[Frontiers in Bioscience S3, 1165-1179, June 1, 2011]

Neural stem cell niches: Roles for the hyaluronan-based extracellular matrix

Marnie Preston[1], Larry S. Sherman[1,2]

[1]Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR, [2]Department of Cell and Developmental Biology, Oregon Health and Science University, Portland, OR

TABLE OF CONTENTS

1. ABSTRACT

Neural stem/progenitor cells capable of differentiating into the neurons and glial cells that populate the mammalian central nervous system (CNS) persist in specific neural stem cell niches that regulate stem cell proliferation, survival and differentiation. There is growing evidence that the extracellular matrix within neural stem cell niches is required for neural stem cell maintenance. Here, we review findings supporting a pivotal role for the glycosaminoglycan hyaluronan (HA) and its transmembrane receptors in neural stem/progenitor cell proliferation, differentiation and maturation. We also outline findings supporting changing roles for HA as cells become committed to distinct lineages in the brain and spinal cord.

2. INTRODUCTION

The mammalian nervous system is comprised of multiple subpopulations of neurons and glial cells whose birth, survival, and cell-cell interactions must be carefully regulated throughout life. Both the central nervous system (CNS) and the peripheral nervous system (PNS) arise in the early embryo during neurulation when a flat sheet of neuroectodermal cells, called the neural plate, undergo a series of divisions and morphological changes, causing the sheet to fold and eventually form the neural tube (Figure 1). Neuroepithelial stem cells within the neural tube will give rise to the CNS, while a population of cells along the dorsal neural tube, called neural crest cells, will become the PNS and numerous other structures.

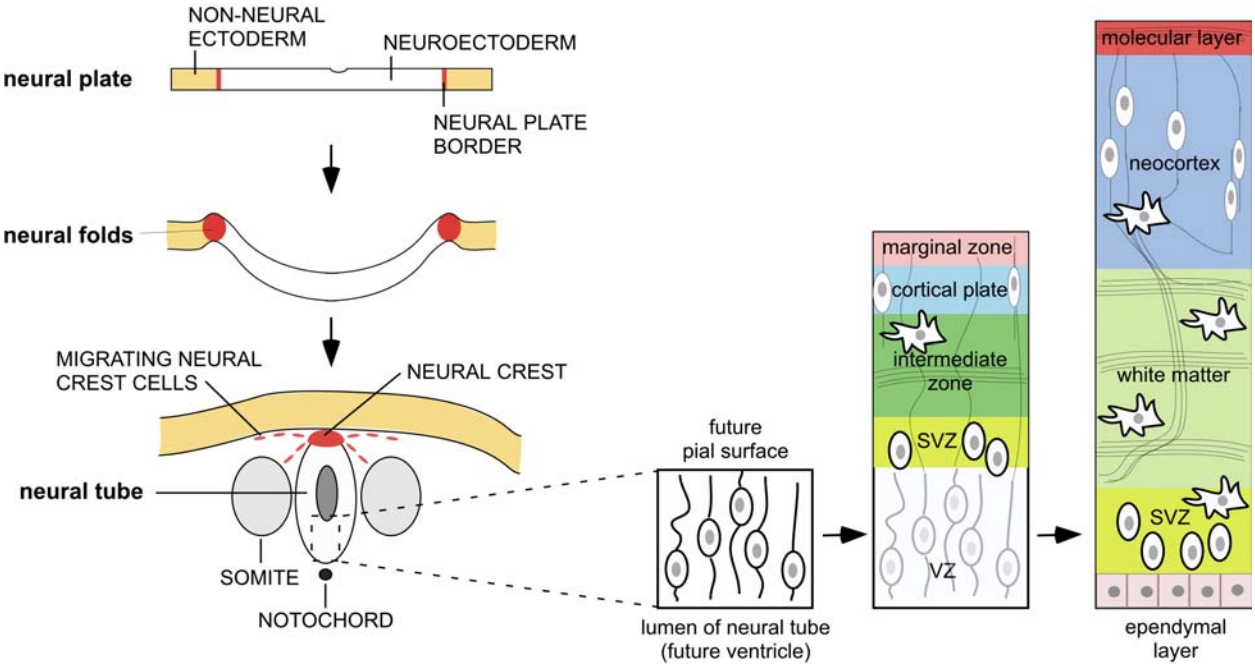

Figure 1. The mammalian nervous system originates from a sheet of neuroepithelial cells (the neural plate). The border between the non-neural ectoderm and the neuroectoderm eventually form the neural folds, whose cells differentiate into neural crest cells that come to reside at the dorsal neural tube. Neural crest cells emigrate from the dorsal neural tube both below the ectoderm and through somites (and, cranially, along branchial arches and other routes) to form the peripheral nervous system and numerous other structures. The cells in the neural tube undergo interkinetic nuclear migration. Symmetrically dividing cells expand the neural tube and eventually undergo asymmetric divisions, giving rise to more neuoepithelial stem cells and neurons that migrate towards the pial surface. These neuroepithelial stem cells come to reside in the ventricular zone (VZ). Some of these cells become radial glia whose processes span from the VZ to the marginal zone at the pial surface, acting as guides for neurons migrating from the VZ. These radial glia also act as a neural stem cell population both in the VZ and the subventricular zone (SVZ) which forms along with the intermediate zone (the future white matter) and the cortical plate, where, in the cerebral cortex, cortical layers occupied by different neurons develop eventually forming the neocortex. By perinatal stages, the adult SVZ has formed with a layer of ependymal cells lining the ventricles. This neural stem cell niche also contains blood vessels, transit-amplifying cells, astrocytes, and extracelluar matrix that all contribute signals that regulate neural stem cell proliferation, differentiation and migration.

As embryonic development proceeds, the neuroepithelial stem cells that make up the neural tube come to reside in the so-called ventricular zone (VZ; Figure 1). These cells initially expand in number and some differentiate into early neuronal populations that migrate toward the pial surface. As development proceeds and the thickness of the early nervous system increases, some of the neuroepithelial cells in the VZ will become radial glial cells that extend their processes from their cell bodies in the VZ to the pial surface. These processes act as guides for later populations of neurons as they differentiate and migrate from the VZ. Later, these radial glia become a neural stem cell population in the VZ and give rise to additional progenitor cells in an area adjacent to the VZ, the so-called subventricular zone (SVZ; Figure 1). While the VZ will eventually disappear, the SVZ (also called the subependymal zone at later stages) persists as a neural stem cell niche throughout adulthood (1). Similarly, in the developing hippocampus, cells in the subgranular zone (SGZ) of the dentate gyrus will form a neural stem cell niche that is a source of new neurons involved in learning and memory throughout life (2).

During the early phase of CNS development, the vast majority of neuroepithelial stem cells and neural stem cells differentiate into neurons. However, by midgestation, neurogenesis declines as neural stem cells shift toward astrocyte and oligodendrocyte generation (3). This timing of differentiation of cells in the CNS is maintained in acutely dissociated cells, such that only neurons arise in cell culture from early embryonic neural tubes, while astrocytes and oligodendrocytes are the predominant population in cultures from later stages. Furthermore, progenitor cells that initially give rise to neurons will differentiate into glial cells if grown for sufficient times *in vitro*. Although cell intrinsic (e.g. epigenetic changes) and cell extrinsic cues have been implicated in the maintenance and the differentiation of neural stem/progenitor cells (NSPCs), both cellular and extracellular elements within neural stem cell microenvironments are critical for regulating neural stem cell behavior during embryogenesis and in adults (4).

Numerous excellent reviews have addressed the roles of growth factors and related extracellular signals as

well as cell-cell interactions in neural stem cell regulation (see (1, (5-7)). Here, we focus on the roles of the glycosaminoglycan hyaluronan in neural stem cell proliferation, differentiation, and survival. We review recent studies from our group and others implicating an instructive role for hyaluronan in nervous system development, maintenance, and repair. Specifically, we will highlight growing evidence that supports a pivotal role for hyaluronan in a number of processes attributed to NSPC quiescence, proliferation and differentiation within neural stem cell niches.

3. THE VENTRICULAR ZONE: AN EARLY NEURAL STEM CELL MICROENVIRONMENT

The VZ of the developing neural tube is a unique microenvironment where the cell bodies of neuroepithelial stem cells move from the luminal side of the neural tube (where the ventricles form) to the future pial surface as they progress through the cell cycle (Figure 1). Mitotic nuclei are located nearest the apical surface, while nuclei undergoing DNA synthesis move towards the basal surface. This movement of cell nuclei by neural epithelial cells was first described based on histological observations (8) and was termed interkinetic nuclear migration (INM) (9). Neuroepithelia with more basal nuclear movements are biased to generate postmitotic daughter cells (10). This may be due to differences in cell signaling as the cells transition from the apical to the basal surface. INM has been proposed to influence the exposure of neuroepithelial stem cells to different gradients of extracellular signals, including Notch signaling, thus balancing the exposure of neuroepithelial cells to neurogenic versus proliferative signals (11). Thus, the micorenvironment of the VZ is one where neuroepithelial cells divide and differentiate in response to localized signals across the expanding neural tube.

4. THE SUBVENTRICULAR AND SUBGRANULAR ZONES: EARLY AND ADULT NEURAL STEM CELL NICHES

By mid-gestation, rodent neuroepithelial cells start to express markers characteristic of astrocytes and they develop a more elongated morphology. These cells, termed radial glial cells, were originally believed to be a transient population in the developing cerebral cortex whose processes guided neurons to different cortical layers. However, more recent findings have indicated that radial glial cells persist and act as a neural stem cell population (12). During later embryonic development, these cells still undergo the INM characteristic of neuroepithelial cells. As the thickness of the nervous tissue increases with the generation of large numbers of neurons, the basal process of radial glia elongate in order to retain attachment to the pial surface (13).

Among the progeny of radial glial cells in the VZ are cells referred to as intermediate progenitor cells and basal progenitor cells that reside in the SVZ ((14); Figure 1). These cells have also been described as radial glial cells (e.g. (15, 16)). The VZ gradually diminishes in rodents such that the SVZ becomes a true neural stem cell niche along the lateral walls of the lateral ventricles. This niche is characterized by a thin area adjacent to the ependymal cell layer that contains infrequently dividing neural stem cells (NSCs), rapidly dividing so-called "transit amplifying precursors" (TaPs) derived from the stem cells; neuroblasts that migrate in chains towards the dorsal and posterior tip of each lateral ventricle then along the rostral migratory stream up to the olfactory bulb glomeruli; and endothelial cell/pericyte-derived blood vessels contacted by the endfeet of astrocytes. A large variety of interactions between these cells types has been implicated in regulating the behaviors of neural stem cells within the niche (see (7, 12, (17)).

Like the adult SVZ, the SGZ niche is comprised of multiple cell types. NSPCs in the SGZ divide only occasionally and produce immature granule neurons through an intermediate progenitor stage. Other cells within this niche include astrocytes, mature granule cell layer neurons, and the endothelial cells and pericytes of nearby blood vessels (18). SGZ NSCs may also produce glial cells although it is unclear under what conditions they do so. Neurons born in the SGZ migrate within the granule cell layers until they reach a final position and integrate into hippocampal circuits (19). Both SGZ neurogenesis and the integration of these new neurons into the hippocampus are required for certain forms of learning and memory (2).

5. THE EXTRACELLULAR MATRIX IS AN ESSENTIAL COMPONENT OF NEURAL STEM CELL NICHES

In addition to the cell-cell interactions that are required to maintain the neural stem cell niche, the extracellular matrix (ECM) also plays a critical role in regulating NSPC proliferation and differentiation. The cell bodies and processes of NSPCs in the SVZ and SGZ are surrounded and positioned in an area comprised of numerous ECM interacting molecules such as laminin chains (20-22), the glycoprotein tenascin-C (23), and a number of proteoglycans (24). The complex ECM of the neural stem cell niche has been shown to direct the proliferation and differentiation of NSPCs. For example, mice lacking laminin γ1, integrin α6 or integrin β1 have a number of deficits including ectopic growth in the cortical marginal zone and retraction of radial glial cell basal endfeet (25-27). Interfering with β1 integrin in neurosphere cultures, an *in vitro* model of the neural stem cell niche, using function-blocking antibodies or genetic ablation resulted in reduced mitogen-activated protein kinase (MAPK) activity, leading to compromised NSPC maintenance (20), although this effect was not observed in a subsequent study using neurospheres grown from cells lacking β1 integrin (28).

Other studies have suggested chondroitin sulfate proteoglycans (CSPGs) may similarly influence NSPCs in the SGZ or SVZ. Enzymatic degradation of CSPGs in neurosphere cultures resulted in reduced self-renewal of radial glia and reduced neurogenesis, as well as reduced NSPC proliferation in the SVZ (29). In other studies, degradation of CSPGs resulted in increased NSPC

proliferation, differentiation and migration through an integrin-dependent mechanism (30). These different outcomes may be the result of differences in the cell types being analyzed (30). Finally, a recent study has indicated that some of the functions of CSPGs in neural stem cell niches may be related to modulation of fibroblast growth factor-2 (FGF-2) and epidermal growth factor (EGF) signaling (31).

Collectively, these findings indicate that the extracellular matrix plays key roles in the maintenance of NSPCs in the SVZ and SGZ. For the rest of this review we will focus on hyaluronan, a major component of the extracellular matrix of all tissues. A growing body of research suggests that in addition to regulating a large variety of cell behaviors in multiple tissue types, hyaluronan may also play critical roles in regulating NSPC proliferation, differentiation and migration in the niche as well as in the developing and injured brain.

6. HYALURONAN IS A MAJOR CONSTITUENT OF THE EXTRACELLULAR MATRIX

Hyaluronan, also known as hyaluronate and hyaluronic acid (HA), is a major constituent of the ECM of most tissues. HA is a large unbranched, non-sulfated glycosaminoglycan (GAG) composed of repeating disaccharide units of N-glucuronic acid and N-acetylglucosamine. While simple in composition, HA polymers can reach upwards of 25,000 disaccharide units, with molecular weights in the 10^7 Dalton range and are capable of organizing into complex secondary and tertiary structures. Different sizes of HA appear to have distinct physiological functions including hydration of tissues, providing elasticity to tissues, and creation of cell free spaces for cell migration. These functions are regulated through transmembrane HA receptors whose activation depends on highly regulated HA synthesis and degradation. Growing evidence suggests specific and critical roles for HA in the nervous system, including the regulation of NSPCs in neural stem cell niches.

7. STRUCTURE, SYNTHESIS AND DEGRADATION OF HA

7.1. HA is synthesized by transmembrane synthases

Unlike most other GAGs, which are processed and extensively modified in the Golgi body, HA is synthesized at the inner face of the plasma membrane and secreted as a linear undecorated polysaccharide directly into the extracellular space by a family of transmembrane proteins known as HA synthases (HASs). The mammalian genome codes for three such synthases, HAS1, HAS2 and HAS3, each of which generate distinct molecular weights of HA. HAS3 generates intermediate sizes of low molecular weight (LMW) HA of $\leq 2.5 \times 10^5$ Daltons while HAS1 and HAS2 are thought to preferentially secrete high molecular weight (HMW) forms of HA ranging from 2.0×10^6 to $\geq 4.0 \times 10^6$ Daltons. (reviewed in (32))

Overall *in vitro* studies have shown that the V_{max}, K_m values, degree of polymerization and stability of HAS enzymes are variable and generally organized as HAS3 > HAS2 > HAS1 (33, 34). This, combined with distinct temporal and spatial expression patterns (35), supports the hypothesis that while the synthases are capable of generating similar sizes of HA, various HASs are probably not functionally redundant in tissues. Consistent with this idea, the transcription of each HAS gene is regulated by distinct growth factors and cytokines (36). To date the generation of homozygous null animals shows that HAS2 knockout is embryonic lethal (37) while HAS1 and HAS3 animals are viable (reviewed in (38)), indicating that conditional or double knockout animals may be required to tease out the physiological importance of each HAS *in vivo (*35).

The kinetics controlling HA synthesis *in vivo* are largely unknown but are likely dependent on available cytosolic monosaccharide concentrations and possible direct and indirect homeostatic feedback mechanisms of newly synthesized HA on HAS activity (34). HAS activity generates large intact linear molecules of HA that are rapidly incorporated into the ECM surrounding cells or may be retained at the cell surface through HA binding receptors and interacting proteins. HA may also be tethered to the cell surface by interactions with HAS proteins themselves (reviewed in (32, 34, 39)).

7.2. HA is catabolized by multiple hyaluronidases

HA degradation is carried out by a family of enzymes called hyaluronidases (HYALs). Genomic analysis reveals that mammals possess multiple hyaluronidase genes, including HYAL-1 through HYAL-5, PH20, and, in humans, a pseudogene designated PHYAL-1 (40). Despite the estimation that approximately 30% of the body's total HA is turned over everyday (41), little is known about the expression, activity and control of HYALs, especially in the nervous system. HA degradation begins with the extracellular processing of the large polysaccharide into smaller cleavage products of about 20 to 50 disaccharides by the GPI-anchored HYAL-2 (42). HA breakdown products are then internalized for further degradation into disaccharides by HYAL-1 in lysosomes (41), while N-acetylglucosaminidase and N-glucuronidase are required to fully degrade the sugar into monosaccharides. Little is known about HYAL-3 and it is still unclear whether HYAL-3 is a functional hyaluronidase (43), although evidence suggests it may regulate the activity of HYAL-1 (44). Similarly, HYAL-4 has no predicted hyaluronidase activity (40).

One of the more interesting hyaluronidases is PH20, also known as sperm adhesion molecule-1 (SPAM-1), which has been studied mostly in the testes where it is localized to the heads of mature sperm and used to penetrate the HA rich zona pellucida during fertilization (45). However, there is growing evidence that PH20 is expressed elsewhere as well, including the kidney (46) and numerous cancer cell types (47, 48). It is unclear whether PH20 is expressed in the nervous system. HYAL-5, not present in humans, is thought to be functionally redundant with PH-20 in the testes of rodents and other mammals (49, 50).

8. PHYSIOLOGICAL RELEVANCE OF HA SIZE AND RECEPTORS IN HA-MEDIATED BIOLOGICAL ACTIVITIES

Multiple reviews have covered the significance of size on HA signaling *in vivo and in vitro* (refer to: (32, 34, 51-53)). Importantly it has been shown that distinct sizes of HA have different physiological effects on cells and that different sizes of HA may have distinct effects on the activation of HA receptors.

8.1. Activities ascribed to HMW HA

The aggregation and structure of HMW HA has been shown to be highly flexible with large molecules folding and aggregating spontaneously into coils, fibers, and nets (34). Due to its high negative charge, HMW HA is capable of holding 10-10,000 times its weight in water and it is proposed that hydration of the HA rich ECM creates cell free pores for migration of cells (32). The structure of HMW HA in the nervous system is likely dependent on binding with HA binding proteins such as hyalectins (54), brain-enriched HA binding protein (BEHAB) (55), and other HA-binding proteins (56). A large body of research has explored the role of HA interactions with these HA-binding proteins and as a structural component in large proteoglycan complexes during development and repair of the CNS but is beyond the scope of this review (for more information see (57, 58)).

HMW HA has also been implicated in the clustering of receptor tyrosine kinases (RTKs) as well as influencing the constitutive activation of multiple growth-associated receptors (59). In particular, several studies have implicated HA in the activation of the erbB2 and erbB3 receptor tyrosine kinases in Schwann cells (60, 61), and erbB1 (the epidermal growth factor receptor) and erbB4 in other cells types via interaction with the transmembrane HA receptor CD44 (62-65). HA-CD44 interactions have also been implicated in regulating cell proliferation by regulating the phosphorylation of the merlin tumor suppressor protein in Schwann cells (65).

8.2. Activities ascribed to LMW HA

LMW HA shows varying binding affinities for different HA receptors as well as strong angiogenic properties, and may function as a signal for CNS tissue damage by inducing pro-inflammatory signaling cascades (66). Small fragments of HA (4-8mers) have been shown to induce anti-apoptotic cascades via activation of NFkB and heat shock proteins (41). Additionally, various breakdown products of HA have been shown to activate toll-like receptors (TLR-2 and TLR-4) (67, 68) and may be one of the molecules responsible for activation of local microglia and astrocytes in response to pathogens and CNS infection (69). Small fragments (<12 disaccharides) or HA oligomers have been used to disrupt HMW and LMW HA signaling *in vitro* by directly competing with the intact sugar for binding sites in HA receptors and binding proteins (reviewed in (70)). Whether HA oligomers produced by endogenous hyaluronidases are capable of competing with large polymers of HA *in vivo* is still unclear.

8.3. Activities of transmembrane HA receptors in the nervous system

Activation of HA receptors has been implicated in tumor metastasis, cell migration and differentiation, and the modulation of signaling cascades associated with cell growth during tissue homeostasis and following injury. Such a multitude of cellular responses reported for HA is likely related to the where, when and how a cell encounters HA. The biological activities of HA in the nervous system are largely mediated by transmembrane receptors including TLR2, TLR4, CD44 and the receptor for hyaluronan-mediated motility (RHAMM). An isoform of the HA receptor for endocytosis (HARE) is also expressed in the brain but its function there is not known (71). Among these receptors, CD44 and RHAMM have been the most extensively characterized in the CNS and PNS.

Multiple isoforms of CD44 are created both by alternative mRNA splicing of 20 exons present in the CD44 gene and extensive posttranslational modification of CD44 proteins including N- and O-linked glycosylation, the addition of heparan sulfate side chains, and the incorporation of chondroitin sulfate side chains (reviewed by (72)). Different modifications occur depending on the presence of particular variant exon-encoded sequences. HA binds to CD44 via an extracellular domain related to cartilage link protein. The binding affinity of HA to CD44 depends on the receptor isoform expressed and the extent of post-translational receptor modifications. In general, CD44 preferentially binds HMW HA although lower MW forms of HA may also signal via CD44. CD44 is expressed throughout both the central and peripheral nervous systems predominantly by glial cells (60, 73). CD44 signaling has been shown to influence multiple cellular behaviors including proliferation, survival, and migration via receptor interactions with a variety of signaling molecules such as ErbB receptors (62, 63), SRC family kinases (74-76), RHO GTPases (77) and proteins in the ezrin-radixing-moesin (ERM) family of actin-associated proteins (78). Additionally, HA uptake and degradation by a CD44-dependent mechanism has been proposed which may also influence tissue integrity, cell growth and homeostasis (41).

RHAMM is also expressed at the cell surface, exists in multiple isoforms and is capable of binding various sizes of HA (79, 80). Like CD44, RHAMM expression has been reported in neurons and glial cells (81). Extracellular binding of HA to RHAMM has been reported to influence cell migration and growth by activating molecules such as focal adhesion kinase (FAK) and inducing changes in actin and microtubule dynamics (82-85). HA binding to RHAMM also activates a variety of signaling molecules such as tyrosine receptor kinases (82), protein kinase C (86) and PI3K (87). Interestingly, RHAMM is also found in the cytoplasm and is associated with nuclear and mitochondrial membranes (88), perhaps mediating intracellular signaling by internalized HA (see (89)).

Finally, HA has been proposed as a ligand for Toll-Like Receptors (TLRs), a family of pattern recognition

receptors activated by pathogenic molecules such as bacterial cell wall components, lipopolysaccharide, DNA and RNA. To date 10 mammalian TLRs have been identified (see (90)). Signaling through TLRs leads to activation of the innate immune system via MyD88-mediated signaling pathways resulting in the nuclear translocation of NFkB and the subsequent upregulation of inflammatory chemokines and cytokines (reviewed in (91)). The endogenous ligands for TLRs are still being investigated but HA oligosaccharides have been proposed as ligands for TLR2 and TLR4 in a variety of cells such as macrophages (92, 93), dendritic (94) and endothelial cells (95). TLR2 and TLR4 expression has been reported in neural stem cells (96), microglia and astrocytes (69). As HA is secreted in large volumes following CNS insults such as stroke, multiple sclerosis attacks and infections, the breakdown products of HA may be capable of inducing TLR2 and TLR4 mediated inflammatory cascades following HA degradation or ECM destruction (see (97)).

9. HA AND THE DEVELOPING AND ADULT CNS

As mentioned above, HA and HA receptors have distinct patterns of expression in the CNS. HA is enriched in the mesenchyme underlying the neural folds in chick, frog, rat and mouse embryos (98-100). It has been proposed that HA lends structural support and tensile strength during neural tube folding and closure (101). Early neurulation and formation of the spinal cord appears to be HA-dependent as enzymatic degradation of the HA matrix in the neural plate with exogenous hyaluronidase leads to incomplete closure of the neural tube in chick spinal cord (102) and altered HA deposition may underlie the hindbrain and spinal cord defects seen in exencephalic loop-tail (Lp/Lp) mice (103) and splotch (sp/sp) mice (104). Additionally, high concentrations (100-500 ug/ml) of HA increase the spreading and distribution of neural crest cells (NCCs) along the quail neural tube (105), perhaps by decreasing the tight cell-cell association of the neuroepithelum and creating a porous ECM for cell migration. Pockets of HA are also observed between neuroepithelial cells of the developing rat neural tube (106).

In later embryonic stages, HA is widely distributed throughout the developing rodent brain including the cortex, cerebellum, striatum, and subpallidian structures (106, 107). As mentioned above, hydration of the HA-rich ECM of the developing CNS may create a 'loose' cell-free matrix to promote neural precursor migration. Consistent with this hypothesis, 90% of HA is associated with water in the developing brain and is expressed in a diffuse global pattern when many cells are moving both tangentially and radially into the rapidly expanding cerebrum (108). Total HA content of the developing CNS declines to approximately 25% of embryonic HA levels by 2 weeks after birth in the rodent (108) and becomes re-organized into dense nets in the corpus callosum and cerebellum, both sites of extensive postnatal progenitor migration (109-111).

HA binding to RHAMM can induce calmodulin-mediated cell signaling that influences cytoskeletal elements such as actin and microtubules (88). It has been proposed that HA-rich fiber tracts may serve as guidance cues for migration of newly born neurons and glia, which express a variety of RHAMM splice variants (80, 81). Astrocytes and migroglia upregulate RHAMM during *in vitro* migration (112) and RHAMM expression is seen on both neurons and oligodendrocytes, *in vivo* (80, 81). The developmental upregulation of RHAMM mRNA in immature neurons and oligodendrocytes also corresponds to periods of progenitor migration (81). To date, HA-induced migration in neural progenitors via RHAMM activation has yet to be tested in the developing CNS.

HA may also influence the organization of distinct structures in the maturing brain. Disruption of the HA matrix leads to lamination defects and inappropriate sprouting in the hippocampus (113). The HA rich matrix may also influence the sorting of axons in fiber tracts such as CD44 positive retinal axons in the developing optic chasm (114-116) and RHAMM positive axons of noradrenergic neurons in the locus coeruleus (117). In the adult CNS, HA is found at especially high levels in dense proteoglycan coats known as perineuronal nets (PNNs). PNN formation is associated with neuronal maturation and thought to correspond to the establishment and maintenance of synaptic circuitry in the adult brain. HA itself, other GAGs and proteoglycans associated with the nets have been proposed to act as sinks for enzymes, neurotransmitters, growth factors and cytokines capable of modulating synaptic transmission. In support of this notion, HA rich nets are concentrated around the dendrites, soma and initial segment of the axon (for a more extensive review see (118)).

A recent report shows that the PNNs around hippocampal neurons can control AMPA receptor trafficking into and out of the synapse, a mechanism thought to influence synaptic plasticity by exchanging desensitized receptors with naïve functional receptors (119). The authors labeled membrane associated GluR1 and GluR2 receptor subunits in cultured primary hippocampal neurons with green fluorescent protein (GFP) and then treated the cells with hyaluronidase to disrupt the PNNs around mature synapases. GFP-labeled AMPA receptors and GPI-linked GFP molecules diffused faster and more widely through the membrane following hyaluronidase treatment. Next, they observed that hyaluronidase treatment increased the speed of recovery of fluorescence following photobleaching of dendritic spines and altered paired pulse ratios following high frequency stimulation. Additionally, they reported no changes in synaptic transmission, receptor expression, or changes in spontaneous transmitter release following hyaluronidase treatment, indicating that altered synaptic plasticity could be affected by AMPA receptor motility alone. These observations are consistent with the hypothesis that HA-rich PNNs may be a way to control the plasticity of individual synapases by creating distinct membrane compartments and controlling the passive diffusion of molecules at the cell surface.

In another study, hippocampal slices from young

rats were treated with hyaluronidase to remove PNNs (120). Scanning electron microscopy (SEM) and *ex vivo* electrophysiological recordings showed that hyaluronidase treatment dramatically reduced the width of the synaptic cleft and increased the amplitude of excitatory postsynaptic potentials in CA1 axodendritic connections. From these studies it is reasonable to conclude that HA rich PNNs could influence both the fidelity and architecture of synapses. Furthermore, the controlled degradation of HA in PNNs may be one mechanism used to alter synaptic plasticity underlying learning and memory.

Although HA is a prominent component of the neural ECM, a comprehensive characterization of the synthesis of HA in the developing rodent nervous system has yet to be done. HAS2 and HAS3 mRNA are found in the embryonic brain (121). Little is known about HAS1 expression or activity in the CNS. In agreement with HA accumulation in PNNs of mature neurons, mRNA for HAS2 and HAS3 is found in neurons during postnatal CNS development (121). HAS2 knockout in mice is lethal by embryonic day 10 due to cardiac and vascular defects (37) so conditional knockout will be required to study loss of HAS2 in the nervous system. HAS1 and HAS3 knockouts are viable but CNS organization in these animals has not been described. (38).

Even less is known about the degradation of HA by endogenous hyaluronidases in the brain. It remains controversial which HYALs are expressed in the mammalian brain and when. Several labs report a conspicuous absence of HYAL2 in the brain (122, 123) and no gross abnormalities in the CNS or PNS are reported in the HYAL2 null mouse (124) . However, a GPI-linked hyaluronidase such as HYAL2 would most likely be needed to degrade HA at the cell surface. Similarly, HYAL3 mRNA is expressed in the brain (125) but conflicting reports exist on the nature of this gene's function. Brain abnormalities in HYAL3 or HYAL1 null mice have not be reported.

10. HA IS ENRICHED IN NEURAL STEM CELL NICHES

While the roles of HA-rich ECM, HA binding proteins and associated proteoglycans, and HA receptors have been studied outside the nervous system for decades, only recently has the effect of HA on NSPC populations been explored. In particular, there is growing evidence that HA itself may directly influence the neural stem cell niche.

HA has been described as a component of the hematopoietic stem cell niche where it is concentrated around hemopoietic stem cells (HSCs) in the endosteal region of bone marrow, a proposed HSC niche (126). HA is thought to be a key molecule in the homing of transplanted HSC to bone marrow niches and it has been demonstrated that interfering with HA interactions in the bone marrow reduces HSC proliferation and granulocyte differentiation (127). HA may play similar roles in the developing and adult nervous system. In the brain, HA synthases and HA receptors appear to be concentrated in the highly proliferative ventricular zones in the frog, where embryonic neurogenesis is occurring (81, 98). We observe discrete concentrations of HA adjacent to GFAP positive B cells, the proposed neural stem cell (128), in the SVZ and SGZ (our own unpublished findings). Additionally when neural stem cells are isolated and grown as free floating 'neurospheres', an *in vitro* model of the niche, we find them to contain both HA and hyaluronidases (unpublished observations).

HA may act to slow proliferation of stem or progenitor cells in neural stem cell niches. A recent report shows that mesenchymal stem cells have increased G0/G1 length when plated on HA coated coverslips leading to slowed proliferation (129). Furthermore, HAS activity, intracellular HA concentrations and RHAMM mRNA synthesis have been shown to fluctuate during mitosis (130-132). There have been several reports linking HA and RHAMM to the formation of the mitotic spindle apparatus suggesting that intercellular binding of HA may directly affect mitosis (84, 133, 134). HMW HA-CD44 interactions at the cell membrane have been shown to organize mitotic spindle orientation and thereby influence asymmetrical versus symmetrical division, a process strongly linked to self-renewal or differentiation of NSCs *in vivo* (135).

HA synthesis and degradation may also influence the proliferation, differentiation and maturation of neural progenitors. For example, enzymatic degradation of HA induces proliferation of O-2A (oligodendrocyte – type2 astrocyte) progenitors isolated from rat brain and in confluent primary rat astrocyte cultures (136). HA also inhibits the maturation of rat oligodendrocyte progenitor cells both in demyelinating lesions and in culture (137). Furthermore, HA degradation induces the proliferation of quiescent astrocytes in the rat spinal cord (138). These data suggest that controlled degradation of HA may be required to allow for proper expansion and maturation of progenitor cells during development and following recruitment to areas of nervous system damage.

11. USE OF HA-BASED HYDROGELS TO PROMOTE CNS REPAIR BY NSPCS

Repairing CNS damage following injury or disease is dependent upon the proper mobilization and maturation of neural progenitor cells to sites of tissue damage (139). Recently, attention has been focused on the generation of biomaterials for the replacement of damaged or necrotic tissue. Tissue engineering has been proposed for the delivery of pharmacological factors and/or neural stem/progenitor cells to sites of injury; as well as, to provide structural support for the migration of endogenous progenitors into areas of damage and the regrowth of axons through damaged tissue. Given the influence of HA on proliferation, migration and differentiation of progenitor cells, HA-based scaffoldings, or HA hydrogels, have been generated to mimic the ECM found in neonatal and adult nervous system. HA-based hydrogels are biodegradable by endogenous hyaluronidases, mechanically and chemically stable, malleable in shape, and show low

immunogenicity. These are all properties desired for bioengineering tissues (for a more extensive review see (140)).

HA-based scaffoldings can be readily modified to alter pore size, hydration, surface charge, and ECM interactions by subtlety adjusting the manufacturing parameters or by the addition of HA interacting proteins such as brain-derived proteoglycans (141). Early studies have shown that implanted HA-based materials minimize glial scar formation, promote vascularization and promote cell migration of astrocytes and microglia into damaged tissue (142, 143). However, pure HA hydrogels have shown limited ability to promote neurite outgrowth, presumably due to low process adhesion to its negatively charged surface (144). Modifications such as crosslinking HA with poly-D-lysine, neural adhesion molecules or peptide sequences commonly found in ECM interacting proteins all appear to increase cell adhesion and promote neurite sprouting, axonal growth and cellular integration into damaged tissue (145-149). Degradation of the implanted HA-hydrogels, presumably by invading cells, correlates temporally with progenitor differentiation and maturation mirroring the loss of HA expression seen during postnatal maturation of the nervous system (150).

HA-hydrogels have also been used to study the effects of 3D scaffoldings on NSPC proliferation, survival and differentiation. NSPCs seeded into HA-based 3D hydrogels survive and mature both *in vitro* and *in vivo* (151). Interestingly, hydrogels made of pure HA promoted the aggregation of cells into clusters of cells reminiscent of neurospheres (152). In another study (151), HA was mixed with collagen I, a molecule enriched in the basal lamina of the SVZ, to form 3D matrices. HA-Collagen I-based 3D cultures showed reduced proliferation and apoptosis of neonatal progenitors as compared to 2D cultures, conditions that strongly promoted the generation of immature neurons following mitogen removal. These studies support the hypothesis that HA is capable of altering proliferation and differentiation of stem cells in the neural stem cell niche.

Modified HA hydrogels also show significantly increased morphogen-induced differentiation of stem cells into neurons as well as increased neurite outgrowth and synapse formation as compared to traditional 2D culture assays (152). Strikingly, in a recent study (153), 'tunable' HA-hydrogels that mimic the mechanical properties of neonatal and adult brain ECM were generated. Isolated NSPCs were encapsulated into these HA-hydrogels during polymerization, transferred to culture medium and allowed to differentiate in the absence of growth factors or morphogens. NSPCs inside 'soft' hydrogels with the flexibility of neonatal brain ECM differentiated primarily into neurons, while NSPCs inside 'medium' hydrogels with the flexibility of adult brain ECM became astrocytes. Here, hydrogel mechanical properties alone were apparently sufficient to drive lineage choices in NSPCs. Again, as seen with endogenous HA, the influence of HA-based hydrogels on progenitor generation and maturation is likely dependent on the conformational organization and degradation of HA and HA binding molecules incorporated into the scaffolding before and after implantation.

12. CONCLUSIONS

While HA has been studied extensively in a variety of tissues comparatively little is known about the role of HA in the developing, mature or injured nervous system. More detailed analysis of HA synthesis, degradation, and signaling in the brain and spinal cord is needed to understand how such a simple molecule is able to modulate such a large variety of complex signaling cascades and generate a plethora of cellular responses. The HA-rich ECM likely provides both structural shape and mechanical support to the developing and adult nervous system as well as acting as core scaffolding for the incorporation of a variety of ECM molecules, growth factors, and signaling molecules known to influence cellular growth and homeostasis. Large molecules of HA are capable of hydration, cushioning and facilitating cellular migration as well as preventing the spread of inflammation in the glial scar. Conversely, HA has been found to signal directly through a variety of receptors, based on size and presentation, and influence a diverse collection of cellular behaviors including progenitor proliferation, differentiation, migration and maturation.

We propose that the controlled synthesis and degradation of HA may be required for proper generation, proliferation and maturation of NSPCs within NSC niches during development and repair of the damaged nervous system. The data that we have reviewed here suggest a model (Figure 2) in which the matrix of neural stem cell niches is enriched with HA, where HA maintains NSPC quiescence and prevents their differentiation. When these cells start to differentiate, they likely express hyaluronidases that either relieve HA-induced signals that inhibit proliferation and differentiation, or which generate LMW HA products that promote proliferation, differentiation and possibly migration. If these cells later encounter an HA-rich matrix at a site of nervous system injury, their proliferation, differentiation and maturation are again inhibited, preventing them from contributing to nervous system repair. It is possible that both HA synthesis and degradation are regulated by signals, such as pro-inflammatory cytokines, found within nervous system lesions. Such signals could influence both cells within lesions as well as progenitor cells within stem cell niches.

It is clear that NSPCs and differentiating cells that are recruited to nervous system lesions respond to multiple signals during development and in response to tissue damage. It is tantalizing to speculate that, in addition to HA-specific signals transduced through HA receptors, the HA-based extracellular matrix also serves

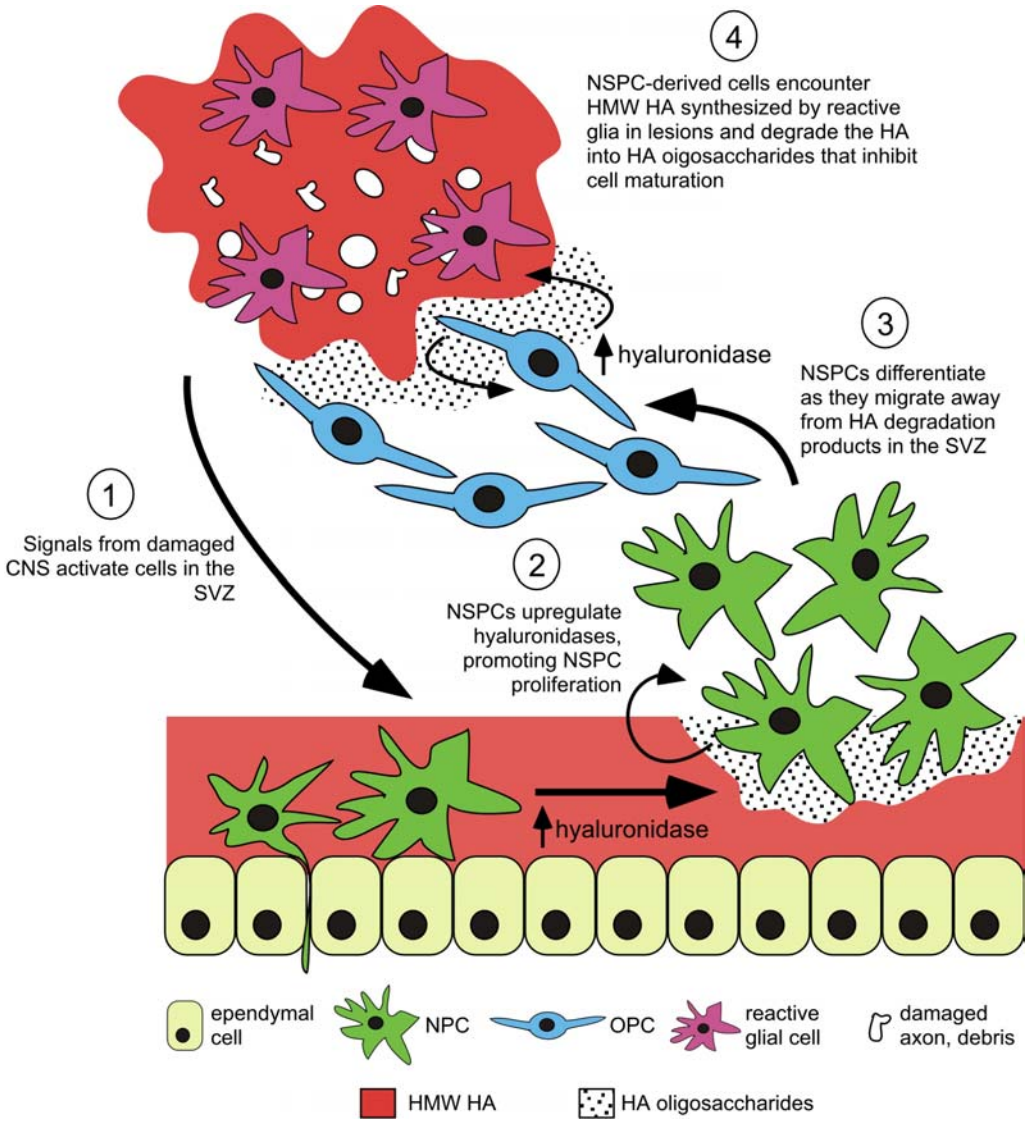

Figure 2. A model of the potential roles played by HA in a demyelinating lesion to illustrate how NSPCs regulate and respond to changes in the HA-based ECM. (1) Following a CNS insult, signals from the site of injury result in the activation of NSPCs in the SVZ. (2) NSPCs or other cells in the niche then increase their hyaluronidase expression and/or activity, leading to (3) the expansion of NSPCs and their initial differentiation and migration away from the niche. If these cells encounter an acute lesion, then they can differentiate into cells that can promote nervous system repair. (4) If they encounter an HA-rich chronic lesion, however, they are blocked from maturation.

as a platform that mediates multiple signals within neural stem cell niches. Future studies focused on the roles of HMW versus LMW HA and their distinct receptors, the regulation of HAS genes and hyaluronidases, and the interactions between HA and other signaling molecules in the nervous system will reveal how HA functions during nervous system development and during the pathogenesis of various nervous system insults and diseases.

13. ACKNOWLEDGEMENTS

We wish to thank Ms. Susan Bond for assistance with the preparation of this manuscript. LS and MP were supported by grants from the National Institutes of Health (AG031892, P51RR000163) and a Collaborative MS Research Center Award from the National Multiple Sclerosis Society.

14. REFERENCES

1. L Bonfanti, P Peretto: Radial glial origin of the adult neural stem cells in the subventricular zone. *Prog Neurobiol* 83, 24-36 (2007)

2. W Deng, JB Aimone, FH Gage: New neurons and new memories: how does adult hippocampal neurogenesis affect

learning and memory? *Nat Rev Neurosci* 11, 339-50 (2010).

3. F Guillemot: Cell fate specification in the mammalian telencephalon. *Prog Neurobiol* 83, 37-52 (2007)

4. V Marthiens, I Kazanis, L Moss, K Long, C Ffrench-Constant: Adhesion molecules in the stem cell niche--more than just staying in shape? *J Cell Sci* 123, 1613-22 (2010)

5. Y Mu, SW Lee, FH Gage: Signaling in adult neurogenesis. *Curr Opin Neurobiol* 20, 416-23 (2010)

6. H Suh, W Deng, FH Gage: Signaling in adult neurogenesis. *Annu Rev Cell Dev Biol* 25, 253-75 (2009)

7. JD Lathia, MS Rao, MP Mattson, C Ffrench-Constant: The microenvironment of the embryonic neural stem cell: lessons from adult niches? *Dev Dyn* 236, 3267-82 (2007)

8. FC Sauer: Using a "Dry" Microscope Objective on Uncovered Objects. *Science* 78, 536 (1933)

9. LM Baye, BA Link: Nuclear migration during retinal development. *Brain Res* 1192, 29-36 (2008)

10. LM Baye, BA Link: Interkinetic nuclear migration and the selection of neurogenic cell divisions during vertebrate retinogenesis. *J Neurosci* 27, 10143-52 (2007)

11. F Del Bene, AM Wehman, BA Link, H Baier: Regulation of neurogenesis by interkinetic nuclear migration through an apical-basal notch gradient. *Cell* 134, 1055-65 (2008)

12. A Kriegstein, A Alvarez-Buylla: The glial nature of embryonic and adult neural stem cells. *Annu Rev Neurosci* 32, 149-84 (2009)

13. P Rakic: Developmental and evolutionary adaptations of cortical radial glia. *Cereb Cortex* 13, 541-9 (2003)

14. LM Farkas, WB Huttner: The cell biology of neural stem and progenitor cells and its significance for their proliferation versus differentiation during mammalian brain development. *Curr Opin Cell Biol* 20, 707-15 (2008)

15. DV Hansen, JH Lui, PR Parker, AR Kriegstein: Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature* 464, 554-61 (2010)

16. F Gubert, C Zaverucha-do-Valle, PM Pimentel-Coelho, R Mendez-Otero, MF Santiago: Radial glia-like cells persist in the adult rat brain. *Brain Res* 1258, 43-52 (2009)

17. JS Goldberg, KK Hirschi: Diverse roles of the vasculature within the neural stem cell niche. *Regen Med* 4, 879-97 (2009)

18. TD Palmer, AR Willhoite, FH Gage: Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* 425, 479-94 (2000)

19. B Seri, JM Garcia-Verdugo, L Collado-Morente, BS McEwen, A Alvarez-Buylla: Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. *J Comp Neurol* 478, 359-78 (2004)

20. LS Campos, DP Leone, JB Relvas, C Brakebusch, R Fassler, U Suter, *et al.*: Beta1 integrins activate a MAPK signalling pathway in neural stem cells that contributes to their maintenance. *Development* 131, 3433-44 (2004)

21. DD Hunter, R Llinas, M Ard, JP Merlie, JR Sanes: Expression of s-laminin and laminin in the developing rat central nervous system. *J Comp Neurol* 323, 238-51 (1992)

22. JD Lathia, B Patton, DM Eckley, T Magnus, MR Mughal, T Sasaki, M Caldwell, *et al.*: Patterns of laminins and integrins in the embryonic ventricular zone of the CNS. *J Comp Neurol* 505, 630-43 (2007)

23. E Garcion, A Halilagic, A Faissner, C ffrench-Constant: Generation of an environmental niche for neural stem cell development by the extracellular matrix molecule tenascin C. *Development* 131, 3423-32 (2004)

24. A von Holst, S Sirko, A Faissner: The unique 473HD-Chondroitinsulfate epitope is expressed by radial glia and involved in neural precursor cell proliferation. *J Neurosci* 26, 4082-94 (2006)

25. HE Beggs, D Schahin-Reed, K Zang, S Goebbels, KA Nave, J Gorski, *et al*: FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. *Neuron* 40, 501-14 (2003)

26. E Georges-Labouesse, M Mark, N Messaddeq, A Gansmuller: Essential role of alpha 6 integrins in cortical and retinal lamination. *Curr Biol* 8, 983-6 (1998)

27. A Niewmierzycka, J Mills, R St-Arnaud, S Dedhar, LF Reichardt: Integrin-linked kinase deletion from mouse cortex results in cortical lamination defects resembling cobblestone lissencephaly. *J Neurosci* 25, 7022-31 (2005)

28. DP Leone, JB Relvas, LS Campos, S Hemmi, C Brakebusch, R Fassler, *et al.*: Regulation of neural progenitor proliferation and survival by beta1 integrins. *J Cell Sci* 118, 2589-99 (2005)

29. S Sirko, A von Holst, A Wizenmann, M Gotz, A Faissner: Chondroitin sulfate glycosaminoglycans control proliferation, radial glia cell differentiation and neurogenesis in neural stem/progenitor cells. *Development* 134, 2727-38 (2007)

30. WL Gu, SL Fu, YX Wang, Y Li, HZ Lu, XM Xu, *et al.*: Chondroitin sulfate proteoglycans regulate the growth, differentiation and migration of multipotent neural precursor cells through the integrin signaling pathway. *BMC Neurosci* 10, 128 (2009)

31. S Sirko, A von Holst, A Weber, A Wizenmann, U Theocharidis, M Gotz, *et al.*: Chondroitin sulfates are required for fibroblast growth factor-2-dependent proliferation and maintenance in neural stem cells and for epidermal growth factor-dependent migration of their progeny. *Stem Cells* 28, 775-87 (2010)

32. BP Toole: Hyaluronan: from extracellular glue to pericellular cue. *Nat Rev Cancer* 4, 528-39 (2004)

33. N Itano, T Sawai, M Yoshida, P Lenas, Y Yamada, M Imagawa, *et al.*: Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties. *J Biol Chem* 274, 25085-92 (1999)

34. AP Spicer, JY Tien: Hyaluronan and morphogenesis. *Birth Defects Res C Embryo Today* 72, 89-108 (2004)

35. JY Tien, AP Spicer: Three vertebrate hyaluronan synthases are expressed during mouse development in distinct spatial and temporal patterns. *Dev Dyn* 233, 130-41 (2005)

36. N Itano, K Kimata: Mammalian hyaluronan synthases. *IUBMB Life* 54, 195-9 (2002)

37. TD Camenisch, AP Spicer, T Brehm-Gibson, J Biesterfeldt, ML Augustine, A Calabrom Jr., *et al.*: Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. *J Clin Invest* 106, 349-60 (2000)

38. AP Spicer, JL Tien, A Joo, RA Bowling Jr.: Investigation of hyaluronan function in the mouse through targeted mutagenesis. *Glycoconj J* 19, 341-5 (2002)

39. R Stern: Hyaluronidases in cancer biology. *Semin Cancer Biol* 18, 275-80 (2008)

40. AB Csoka, GI Frost, R Stern: The six hyaluronidase-like genes in the human and mouse genomes. *Matrix Biol* 20, 499-508 (2001)

41. R Stern: Hyaluronan catabolism: a new metabolic pathway. *Eur J Cell Biol* 83, 317-25 (2004)

42. SK Rai, FM Duh, V Vigdorovich, A Danilkovitch-Miagkova, MI Lerman, AD Miller: Candidate tumor suppressor HYAL2 is a glycosylphosphatidylinositol (GPI)-anchored cell-surface receptor for jaagsiekte sheep retrovirus, the envelope protein of which mediates oncogenic transformation. *Proc Natl Acad Sci U S A* 98, 4443-8 (2001)

43. V Atmuri, DC Martin, R Hemming, A Gutsol, S Byers, S Sahebjam, *et al.*: Hyaluronidase 3 (HYAL3) knockout mice do not display evidence of hyaluronan accumulation. *Matrix Biol* 27, 653-60 (2008)

44. R Hemming, DC Martin, E Slominski, JI Nagy, AJ Halayko, S Pind, B Triggs-Raine: Mouse Hyal3 encodes a 45- to 56-kDa glycoprotein whose overexpression increases hyaluronidase 1 activity in cultured cells. *Glycobiology* 18, 280-9 (2008)

45. Y Zheng, PA Martin-Deleon: Characterization of the genomic structure of the murine Spam1 gene and its promoter: evidence for transcriptional regulation by a cAMP-responsive element. *Mol Reprod Dev* 54, 8-16 (1999)

46. L Sun, E Feusi, A Sibalic, B Beck-Schimmer, RP Wuthrich: Expression profile of hyaluronidase mRNA transcripts in the kidney and in renal cells. *Kidney Blood Press Res* 21, 413-8 (1998)

47. LP Wang, XM Xu, HY Ning, SM Yang, JG Chen, JY Yu, *et al.*: Expression of PH20 in primary and metastatic breast cancer and its pathological significance. *Zhonghua Bing Li Xue Za Zhi* 33, 320-3 (2004)

48. DA Godin, PC Fitzpatrick, AB Scandurro, PC Belafsky, BA Woodworth, RG Amedee, *et al.*: PH20: a novel tumor marker for laryngeal cancer. *Arch Otolaryngol Head Neck Surg* 126, 402-4 (2000)

49. M Kimura, E Kim, W Kang, M Yamashita, M Saigo, T Yamazaki, *et al.*: Functional roles of mouse sperm hyaluronidases, HYAL5 and SPAM1, in fertilization. *Biol Reprod* 81, 939-47 (2009)

50. E Kim, D Baba, M Kimura, M Yamashita, S Kashiwabara, T Baba: Identification of a hyaluronidase, Hyal5, involved in penetration of mouse sperm through cumulus mass. *Proc Natl Acad Sci U S A* 102, 18028-33 (2005)

51. R Stern: Hyaluronan metabolism: a major paradox in cancer biology. *Pathol Biol (Paris)* 53, 372-82 (2005)

52. R Stern, G Kogan, MJ Jedrzejas, L Soltes: The many ways to cleave hyaluronan. *Biotechnol Adv* 25, 537-57 (2007)

53. N Itano: Simple primary structure, complex turnover regulation and multiple roles of hyaluronan. *J Biochem* 144, 131-7 (2008)

54. ML Lemons, S Barua, ML Abanto, W Halfter, ML Condic: Adaptation of sensory neurons to hyalectin and decorin proteoglycans. *J Neurosci* 25, 4964-73 (2005)

55. DM Jaworski, GM Kelly, S Hockfield: BEHAB, a new member of the proteoglycan tandem repeat family of hyaluronan-binding proteins that is restricted to the brain. *J Cell Biol* 125, 495-509 (1994)

56. SS Deepa, D Carulli, C Galtrey, K Rhodes, J Fukuda, T Mikami, *et al.*: Composition of perineuronal net extracellular matrix in rat brain: a different disaccharide composition for the net-associated proteoglycans. *J Biol Chem* 281, 17789-800 (2006)

57. LS Sherman, SA Back: A 'GAG' reflex prevents repair of the damaged CNS. *Trends Neurosci* 31, 44-52 (2008)

58. JC Kwok, F Afshari, G Garcia-Alias, JW Fawcett: Proteoglycans in the central nervous system: plasticity, regeneration and their stimulation with chondroitinase ABC. *Restor Neurol Neurosci* 26, 131-45 (2008)

59. S Misra, BP Toole, S Ghatak: Hyaluronan constitutively regulates activation of multiple receptor tyrosine kinases in epithelial and carcinoma cells. *J Biol Chem* 281, 34936-41 (2006)

60. A Gorlewicz, J Wlodarczyk, E Wilczek, M Gawlak, A Cabaj, H Majczynski, *et al.*: CD44 is expressed in non-myelinating Schwann cells of the adult rat, and may play a role in neurodegeneration-induced glial plasticity at the neuromuscular junction. *Neurobiol Dis* 34, 245-58 (2009)

61. LS Sherman, TA Rizvi, S Karyala, N Ratner: CD44 enhances neuregulin signaling by Schwann cells. *J Cell Biol* 150, 1071-84 (2000)

62. S Ghatak, S Misra, BP Toole: Hyaluronan constitutively regulates ErbB2 phosphorylation and signaling complex formation in carcinoma cells. *J Biol Chem* 280, 8875-83 (2005)

63. Z Palyi-Krekk, M Barok, T Kovacs, H Saya, O Nagano, J Szollosi, *et al.*: EGFR and ErbB2 are functionally coupled to CD44 and regulate shedding, internalization and motogenic effect of CD44. *Cancer Lett* 263, 231-42 (2008)

64. D Tsatas, V Kanagasundaram, A Kaye, U Novak: EGF receptor modifies cellular responses to hyaluronan in glioblastoma cell lines. *J Clin Neurosci* 9, 282-8 (2002)

65. H Morrison, LS Sherman, J Legg, F Banine, C Isacke, CA Haipek, *et al.*: The NF2 tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44. *Genes Dev* 15, 968-80 (2001)

66. F Gao, CX Yang, W Mo, YW Liu, YQ He: Hyaluronan oligosaccharides are potential stimulators to angiogenesis via RHAMM mediated signal pathway in wound healing. *Clin Invest Med* 31, E106-16 (2008)

67. GM Campo, A Avenoso, S Campo, A D'Ascola, G Nastasi, A Calatroni: Small hyaluronan oligosaccharides induce inflammation by engaging both toll-like-4 and CD44 receptors in human chondrocytes. *Biochem Pharmacol* 80, 480-90 (2010)

68. S Gariboldi, M Palazzo, L Zanobbio, S Selleri, M Sommariva, L Sfondrini, *et al.*: Low molecular weight hyaluronic acid increases the self-defense of skin epithelium by induction of beta-defensin 2 via TLR2 and TLR4. *J Immunol* 181, 2103-10 (2008)

69. C Gurley, J Nichols, S Liu, NK Phulwani, N Esen, T Kielian: Microglia and Astrocyte Activation by Toll-Like Receptor Ligands: Modulation by PPAR-gamma Agonists. *PPAR Res* 2008, 453120 (2008)

70. R Stern, AA Asari, KN Sugahara: Hyaluronan fragments: an information-rich system. *Eur J Cell Biol* 85, 699-715 (2006)

71. SV Kyosseva, EN Harris, PH Weigel: The hyaluronan receptor for endocytosis mediates hyaluronan-dependent signal transduction via extracellular signal-regulated kinases. *J Biol Chem* 283, 15047-55 (2008)

72. H Ponta, L Sherman, PA Herrlich: CD44: from adhesion molecules to signalling regulators. *Nat Rev Mol Cell Biol* 4, 33-45 (2003)

73. Y Liu, SS Han, Y Wu, TM Tuohy, H Xue, J Cai, *et al.*: CD44 expression identifies astrocyte-restricted precursor cells. *Dev Biol* 276, 31-46 (2004)

74. S Ilangumaran, A Briol, DC Hoessli: CD44 selectively associates with active Src family protein tyrosine kinases Lck and Fyn in glycosphingolipid-rich plasma membrane domains of human peripheral blood lymphocytes. *Blood* 91, 3901-8 (1998)

75. NK Wong, JC Lai, D Birkenhead, AS Shaw, P Johnson: CD45 down-regulates Lck-mediated CD44 signaling and modulates actin rearrangement in T cells. *J Immunol* 181, 7033-43 (2008)

76. C Jurzynski, J Gysin, B Pouvelle: CD44, a signal receptor for the inhibition of the cytoadhesion of CD36-binding Plasmodium falciparum-infected erythrocytes by CSA-binding infected erythrocytes. *Microbes Infect* 9, 1463-70 (2007)

77. M Shi, K Dennis, JJ Peschon, R Chandrasekaran, K Mikecz: Antibody-induced shedding of CD44 from adherent cells is linked to the assembly of the cytoskeleton. *J Immunol* 167, 123-31 (2001)

78. GM Harrison, G Davies, TA Martin, WG Jiang, MD Mason: Distribution and expression of CD44 isoforms and Ezrin during prostate cancer-endothelium interaction. *Int J Oncol* 21, 935-40 (2002)

79. S Zhang, MC Chang, D Zylka, S Turley, R Harrison, EA Turley: The hyaluronan receptor RHAMM regulates extracellular-regulated kinase. *J Biol Chem* 273, 11342-8 (1998)

80. BD Lynn, X Li, PA Cattini, EA Turley, JI Nagy: Identification of sequence, protein isoforms, and distribution of the hyaluronan-binding protein RHAMM in adult and developing rat brain. *J Comp Neurol* 439, 315-30 (2001)

81. P Casini, I Nardi, M Ori. RHAMM mRNA expression in proliferating and migrating cells of the developing central nervous system. *Gene Expr Patterns* 10, 93-7 (2010)

82. CL Hall, C Wang, LA Lange, EA Turley: Hyaluronan and the hyaluronan receptor RHAMM promote focal adhesion turnover and transient tyrosine kinase activity. *J Cell Biol* 126, 575-88 (1994)

83. VB Lokeshwar, MG Selzer: Differences in hyaluronic acid-mediated functions and signaling in arterial, microvessel, and vein-derived human endothelial cells. *J Biol Chem* 275, 27641-9 (2000)

84. SP Evanko, WT Parks, TN Wight: Intracellular hyaluronan in arterial smooth muscle cells: association with microtubules, RHAMM, and the mitotic spindle. *J Histochem Cytochem* 52, 1525-35 (2004)

85. CL Hall, EA Turley: Hyaluronan: RHAMM mediated cell locomotion and signaling in tumorigenesis. *J Neurooncol* 26, 221-9 (1995)

86. CL Hall, LA Collis, AJ Bo, L Lange, A McNicol, JM Gerrard, *et al.*: Fibroblasts require protein kinase C activation to respond to hyaluronan with increased locomotion. *Matrix Biol* 20, 183-92 (2001)

87. Y Goueffic, C Guilluy, P Guerin, P Patra, P Pacaud, G Loirand: Hyaluronan induces vascular smooth muscle cell migration through RHAMM-mediated PI3K-dependent Rac activation. *Cardiovasc Res* 72, 339-48 (2006)

88. BD Lynn, EA Turley, JI Nagy: Subcellular distribution, calmodulin interaction, and mitochondrial association of the hyaluronan-binding protein RHAMM in rat brain. *J Neurosci Res* 65, 6-16 (2001)

89. J Entwistle, CL Hall, EA Turley: HA receptors: regulators of signalling to the cytoskeleton. *J Cell Biochem* 61, 569-77 (1996)

90. IP Fraser, L Stuart, RA Ezekowitz: TLR-independent pattern recognition receptors and anti-inflammatory mechanisms. *J Endotoxin Res* 10, 120-4 (2004)

91. JA Sloane, D Blitz, Z Margolin, T Vartanian: A Clear and Present Danger: Endogenous Ligands of Toll-like Receptors. *Neuromolecular Med* 12, 149-63 (2010)

92. Y Iwata, A Yoshizaki, K Komura, K Shimizu, F Ogawa, T Hara, *et al.*: CD19, a response regulator of B lymphocytes, regulates wound healing through hyaluronan-induced TLR4 signaling. *Am J Pathol* 175, 649-60 (2009)

93. I Ernens, F Leonard, M Vausort, M Rolland-Turner, Y Devaux, DR Wagner: Adenosine up-regulates vascular endothelial growth factor in human macrophages. *Biochem Biophys Res Commun* 392, 351-6 (2010)

94. C Termeer, F Benedix, J Sleeman, C Fieber, U Voith, T Ahrens, *et al.*: Oligosaccharides of Hyaluronan activate dendritic cells via toll-like receptor 4. *J Exp Med* 195, 99-111 (2002)

95. V Voelcker, C Gebhardt, M Averbeck, A Saalbach, V Wolf, F Weih, *et al.*: Hyaluronan fragments induce cytokine and metalloprotease upregulation in human melanoma cells in part by signalling via TLR4. *Exp Dermatol* 17, 100-7 (2008)

96. A Rolls, R Shechter, A London, Y Ziv, A Ronen, R Levy, *et al.*: Toll-like receptors modulate adult hippocampal neurogenesis. *Nat Cell Biol* 9, 1081-8 (2007)

97. D Jiang, J Liang, PW Noble: Hyaluronan in tissue injury and repair. *Annu Rev Cell Dev Biol* 23, 435-61 (2007)

98. J Mullegger, G Lepperdinger: Hyaluronan is an abundant constituent of the extracellular matrix of Xenopus embryos. *Mol Reprod Dev* 61, 312-6 (2002)

99. JA Ripellino, MM Klinger, RU Margolis, RK Margolis: The hyaluronic acid binding region as a specific probe for the localization of hyaluronic acid in tissue sections. Application to chick embryo and rat brain. *J Histochem Cytochem* 33, 1060-6 (1985)

100. J Morris-Wiman, LL Brinkley: Changes in mesenchymal cell and hyaluronate distribution correlate with *in vivo* elevation of the mouse mesencephalic neural folds. *Anat Rec* 226, 383-95 (1990)

101. J Morris-Wiman, LL Brinkley: The role of the mesenchyme in mouse neural fold elevation. II. Patterns of hyaluronate synthesis and distribution in embryos developing *in vitro*. *Am J Anat* 188, 133-47 (1990)

102. GC Schoenwolf, M Fisher: Analysis of the effects of Streptomyces hyaluronidase on formation of the neural tube. *J Embryol Exp Morphol* 73, 1-15 (1983)

103. DB Wilson: Histochemical analysis of the neural basal lamina in the hindbrain region of exencephalic mutant mice. *J Craniofac Genet Dev Biol* 5, 363-71 (1985)

104. DG McLone, PA Knepper: Role of complex carbohydrates and neurulation. *Pediatr Neurosci* 12, 2-9 (1985)

105. L Luckenbill-Edds, JL Carrington: Effect of hyaluronic acid on the emergence of neural crest cells from the neural tube of the quail, Coturnix coturnix japonica. *Cell Tissue Res* 252, 573-9 (1988)

106. A Bignami, R Asher: Some observations on the localization of hyaluronic acid in adult, newborn and embryonal rat brain. *Int J Dev Neurosci* 10, 45-57 (1992)

107. O Yasuhara, H Akiyama, EG McGeer, PL McGeer: Immunohistochemical localization of hyaluronic acid in rat and human brain. *Brain Res* 635, 269-82 (1994)

108. RU Margolis, RK Margolis, LB Chang, C Preti: Glycosaminoglycans of brain during development. *Biochemistry* 14, 85-8 (1975)

109. J Kappler, O Hegener, SL Baader, S Franken, V Gieselmann, H Haberlein, *et al.*: Transport of a hyaluronan-binding protein in brain tissue. *Matrix Biol* 28, 396-405 (2009)

110. B Delpech, A Delpech, G Bruckner, N Girard, C Maingonnat: Hyaluronan and hyaluronectin in the nervous system. *Ciba Found Symp* 143, 208-20 (1989)

111. C Baier, SL Baader, J Jankowski, V Gieselmann, K Schilling, U Rauch, *et al.*: Hyaluronan is organized into fiber-like structures along migratory pathways in the developing mouse cerebellum. *Matrix Biol* 26, 348-58 (2007)

112. EA Turley, MZ Hossain, T Sorokan, LM Jordan, JI Nagy: Astrocyte and microglial motility *in vitro* is functionally dependent on the hyaluronan receptor RHAMM. *Glia* 12, 68-80 (1994)

113. E Forster, S Zhao, M Frotscher: Hyaluronan-associated adhesive cues control fiber segregation in the hippocampus. *Development* 128, 3029-39 (2001)

114. CK Chan, J Wang, L Lin, Y Hao, SO Chan: Enzymatic removal of hyaluronan affects routing of axons in the mouse optic chiasm. *Neuroreport* 18, 1533-8 (2007)

115. L Lin, SO Chan: Perturbation of CD44 function affects chiasmatic routing of retinal axons in brain slice preparations of the mouse retinofugal pathway. *Eur J Neurosci* 17, 2299-312 (2003)

116. DW Sretavan, L Feng, E Pure, LF Reichardt: Embryonic neurons of the developing optic chiasm express L1 and CD44, cell surface molecules with opposing effects on retinal axon growth. *Neuron* 12, 957-75 (1994)

117. JI Nagy, ML Price, WA Staines, BD Lynn, AC Granholm: The hyaluronan receptor RHAMM in noradrenergic fibers contributes to axon growth capacity of locus coeruleus neurons in an intraocular transplant model. *Neuroscience* 86, 241-55 (1998)

118. R Frischknecht, CI Seidenbecher: The crosstalk of hyaluronan-based extracellular matrix and synapses. *Neuron Glia Biol* 4, 249-57 (2008)

119. R Frischknecht, M Heine, D Perrais, CI Seidenbecher, D Choquet, ED Gundelfinger: Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. *Nat Neurosci* 12, 897-904 (2009)

120. SV Kul'chitskii, NV Yakubovich, AA Emel'yanova, YS Garkun, SG Pashkevich, VA Kul'chitskii: Changes in neuropil ultrastructure in hippocampal field CA1 in rat pups after application of hyaluronidase. *Neurosci Behav Physiol* 39, 517-21 (2009)

121. D Carulli, KE Rhodes, DJ Brown, TP Bonnert, SJ Pollack, K Oliver, P Strata, *et al.*: Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components. *J Comp Neurol* 494, 559-77 (2006)

122. G Lepperdinger, J Mullegger, G Kreil: Hyal2--less active, but more versatile? *Matrix Biol* 20, 509-14 (2001)

123. B Strobl, C Wechselberger, DR Beier, G Lepperdinger: Structural organization and chromosomal localization of Hyal2, a gene encoding a lysosomal hyaluronidase. *Genomics* 53, 214-9 (1998)

124. L Jadin, X Wu, H Ding, GI Frost, C Onclinx, B Triggs-Raine, *et al.*: Skeletal and hematological anomalies in HYAL2-deficient mice: a second type of mucopolysaccharidosis IX? *FASEB J* 22, 4316-26 (2008)

125. AB Csoka, SW Scherer, R Stern: Expression analysis of six paralogous human hyaluronidase genes clustered on chromosomes 3p21 and 7q31. *Genomics* 60, 356-61 (1999)

126. SL Ellis, B Williams, S Asquith, I Bertoncello, SK Nilsson: An innovative triple immunogold labeling method to investigate the hemopoietic stem cell niche *in situ*. Microsc Microanal 15, 403-14 (2009)

127. SK Nilsson, DN Haylock, HM Johnston, T Occhiodoro, TJ Brown, PJ Simmons: Hyaluronan is synthesized by primitive hemopoietic cells, participates in their lodgment at the endosteum following transplantation, and is involved in the regulation of their proliferation and differentiation *in vitro*. Blood 101, 856-62 (2003)

128. RA Ihrie, A Alvarez-Buylla: Cells in the astroglial lineage are neural stem cells. Cell Tissue Res 331, 179-91 (2008)

129. CM Liu, CH Yu, CH Chang, CC Hsu, LL Huang: Hyaluronan substratum holds mesenchymal stem cells in slow-cycling mode by prolonging G1 phase. Cell Tissue Res 334, 435-43 (2008)

130. SP Evanko, TN Wight: Intracellular localization of hyaluronan in proliferating cells. J Histochem Cytochem 47, 1331-42 (1999)

131. P Prehm: Identification and regulation of the eukaryotic hyaluronate synthase. Ciba Found Symp 143, 21-30 (1989)

132. S Sohr, K Engeland: RHAMM is differentially expressed in the cell cycle and downregulated by the tumor suppressor p53. Cell Cycle 7, 3448-60 (2008)

133. CA Maxwell, JJ Keats, M Crainie, X Sun, T Yen, E Shibuya, *et al.*: RHAMM is a centrosomal protein that interacts with dynein and maintains spindle pole stability. *Mol Biol Cell* 14, 2262-76 (2003)

134. CA Maxwell, J McCarthy, E Turley: Cell-surface and mitotic-spindle RHAMM: moonlighting or dual oncogenic functions? *J Cell Sci* 121, 925-32 (2008)

135. T Fujiwara, T Kawakatsu, S Tayama, Y Kobayashi, N Sugiura, K Kimata, *et al.*: Hyaluronan-CD44 pathway regulates orientation of mitotic spindle in normal epithelial cells. *Genes Cells* 13, 759-70 (2008)

136. S Marret, B Delpech, A Delpech, H Asou, N Girard, MN Courel, *et al.*: Expression and effects of hyaluronan and of the hyaluronan-binding protein hyaluronectin in newborn rat brain glial cell cultures. *J Neurochem* 62, 1285-95 (1994)

137. SA Back, TM Tuohy, H Chen, N Wallingford, A Craig, J Struve, *et al.*: Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. *Nat Med* 11, 966-72 (2005)

138. J Struve, PC Maher, YQ Li, S Kinney, MG Fehlings, C Kuntz, *et al.*: Disruption of the hyaluronan-based extracellular matrix in spinal cord promotes astrocyte proliferation. *Glia* 52, 16-24 (2005)

139. PJ Horner, FH Gage: Regenerating the damaged central nervous system. *Nature* 407, 963-70 (2000)

140. G Orive, E Anitua, JL Pedraz, DF Emerich: Biomaterials for promoting brain protection, repair and regeneration. *Nat Rev Neurosci* 10, 682-92 (2009)

141. TW Wang, M Spector: Development of hyaluronic acid-based scaffolds for brain tissue engineering. *Acta Biomater* 5, 2371-84 (2009)

142. CM Riley, PW Fuegy, MA Firpo, XZ Shu, GD Prestwich, RA Peattie: Stimulation of *in vivo* angiogenesis using dual growth factor-loaded crosslinked glycosaminoglycan hydrogels. *Biomaterials* 27, 5935-43 (2006)

143. WM Tian, SP Hou, J Ma, CL Zhang, QY Xu, IS Lee, *et al.*: Hyaluronic acid-poly-D-lysine-based three-dimensional hydrogel for traumatic brain injury. *Tissue Eng* 11, 513-25 (2005)

144. S Carbonetto, MM Gruver, DC Turner: Nerve fiber growth in culture on fibronectin, collagen, and glycosaminoglycan substrates. *J Neurosci* 3, 2324-35 (1983)

145. FZ Cui, WM Tian, SP Hou, QY Xu, IS Lee: Hyaluronic acid hydrogel immobilized with RGD peptides for brain tissue engineering. *J Mater Sci Mater Med* 17, 1393-401 (2006)

146. S Hou, Q Xu, W Tian, F Cui, Q Cai, J Ma, *et al.*: The repair of brain lesion by implantation of hyaluronic acid hydrogels modified with laminin. *J Neurosci Methods* 148, 60-70 (2005)

147. J Ma, WM Tian, SP Hou, QY Xu, M Spector, FZ Cui: An experimental test of stroke recovery by implanting a hyaluronic acid hydrogel carrying a Nogo receptor antibody in a rat model. *Biomed Mater* 2, 233-40 (2007)

148. WM Tian, CL Zhang, SP Hou, X Yu, FZ Cui, QY Xu, *et al.*: Hyaluronic acid hydrogel as Nogo-66 receptor antibody delivery system for the repairing of injured rat brain: *in vitro*. *J Control Release* 102, 13-22 (2005)

149. YT Wei, WM Tian, X Yu, FZ Cui, SP Hou, QY Xu, *et al.*: Hyaluronic acid hydrogels with IKVAV peptides for tissue repair and axonal regeneration in an injured rat brain. *Biomed Mater* 2, S142-6 (2007)

150. J Zhong, A Chan, L Morad, HI Kornblum, G Fan, ST Carmichael: Hydrogel Matrix to Support Stem Cell Survival After Brain Transplantation in Stroke. *Neurorehabil Neural Repair* 24, 636-44 (2010)

151. K Brannvall, K Bergman, U Wallenquist, S Svahn, T Bowden, J Hilborn, *et al.*: Enhanced neuronal differentiation in a three-dimensional collagen-hyaluronan matrix. *J Neurosci Res* 85, 2138-46 (2007)

152. L Pan, Y Ren, F Cui, Q Xu: Viability and differentiation of neural precursors on hyaluronic acid hydrogel scaffold. *J Neurosci Res* 87, 3207-20 (2009)

153. SK Seidlits, ZZ Khaing, RR Petersen, JD Nickels, JE Vanscoy, JB Shear, *et al.*: The effects of hyaluronic acid hydrogels with tunable mechanical properties on neural progenitor cell differentiation. *Biomaterials* 31, 3930-40 (2010)

Abbreviations: CNS: Central Nervous System, CSPG: Chondroitin sulfate proteoglycans, GAG: Glycosaminoglycan, ECM: Extracellular matrix, HA: Hyaluronan or Hyaluronic Acid, HAS: Hyaluronan synthase, HMW: High molecular weight, HYAL: hyaluronidase, INM: Interkinetic nuclear migration , LMW: Low molecular weight, NSC: Neural stem cell, NSPC: Neural stem/progenitor cell, PNS: Peripheral Nervous System, SGZ: Subgranular zone, SVZ: Subventricular zone, VZ: Ventricular zone

Key Words: Neural Stem Cell Niches, Ventricular Zone, Subventricular Zone, Subgranular Zone, Hyaluronan, Review

Send correspondence to: Larry S. Sherman, Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR, Tel:503-690-5217 Fax: 503-690-5384, E-mail: shermanl@ohsu.edu

http://www.bioscience.org/current/vol3S.htm