mediated by integrins via focal adhesions (Schwartz and DeSimone, 2008).

Paper II – Enlarged Ventricles and Aberrant Behavior in Mice Overexpressing PDGF-B in Embryonic Neural Stem Cells

It has been well established that deregulated PDGF signaling is involved in developmental defects and brain tumorigenesis (reviewed by Heldin & Westermark (1999)). Also, it has been shown that neural stem cells in culture respond to PDGF signaling (Erlandsson et al., 2001; 2006). Therefore we set out to investigate the effects of overexpressing PDGF-B in neural
stem cells during development in vivo. A transgenic mouse, nes/tk-PdgfB-lacZ, was created with PDGF-B expressed under the control of the minimal nestin enhancer in the second intron of the Nestin gene. Postnatal transgenic mice had enlarged ventricles, more pronounced in males, which remained into adulthood. In addition, adult male transgenic mice showed behavioral aberrations and locomotor dysfunction.

Enlarged ventricles can be found in other transgenic mice models, all of which in addition display behavioral defects. The Waved-1 mutant (Burrows et al., 2000) is reported to have a reduced ability of fear conditioning, the NCAM-180 knockout mouse (Wood et al., 1998) have a decreased prepulse inhibition of startle and the Disc-1 transgenic mouse display signs of schizophrenia (Shen et al., 2008). All of the models above in addition demonstrate enlarged ventricles. This further supports the correlation between ventricle size and behavioural defects seen in the PDGF-B transgenic mice and suggests a novel correlation between embryonic PDGF levels and behaviour.

The nes/tk-PdgfB-lacZ transgenic mice also displayed an increased apoptosis in the developing striatum suggesting an altered regulation of progenitor populations. However, we found a normal composition of cell numbers and types in the brain and the transgenic mice were viable and fertile. There were no signs of hyperplasia or tumors in the transgenic brains. This probably has several explanations. First, due to the use of a minimal nestin enhancer, PDGF-B expression is expressed in a narrow temporal window. When the extensive gliogenesis commences at around embryonic day 16 in the mouse, the transgenic expression of PDGF-B is limited. Second, our approach, in contrast to retroviral overexpression, rarely introduces secondary mutations (Rijkers et al., 1994), which most likely are needed for neoplastic development.

We also found that nes/tk-PdgfB-lacZ mice displayed a severely disturbed development of the retina, with disorganization of retinal layers. This has later been investigated in detail as described in Edqvist et al. (2012). Due to PDGF-B overexpression, regression of the hyaloid vasculature was delayed, preventing astrocyte precursor cells to populate the retina. This resulted in randomly distributed capillary-like vessels but no formation of large trunk vessels, and the intraocular pressure was reduced.

Paper III – Modeling Human Glioblastoma Subtypes in vitro using Stem Cell Culture Conditions

Hallmarks of GBM include extensive tumor cell infiltration, microvascular proliferation and necrosis. These are features not well represented in xenografts of traditional serum-derived cell lines. In Paper III we describe the establishment and characterization of a novel panel of glioma stem cells (GSC), where tumor biopsies from patients with high-grade glioma were
explanted and propagated under stem cell culture conditions. Cultured under serum-free conditions on laminin and with EGF and FGF-2, cells displayed neural stem cell marker expression in vitro and a capacity to induce brain tumors in vivo. A subset of GSC lines and corresponding tumor tissue were categorized according to TCGA molecular subclasses (Verhaak et al., 2010) by NanoString analysis. We also performed Affymetrix Exon microarray transcriptional profiling of a larger set of the cell lines. By this, we demonstrate that GBM molecular subclasses can be recapitulated in GSC cultures, and that a majority of cell lines retain their parent tumor molecular subclass. We show for the first time that molecular subclasses can be modeled in vitro which provides a useful tool in GBM research.

The discovery that GBM can be divided in molecular subclasses raises interesting questions on the future of GBM diagnostics. From a diagnostic point-of-view, molecular subclasses are only useful if they are clinically relevant. As an example, it is well established that mutated EGFR is a driver in glioma development (Shinojima et al., 2003) but this knowledge has not yet translated into therapies (Huse et al., 2013). One of few examples where molecular analysis adds a valuable clinical parameter is MGMT promoter methylation status that has been shown to be important for temozolomide resistance (Wick et al., 2009). In a recent study, quantitative evaluation of immunostainings of EGFR, PDGFRA and TP53 was used to categorize tumor samples into proneural-like or classical-like subtypes with implications for overall survival (Le Mercier et al., 2012). Although seemingly too simplistic, the approach hints at a possible extension of already established diagnostic paradigms based on immunohistochemical methods. We will therefore continue our analysis of GSC line expression profiles to refine the classification paradigm. The choice of classifier genes and the size of the reference sample sets are important parameters that will greatly influence classification of tumor samples and cell lines. Also, it is not known how stable the molecular subclasses are, i.e. if they could shift due to changes in culture conditions. In an attempt to determine any potential plasticity between subclasses, nanostring analysis of mRNA from GSC lines after growth factor withdrawal, or upon substitution of one growth factor for another, will be performed.

Paper IV – Glioma-Derived Macrophage Migration Inhibitory Factor (MIF) Affects Mast Cell Migration in a STAT5-Dependent Manner

Interactions with the immune system are an important part of tumorigenesis, both evasion of immune response, and the possible detrimental effects of inflammation affects disease progression. In Paper IV we explore grade-dependent mast cell accumulation in glioma and possible mechanisms be-
hind their recruitment. In a previous study (Polajeva et al., 2011), grade-dependent accumulation of mast cells in glioma was described for the first time. The present study confirms the finding in a larger set of patient samples and attempts to delineate the mechanisms behind mast cell migration to glioma. We demonstrate that mast cells migrate toward glioma cell line conditioned medium from cells grown in either defined serum-free medium or serum-containing medium. Cytokine array analysis of the media revealed a number of candidate chemotaxins secreted from glioma cells. We chose to focus on one of these candidates, macrophage migration inhibitory factor (MIF), because of its pro-inflammatory and pro-tumorigenic abilities. Furthermore, we reported a positive correlation between the number of mast cells and the level of MIF, evaluated on a high-grade glioma tissue microarray (TMA).

Mast cell migration was attenuated by blocking antibodies targeting MIF, demonstrating a possible role for MIF in attracting mast cells in the context of glioma. To better understand the mechanism behind MIF-mediated mast cell attraction to glioma cells we performed a phospho-kinase array. Both purified MIF and conditioned medium from glioma cells gave rise to distinct phosphorylation of a number of signaling molecules. One of these was signal transducer and activator of transcription 5 (STAT5). Blocking the phosphorylation of STAT5 lead to an attenuation of MC migration toward conditioned medium from glioma cells. In addition, immunofluorescent analysis of the TMA showed a correlation between levels of phosphorylated STAT5 and MIF in mast cells.

CXCR4 is a receptor for MIF (Bernhagen et al., 2007), and was previously found on mast cells (Polajeva et al., 2011). This offers a possible mechanism for how glioma cells recruit mast cells. Whether mast cell recruitment is a cause of disease progression or a result thereof is not clear at the moment. We hypothesize that the low numbers of mast cell recruited in low grade glioma is associated with an activated antitumorigenic machinery. With increased tumor grade, glioma cells secrete higher levels of cytokines, including MIF, and thus attract more mast cells. In high-grade gliomas, with a higher number of infiltrating mast cells, the effects of mast cells become detrimental.

The possible CXCR4/MIF signaling offers a way to interfere with mast cell recruitment in a therapeutic setting. A specific CXCR4 inhibitor was recently shown to inhibit metastasis and tumor growth in an in vivo breast cancer model (Ling et al., 2013). Furthermore, blocking MIF has been suggested as a potential strategy for treatment of glioma (Wang et al., 2012). Finally, as we demonstrate in the present study, inhibition of phosphorylation of STAT5 lead to decreased mast cell migration in vitro. This may prevent mast cell recruitment and thereby prevent detrimental effects in glioma.
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Back in the days I was a humble, but great, guitar player. When applying for Upper Secondary School I wanted to become a musician but Britt-Marie (my dear mother, I miss you every day) mumbled something about “a waste of resources”. Thus, I ended up at the Natural Science Programme. Coming to Uppsala, I was supposed to become a teacher in mathematics and biology. However, a great series of lectures on the topic of neuroscience by Dan Larhammar made me decide to pursue a scientific career instead. In Åsa Fex Svenningsen’s lab I met Grzegorz Wicher. He taught me how to pronounce his name and how to culture primary cells, sterile. Grzegorz introduced me to the lab of Karin Forsberg Nilsson who accepted me as a PhD student. My co-supervisor Staffan Johansson turned out to be a valuable source of knowledge. One of my colleagues at that time, Jimmy Larsson, inspired me to do experiments instead of just fiddle around. I enjoyed the company of Anna Eriksson and missed her when we moved to the Rudbeck Laboratory. At Rudbeck, luckily, there are many nice persons to compensate for the bad coffee. During my time here I have enjoyed working with and around Lucy, Marianne, Annika, Demet, Lotta, Pernilla, Sara B, Soumi, Mimmi, Anqi and Grzegorz just to name a few. To be honest, I couldn’t have done this without the help and support from you Karin. Thanks for everything! I also want to take the opportunity to thank for the support from my families in Piteå and Sösdala.

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