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Developmentally regulated collagen/integrin interactions confer adhesive properties to early postnatal neural stem cells ☆

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ABSTRACT

Background: It is becoming increasingly apparent that the extracellular matrix acts as an important regulator of the neural stem niche. Previously we found that neural stem and progenitor cells (NSPCs) derived from the early postnatal subventricular zone of mice adhere to a collagen/hyaluronan hydrogel, whereas NSPCs from the adult and embryonic brain do not.
Methods: To examine the specific adhesive properties of young stem cells in more detail, NSPCs isolated from embryonic, postnatal day 6 (P6), and adult mouse brains were cultured on collagen I.
Results: Early postnatal NSPCs formed paxillin-positive focal adhesions on collagen I, and these adhesions could be prevented by an antibody that blocked integrin β1. Furthermore, we found the corresponding integrin alpha subunits α2 and α11 levels to be highest at the postnatal stage. Gene ontology analysis of differentially expressed genes showed higher expression of transcripts involved in vasculature development and morphogenesis in P6 stem cells, compared to adult.
Conclusions: The ability to interact with the extracellular matrix differs between postnatal and adult NSPCs.
General significance: Our observations that the specific adhesive properties of early postnatal NSPCs, which are lost in the adult brain, can be ascribed to the integrin subunits expressed by the former furthering our understanding of the developing neurogenic niche. This article is part of a Special Issue entitled Matrix-mediated cell behaviour and properties.

1. Introduction

The growth, differentiation and migration of neural stem cells are regulated by intricate signaling patterns involving both soluble factors and interactions with the extracellular matrix (ECM) in the subventricular zone (SVZ). These interactions play a key role in positioning cells in relation to their neighbors, as well as in the movement of progenitor cells during development to their final positions in the brain. In addition to such spatial aspects, cues from the environment influence self-renewal, proliferation and differentiation. Despite their emerging importance, interactions between the ECM and neural stem cells are not fully understood [1].

These interactions can also be exploited for purposes of regeneration, and biomolecules such as collagen and hyaluronan are commonly used as scaffolds for neural stem and progenitor cells (NSPCs) [2]. Attachment of cells to such scaffolds is mediated by specific adhesion receptors, among which integrins are the major receptors for collagen. Attempts to modulate NSPC behavior by employing matrices of different composition have met with some success [3,4] but a detailed understanding of cell–ECM interactions is needed to further develop tissue-engineering efforts.

Previously, we found that NSPCs isolated from embryonic, early postnatal and adult mouse brains can be cultured and differentiated in a collagen/hyaluronan hydrogel [5]. This matrix supports the survival and proliferation of all of the NSPCs, but only those isolated from postnatal day 6 attach to the hydrogel; despite seeding as single-cell suspensions, cells from other ages aggregate in this gel. In light of the importance of ECM–NSPC interactions, this notable difference motivates exploration of the underlying mechanisms.

In the mouse brain, collagen I that belongs to the fibrillar subfamily of collagens [6] is present in structures called fractones [7]. Those are basal lamina extensions from blood vessels in the SVZ proposed to support stem cell proliferation locally through sequestration of growth factors by heparan sulfate proteoglycans. Collagen I positive vessels constitute a substantial part of the meninges, but parenchymal collagen I is found only in the SVZ [7]. Cell attachment to collagen is mediated by integrin heterodimers. Integrins α2β1 and α11β1 bind fibrillar collagen I with high affinity, whereas α1β1 and α10β1 prefer collagen IV [6]. Integrin α1β1 can also bind collagen I, although with lower affinity [8]. When bound to the ECM, integrins rapidly attract kinases, adaptor molecules

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and other proteins to form focal adhesions that promote various biological responses, including migration, proliferation and differentiation [9].

There is growing evidence for the existence of a neurovascular niche where interactions between neural stem cells and the blood vessels of the SVZ regulate proliferation of stem cells. Investigations on neurogenesis in various regions of the brain [10–13] as well as on stem cells in other tissues [14,15] have revealed the important role played by interactions with blood vessels in proliferation, self-renewal and differentiation of neural stem cells.

The present investigation reveals that NSPCs isolated from the SVZ of postnatal day 6 mice can adhere to collagen and establish focal adhesions in an integrin-dependent manner, whereas the corresponding cells isolated from the adult SVZ or embryonic cortex cannot. We suggest that this developmental difference in adhesive behavior reflects the higher expression of integrin α2 and integrin α11 by NSPCs on postnatal day 6.

2. Results

2.1. Organization of the subventricular zone in neonatal and adult mice

The periventricular germinal zones in the postnatal day 6 and adult mouse brains differ considerably, with many more cells in the neonate [16]. Accordingly, H/E staining of coronal sections here revealed a much denser cell population on postnatal day 6 (P6) (Fig. 1A) than in the adult SVZ (Fig. 1E).

Furthermore, during the first week of postnatal life, the SVZ contains heterogeneous populations of immature cells (largely radial glia, that will populate both glial and neuronal tracts), the sizes of which are greatly reduced one week later [17]. Our immunofluorescent staining showed a more pronounced expression of nestin in P6 than adult brain sections (Fig. 1B,F), with GFAP-positive cells in contact with the ventricular side in both cases (Fig. 1C,G). The GFAP-positive cells in the postnatal SVZ had long processes extending into the subependymal layers, and were probably radial glia differentiating into SVZ astrocytes (neural stem cells) a process that occurs at approximately postnatal day 7 in the mouse [17]. As an indication of the important interactions with the extracellular matrix, we observed collagen I in the vicinity of lectin-positive blood vessels in the subventricular zone of both the adult and P6 mouse brains, but not elsewhere in the parenchyma (Fig. 1D,H).

2.2. NSPCs isolated on P6 adhere to collagen

We previously employed a three-dimensional culture system with a hydrogel of collagen and hyaluronan [5], but for more detailed monitoring of adhesive properties we here used two-dimensional cultures on collagen. NSPCs isolated from E14, P6, and adult mouse brains were seeded onto collagen-coated tissue culture dishes and analyzed already 1 h later in order to eliminate the possibility that the cells might deposit their own ECM components and thereby confound interpretation of the results [18]. At this time, most of the P6 cells had attached to the surface displaying a flat morphology indicative of strong adhesion (Fig. 2B) whereas almost all of the E14 and adult NSPCs remained floating in the medium (Fig. 2A,C). Extending the incubation period for as long as 24 h did not enhance the adhesion of E14 and adult NSPCs to collagen I (not shown). To confirm that the E14 and adult NSPCs were viable, these were cultured in parallel on fibronectin (Fig. 2D–F) to which substrate all of the cell preparations attached. As a further control, P6 NSPCs were also seeded onto laminin, and found to adhere after 1 h (Fig. S1).

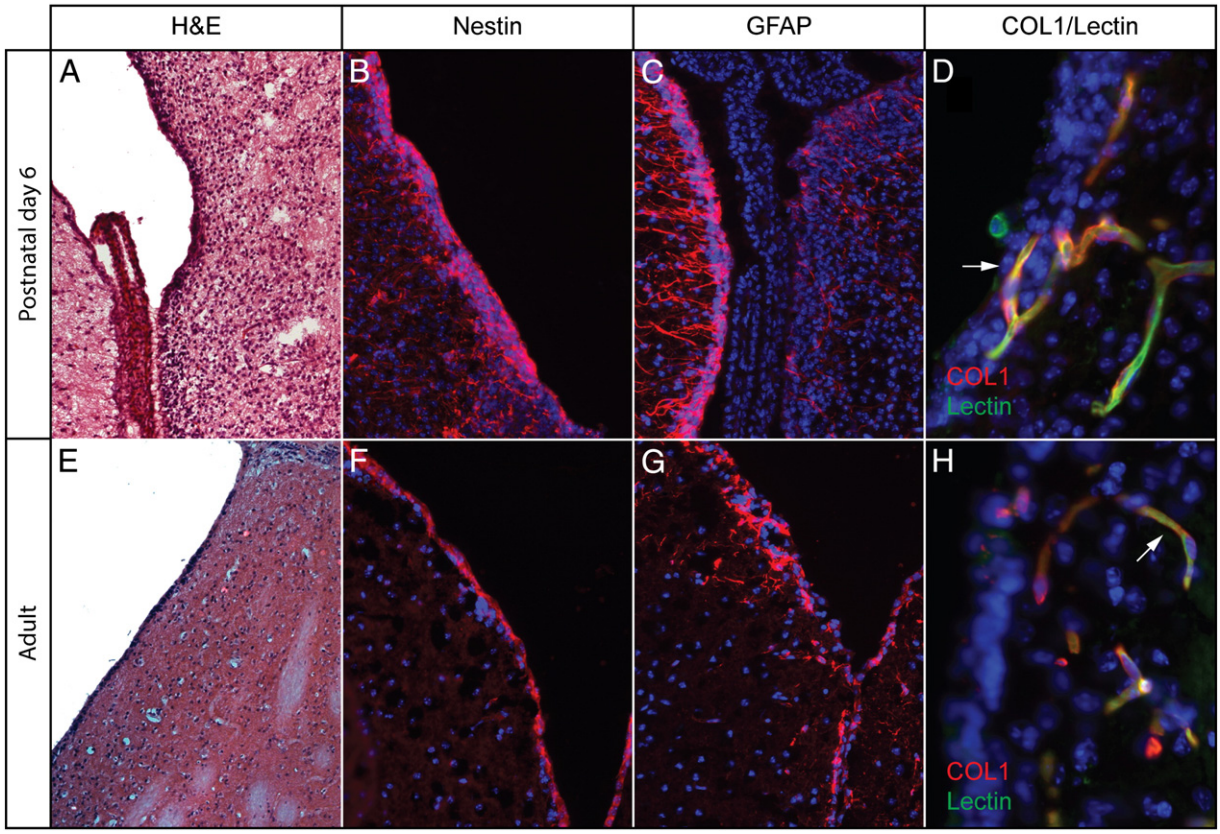

Fig. 1. Comparison of the subventricular zone between postnatal day 6 (P6) and adult mice. (A, E) Hematoxylin and eosin staining demonstrating a higher cellular density in the P6 (A) compared to the adult (E) SVZ. (B, F) Immunostaining for the neural stem and progenitor marker nestin (C, G) and GFAP identifying radial glia in the postnatal brain (D) and later on, stem cells in the adult SVZ (H). Immunostaining for collagen I (red) and lectin (green). Arrows indicate blood vessels positive for collagen I.

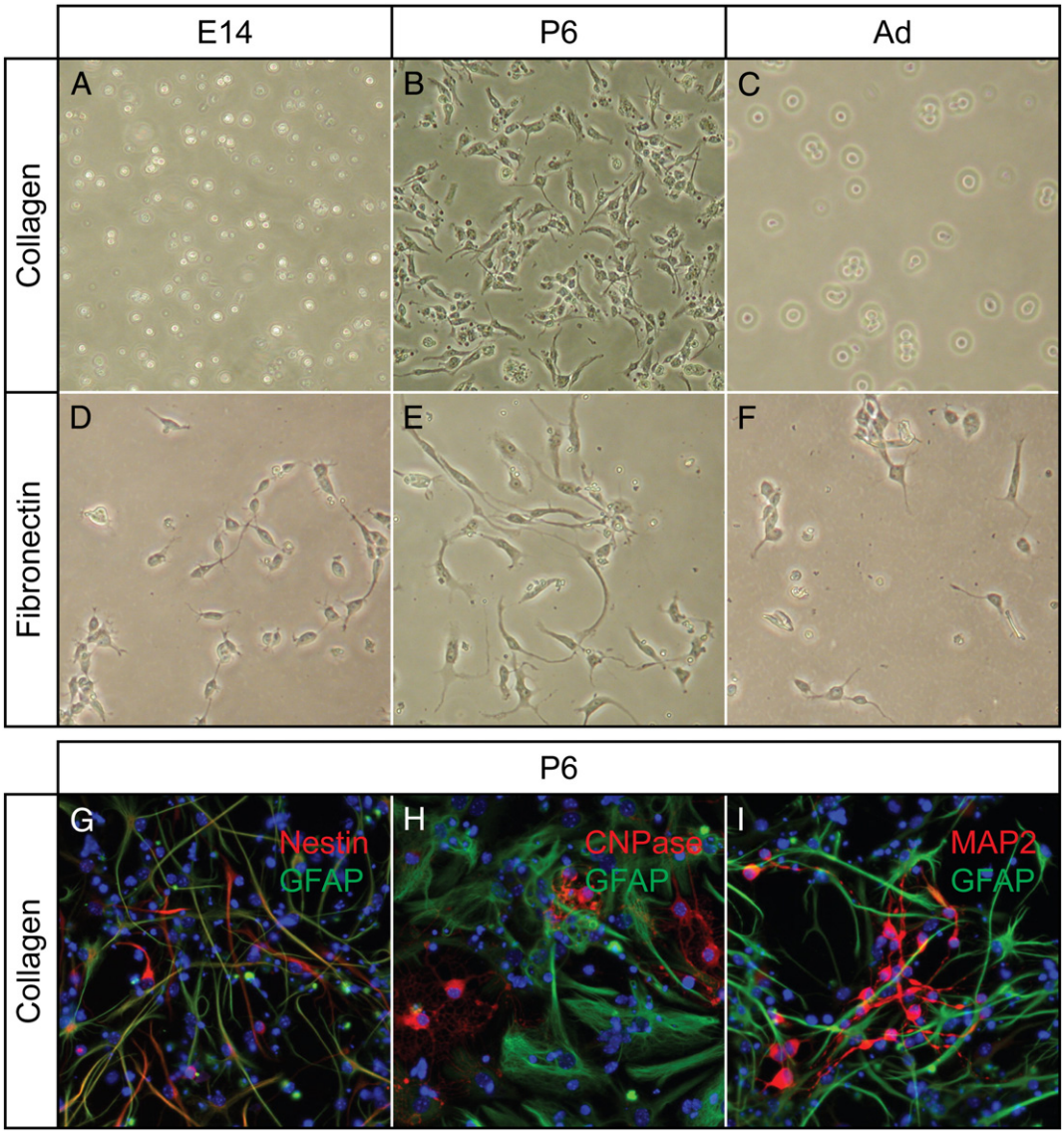

Fig. 2. Adhesion of NSPCs to collagen I and/or fibronectin. (A–C) E14, P6 and adult NPSC were seeded onto collagen I. Note that only the P6 cells (B) exhibit flattened morphology on collagen indicating adhesion. In comparison, E14, P6 and adult NPSC seeded onto fibronectin adhered to the substrate (D–F). The photographs were taken already 1 h after seeding to avoid ECM-production from the NSPCs, which might influence the result. (G–I) P6 NSPC cultured on collagen I for 8 days under differentiating conditions, and stained for markers of the neural lineages. The presence of GFAP, MAP-2 and CNPase positive cells indicated that differentiation had taken place.

To investigate if postnatal day 6 NSPCs cultured on collagen retained the capacity to form neurons and glia, they were subjected to differentiation by withdrawal of growth factors. After 8 days in differentiating conditions they expressed markers for neurons, astrocytes and oligodgendrocytes, and displayed morphologies indicative of mature cell types of all three lineages (Fig. 2G–I). This shows that proper differentiation has occurred, and thus attachment on collagen has not altered cell fate choice.

2.3. NSPCs from P6 form focal adhesions on collagen

In order to elicit a biological response in the cell, integrin binding to collagen leads to rapid formation of assemblies of various proteins, termed focal adhesions. Next, we therefore used staining with paxillin antibodies to determine whether the P6 NSPCs that adhered to collagen I have initiated focal adhesions. Detection of paxillin-positive focal adhesions in nestin-positive P6 cells 2.5 h after seeding (Fig. 3A) provided a strong indication that the adhesion was indeed mediated by integrins. This is further visualized in Fig. 3B where paxillin and nestin double positive cells are shown. This formation of focal adhesions also indicated

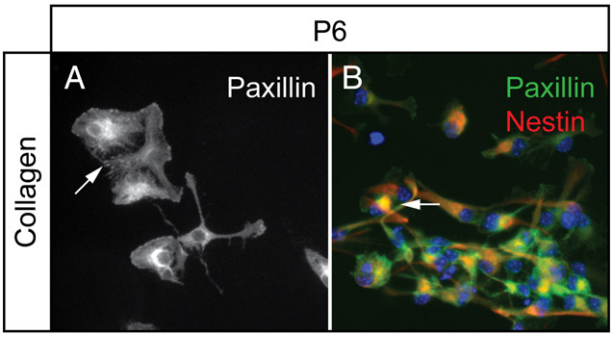

Fig. 3. NSPCs from postnatal day 6 formed focal adhesions on collagen I. (A) Immunostaining of paxillin-positive focal adhesions (arrow). The presence of focal adhesions indicate that integrins are involved in P6 NSPC adhesion to collagen I. (B) Immunostaining of nestin-positive P6 NSPCs on collagen with paxillin-positive focal adhesions (arrow). The analysis was performed 2.5 h after seeding the cells.

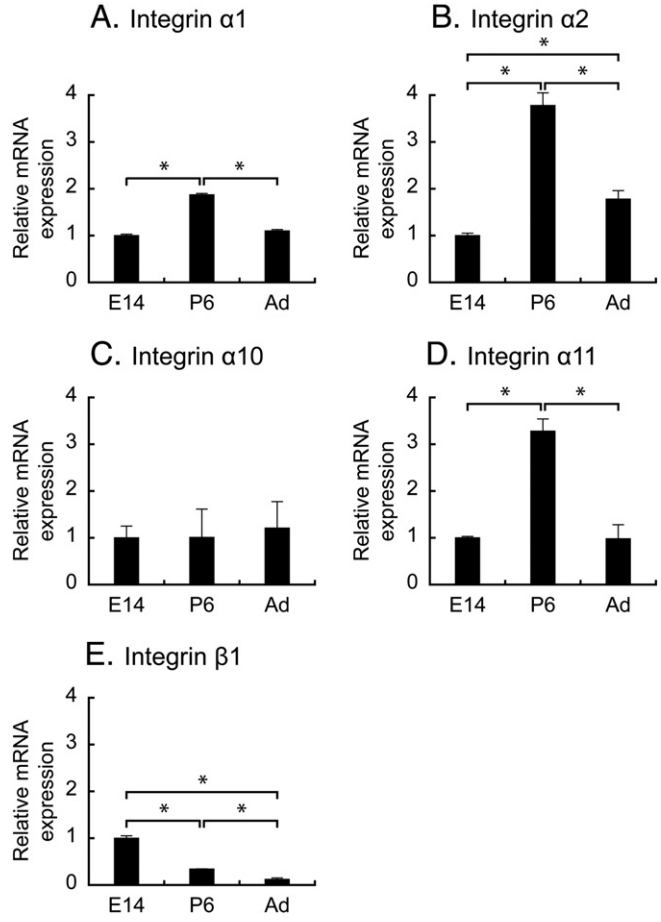

Fig. 4. Quantitative real-time PCR analysis of the levels of mRNA encoding integrin subunits. (A–E) mRNA expression of integrin subunits α1, α2, α10, α11 and β1 in embryonic (E14), postnatal day 6 (P6) and adult NSPCs. The bars illustrate the relative Ct values normalized against the level of β-actin mRNA, and expressed relative to the E14 level, which was defined as 1. Error bars indicate standard deviation and stars indicate statistical significance, $p \leq 0.01$.

that interactions with collagen I via integrins might be involved in signaling in the neural stem cell niche because collagen I is expressed on blood vessels in the postnatal day 6 SVZ (Fig. 1D).

2.4. Differential expression of integrin subunits by P6 NSPCs

Subsequently we employed qPCR to determine the levels of mRNA encoding the four integrin α subunits (α1, α2, α10 and α11) that, together with integrin β1, mediate binding to collagen (Fig. 4). Since integrins α2 and α11 exhibit the highest affinities for fibrillar collagen I [6,8], these subunits would most likely be involved in the adhesion of postnatal NSPCs to collagen I. Indeed, the levels of α2 and α11 mRNAs were higher in P6 than E14 and adult NSPCs (Fig. 4B and D). There was slightly more integrin α1 mRNA (Fig. 4A), but no difference in α10 mRNA (Fig. 4C) in P6 compared to embryonic and adult cultures lending further support to the conclusion that these integrin subunits were not responsible for the adhesion. The level of integrin β1 mRNA was found to be highest in the embryonic, intermediate in the postnatal, and lowest in the adult NSPCs (Fig. 4E). Thus, the β1 subunit, reportedly important in neural stem cell maintenance [19] was present in NSPCs at the time point for analysis of collagen adhesion.

2.5. Adhesion of P6 NSPCs to collagen is mediated by integrins

Having analyzed the integrin subunits that can confer collagen attachment we next set out to test their functionality. When a suspension of P6 NSPCs was pre-incubated with different concentrations of an antibody that blocked integrin β1, attachment to collagen was blocked in a dose-dependent fashion (Fig. 5). Since β1 is involved

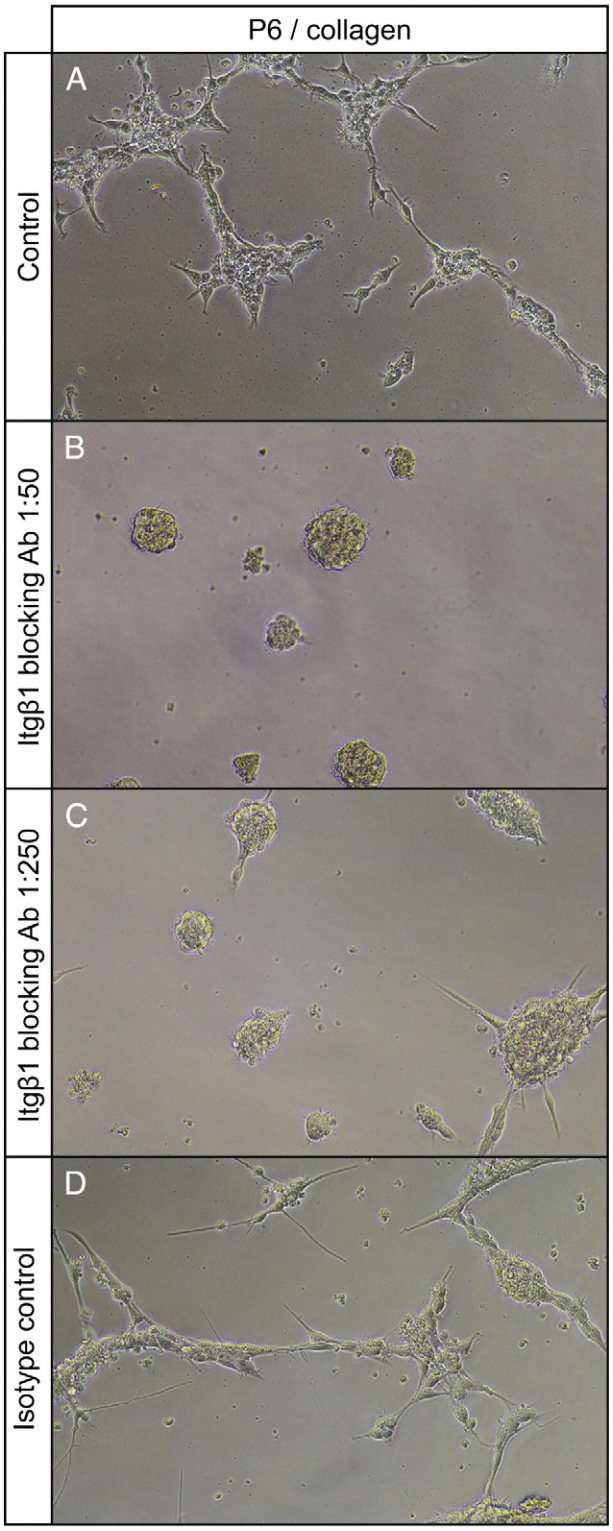

Fig. 5. Blocking antibodies to integrin β1 inhibited adhesion of P6 NSPCs to collagen I. (A) Control culture showing adhesion of P6 NSPCs to the collagen I substrate. (B) The adhesion was blocked by pre-incubating P6 NSPCs in the presence of an integrin β1 blocking antibody, diluted 1:50. (C) A 1:250 dilution of the integrin β1-blocking antibody prevented adhesion to collagen but not to the same extent. (D) An isotype control antibody did not block adhesion. The photographs were taken 72 h after seeding.

in all of the combinations of integrin subunits that bind collagen [6], we can conclude that the adhesion to collagen I is dependent on integrins.

2.6. Gene expression in neonatal and adult murine SVZ differs

To obtain an overall view of differentially expressed genes between P6 and adult NSPCs we analyzed their transcriptome. An Affymetrix Mouse Gene ST 1.0 microarray revealed that 145 genes were more highly expressed and 136 expressed at a lower level in postnatal than adult NSPCs. Fig. S2 represents a heat-map of genes that were up- (A) or down-regulated (B) at least 4-fold in the postnatal relative to the adult NSPCs. Among the genes with highest expression in P6 NSPCs relative to adult are retinol-binding protein (Rbp1), thrombospondin 2 (Thbs2) and angiopoietin 2 (Angpt2).

Table 1 documents enrichment clusters identified by a DAVID gene ontology analysis [20,21]. The annotation cluster with the highest score for enrichment in postnatal NSPCs included genes involved in blood vessel development, and morphogenesis. This suggests that significant regulation of neurovascular niche development occurs at early postnatal stages.

Table 1
Gene ontology enrichment clusters.
Enrichment clusters were identified by a DAVID gene ontology analysis (with a score ≥ 1.3 and a p value ≤ 0.05). The highest ranked enrichment cluster contained genes important for blood vessel development and morphogenesis, which were expressed at higher levels in the postnatal day 6 NSPCs than in stem cells from the adult SVZ.

Term	Count	%	P value
A — P6 higher			
Annotation cluster 1 (enrichment score: 6.556)			
Blood vessel development	13	9.35	1.06E−07
Blood vessel morphogenesis	12	8.63	1.10E−07
Vasculature development	13	9.35	1.38E−07
Angiogenesis	9	6.47	3.68E−06
Annotation cluster 2 (enrichment score: 2.714)			
Tissue development	16	11.51	0.00003
Epithelium development	9	6.47	0.00056
Organ morphogenesis	13	9.35	0.00077
Tissue morphogenesis	7	5.04	0.0061
Morphogenesis of an epithelium	6	4.32	0.00695
Annotation cluster 3 (enrichment score: 2.047)			
Regulation of signal transduction	14	10.07	0.00058
Negative regulation of signal transduction	5	3.6	0.03083
Negative regulation of cell communication	5	3.6	0.04007
Annotation cluster 4 (enrichment score:1.598)			
Striated muscle tissue development	5	3.6	0.01163
Skeletal muscle organ development	4	2.88	0.0146
Muscle tissue development	5	3.6	0.01464
Skeletal muscle fiber development	3	2.16	0.02056
Striated muscle cell differentiation	4	2.88	0.02377
Muscle fiber development	3	2.16	0.02837
Muscle organ development	5	3.6	0.03375
B — Adult higher			
Annotation cluster 1 (enrichment score: 2.058)			
Synaptic transmission	8	6.11	0.00012
Regulation of neurotransmitter levels	4	3.05	0.00689
Neurotransmitter secretion	3	2.29	0.02133
Annotation cluster 2 (enrichment score: 1.797)			
Ion homeostasis	7	5.34	0.01011
Cellular cation homeostasis	5	3.82	0.01523
Cellular chemical homeostasis	6	4.58	0.02644
Annotation cluster 3 (enrichment score: 1.618)			
Biopolymer glycosylation	4	3.05	0.01945
Protein amino acid glycosylation	4	3.05	0.01945
Glycoprotein biosynthetic process	4	3.05	0.03712
Annotation cluster 4 (enrichment score: 1.534)			
Regulation of synaptic transmission	4	3.05	0.02487
Regulation of transmission of nerve impulse	4	3.05	0.0296
Regulation of neurological system process	4	3.05	0.03401
Annotation cluster 5 (enrichment score: 1.480)			
Metal ion transport	8	6.11	0.02067
Monovalent inorganic cation transport	6	4.58	0.04152
Cation transport	8	6.11	0.04235

3. Discussion

Here we have attempted to explain why early postnatal NSPCs attach to collagen I whereas embryonic and adult NSPCs do not, and to relate this difference to the development of the CNS. The formation of focal adhesions by the postnatal cells indicated that the attachment to collagen I was dependent on integrins. The finding that blocking antibodies to β1 integrin inhibited stem cell attachment supported this notion as well as the temporally regulated expression of appropriate integrin alpha subunits.

Integrins are important regulators of adult neural stem cells in the neurovascular niche. For example, Shen et al. [12] found that integrin α6β1 holds these cells close to blood vessels, where interactions with endothelial cells support their proliferation. To characterize the adhesion of postnatal NSPCs to collagen I, we evaluated expression of the collagen-binding integrins. We observed that the pattern of mRNAs encoding subunits of integrins differs between postnatal and adult NSPCs. The relative levels of the α1, α2 and α11 integrin subunits appeared to explain the observation that only the P6 NSPCs adhered to collagen I, both in two-dimensional cultures (present study) and in a collagen/hyaluronan hydrogel [5]. In this context, α2 and α11 are the most likely partners for β1 because of their high and specific affinities for fibrillar collagen [6,8].

Integrin α2β1 is of particular interest, since apart from binding to collagen, this heterodimer also binds to laminin chains present on blood vessels and fractones in the SVZ [1,22]. It is of interest to note that the survival of mesenchymal stem cells has been reported to depend on interactions between integrins α2β1 and α11β1 and collagen I, but establishing the relative contributions of these two subunits proved to be difficult [23].

The subventricular zone is a highly organized compartment in which growth factors, neurotransmitters, morphogens and extracellular matrix molecules form the neural stem cell niche [24]. In the adult mouse this niche contains collagen I, as previously described [7]. In the current study we found that staining for collagen I co-localized with staining for the endothelium of blood vessels in the early postnatal SVZ, indicating that collagen I is part of the neurovascular niche at this developmental stage as well.

Microarray analysis data, although they should be interpreted with caution, revealed differences in the gene expression profiles of adult and postnatal NSPCs. Among the genes that were more highly expressed in P6 NSPCs were Rbp1, Thbs2 and Angpt2. Rbp1 binds retinol and acts as a chaperone, and can as such influence the levels of all-trans retinoic acid, which is of crucial importance in neural differentiation [25], and is thus an example of a gene whose expression should be higher than the developing brain. In contrast, DPP6, an auxiliary subunit potassium channel associated with the mature brain [26] showed higher expression in adult neural stem cells. Several genes that were more highly expressed in postnatal day 6 NSPC encoded proteins related to the ECM, e.g. Thbs2 and Angpt2. Thbs2, a regulatory protein of the neurovascular niche and involved in collagen remodeling [27], is thus highly interesting in the context of the current study. Angpt2 has been suggested to play a role in the neurovascular niche [28] and its expression is lost upon neural stem cell differentiation [29]. Because Angpt2 has a pro-angiogenic function keeping its levels high during development may be important for expansion of the neurovascular niche.

Our finding that the annotation cluster in P6 NSPCs with the highest enrichment score was related to “blood vessel development”, “blood vessel morphogenesis” and “vasculature development” underscores the importance of the developing neurovascular niche. Taken together, our findings might reflect a mechanism for attaching NSPCs to blood vessels at specific time-points during development.

4. Materials and methods

4.1. Animals

The animal protocol was pre-approved by the Local Ethical Committee for Animal Experimentation in accordance with Swedish law. Tissues taken from C57BL6 mice on embryonic day 14.5 (E14.5), postnatal day 6 (P6), and as adults (60–90 days) were used to prepare brain cryosections and neurosphere cultures of NSPCs.

4.2. Cell culture

Neurosphere cultures were prepared as previously described [5]. In brief, the cerebral cortices (E14.5) and subventricular zones (SVZ; P6 and adult) dissected from C57Bl6 mice (Taconic M&B, Ejby, Denmark) were dissociated mechanically to obtain single cells in Hank's balanced salt solution, pH 7.4 (Invitrogen, Stockholm, Sweden). NSPCs were cultured as neurospheres in DMEM/F12-GlutaMAX (Invitrogen) supplemented with 2% B27 (Invitrogen), 1% penicillin/streptomycin (Invitrogen) and EGF and FGF-2 (PeproTech, Rocky Hill, NJ; 20 ng/mL and 10 ng/mL, respectively). The medium was changed during passage of the neurospheres once every 4–5 days. EGF and FGF-2 were added to the medium in connection with every passage and, in addition to every other day in the case of the P6 and adult NSPCs.

4.3. Adhesion to collagen I and antibody blocking

Following their second passage E14, P6 and Adult neurospheres ($n = 3$ per age) were dissociated mechanically, seeded at 50,000 cells/cm^2 onto 6-well culture dishes coated with 10 μg/mL polyornithine and either 100 μg/mL collagen I (PureCol, Nutacon BV, Leimuiden, The Netherlands), 1 μg/mL fibronectin (Invitrogen, 33010018) or 10 μg/mL laminin I (Sigma-Aldrich, Stockholm, Sweden, L2020) and fixed 1 h later for assessment of adhesion. For blocking, after mechanical dissociation of P6 neurospheres ($n = 3$), the single cells were preincubated for 30 min at 37 °C with an integrin β1-blocking antibody (BD Pharmingen, 555002, diluted 1:50 and 1:250) or an isotype IgM control antibody (DAKO, Stockholm, Sweden, X0942, diluted 1:50) prior to seeding on polyornithine/collagen I coated 96-well culture dishes. In both cases photographs were taken 72 h after seeding.

4.4. Immunofluorescent staining

For immunofluorescent and hematoxylin/eosin staining, P6 and adult brains were fixed (4% paraformaldehyde in PBS, 2 h room temperature) and incubated in sucrose (30%, overnight) prior to OCT embedding and cryosectioning (10 μm). P6 NSPC cultures adhered to collagen I were fixed (4% paraformaldehyde in PBS, 15 min room temperature) and washed twice with PBS before being stained.

For immunofluorescent staining, the sections or fixed cultures were first blocked (5%NGS and 0.1% Triton-X-100 in PBS, 1 h) and then incubated overnight at 4 °C with primary antibodies (in blocking solution). The primary antibodies employed were rabbit anti-nestin (Covance PRB-315C, diluted 1:200), rabbit anti-GFAP (DAKO Z0334, diluted 1:200), mouse anti-GFAP (Sigma G3893, diluted 1:200), rabbit anti-MAP2 (Abcam, ab32454, diluted 1:200), mouse anti-CNP (Sigma C5922, diluted 1:200), mouse anti-paxillin (BD Transduction Labs 610052, diluted 1:200) and rabbit anti-collagen I (Abcam ab292, 1:200). After subsequent washing with PBS, the samples were incubated with fluorescent secondary antibodies (in blocking solution) for 2 h at room temperature. These secondary antibodies used were Alexa 555-conjugated goat anti-rabbit (Invitrogen A21430, diluted 1:400), Cy3-conjugated goat anti-mouse (Jackson 115-165-071, diluted 1:200), Alexa 488-conjugated goat anti-mouse (Invitrogen A11029, diluted 1:400) and FITC-conjugated goat anti-rabbit (Jackson 111-095-144, diluted 1:200). Blood vessels were stained with fluorescein-conjugated tomato lectin (Vector Laboratories, FL-1171, diluted 1:100). Finally the sections and fixed cultures were mounted in Fluoromount (DAKO).

In addition, other, sections were stained with hematoxylin for 3 min before developing, for 10 min in running tap water and then stained for 30 s in eosin solution before being mounted in Fluoromount (DAKO). Pictures were taken at 50× and 200× with a Zeiss AxioImager fluorescence microscope.

4.5. Quantitative real-time PCR and microarray analysis

For these experiments total RNA was extracted from E14, P6 and adult ($n = 3$ per age) neurosphere cultures after the second passage. The RNA was treated with the RNase-Free DNase Set (QIAGEN, Hilden, Germany) and 1 μg of RNA was then reverse-transcribed into cDNA with the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA). Quantitative RT-PCR was performed in triplicate with a StepOnePlus Real-Time PCR System (Applied Biosystems) utilizing primers and Taqman probes that recognized integrin subunits α1, α2, α10, α11 and β1. Quantitative values were calculated employing the $2^{-\Delta\Delta C(t)}$ method [30]; transcript levels were normalized against the level of β-actin transcript; and changes in transcript levels were expressed relative to the E14 level (designated as 1). Statistical tests were performed with GraphPad Prism software using t-tests corrected for multiple testing (Holm–Sidak).

An Affymetrix Gene ST 1.0 gene expression array was performed in triplicate on the P6 and adult RNA. Principal component analysis identified one P6 and one adult outlier and these were excluded from further analysis. The heat-map was produced using Genesis software [31] and the GO analysis was carried out with the online DAVID tool [20,21].

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbagen.2014.01.021.

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References

[1] I. Kazanis, J.D. Lathia, T.J. Vadakkan, E. Raborn, R. Wan, M.R. Mughal, D.M. Eckley, T. Sasaki, B. Patton, M.P. Mattson, K.K. Hirschi, M.E. Dickinson, C. ffrench-Constant, Quiescence and activation of stem and precursor cell populations in the subependymal zone of the mammalian brain are associated with distinct cellular and extracellular matrix signals, J. Neurosci. 30 (2010) 9771–9781.
[2] W.M. Tian, S.P. Hou, J. Ma, C.L. Zhang, Q.Y. Xu, I.-S. Lee, H.D. Li, M. Spector, F.Z. Cui, Hyaluronic acid–poly-D-lysine-based three-dimensional hydrogel for traumatic brain injury, Tissue Eng. 11 (2005) 513–525.
[3] A.I. Teixeira, J.K. Duckworth, O. Hermanson, Getting the right stuff: controlling neural stem cell state and fate in vivo and in vitro with biomaterials, Cell Res. 17 (2007) 56–61.
[4] Y. Wang, M. Yao, J. Zhou, W. Zheng, C. Zhou, D. Dong, Y. Liu, Z. Teng, Y. Jiang, G. Wei, X. Cui, The promotion of neural progenitor cells proliferation by aligned and randomly oriented collagen nanofibers through beta1 integrin/MAPK signaling pathway, Biomaterials 32 (2011) 6737–6744.
[5] K. Brännvall, K. Bergman, U. Wallenquist, S. Svahn, T. Bowden, J. Hilborn, K. Forsberg-Nilsson, Enhanced neuronal differentiation in a three-dimensional collagen-hyaluronan matrix, J. Neurosci. Res. 85 (2007) 2138–2146.
[6] B. Leitinger, Transmembrane collagen receptors, Annu. Rev. Cell Dev. Biol. 27 (2011) 265–290.
[7] F. Mercier, J.T. Kitasako, G.I. Hatton, Anatomy of the brain neurogenic zones revisited: fractones and the fibroblast/macrophage network, J. Comp. Neurol. 451 (2002) 170–188.
[8] J. Jokinen, E. Dadu, P. Nykvist, J. Käpylä, D.J. White, J. Ivaska, P. Vehviläinen, H. Reunanen, H. Larjava, L. Häkkinen, J. Heino, Integrin-mediated cell adhesion to type I collagen fibrils, J. Biol. Chem. 279 (2004) 31956–31963.
[9] B. Geiger, J. Spatz, A. Bershadsky, Environmental sensing through focal adhesions, Nat. Rev. Mol. Cell Biol. 10 (2009) 21–33.
[10] T.D. Palmer, A.R. Willhoite, F.H. Gage, Vascular niche for adult hippocampal neurogenesis, J. Comp. Neurol. 425 (2000) 479–494.
[11] A. Louissaint Jr., S. Rao, C. Leventhal, S.A. Goldman, Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain, Neuron 34 (2002) 945–960.

[12] Q. Shen, Y. Wang, E. Kokovay, G. Lin, S.-M. Chuang, S.K. Goderie, B. Roysam, S. Temple, Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell–cell interactions, Cell Stem Cell 3 (2008) 289–300.
[13] M. Tavazoie, L. van der Veken, V. Silva-Vargas, M. Louissaint, L. Colonna, B. Zaidi, J.M. Garcia-Verdugo, F. Doetsch, A specialized vascular niche for adult neural stem cells, Cell Stem Cell 3 (2008) 279–288.
[14] E. Fuchs, T. Tumbar, G. Guasch, Socializing with the neighbors: stem cells and their niche, Cell 116 (2004) 769–778.
[15] M.J. Kiel, O.H. Yilmaz, T. Iwashita, O.H. Yilmaz, C. Terhorst, S.J. Morrison, SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells, Cell 121 (2005) 1109–1121.
[16] P. Peretto, A. Merighi, A. Fasolo, L. Bonfanti, The subependymal layer in rodents: a site of structural plasticity and cell migration in the adult mammalian brain, Brain Res. Bull. 49 (1999) 221–243.
[17] A.D. Tramontin, J.-M. Garcia-Verdugo, D.A. Lim, A. Alvarez-Buylla, Postnatal development of radial glia and the ventricular zone (VZ): a continuum of the neural stem cell compartment, Cereb. Cortex 13 (2003) 580–587(New York, NY: 1991).
[18] C. Roberts, C.S. Chen, M. Mrksich, V. Martichonok, D.E. Ingber, G.M. Whitesides, Using mixed self-assembled monolayers presenting RGD and (EG)3OH groups to characterize long-term attachment of bovine capillary endothelial cells to surfaces, J. Am. Chem. Soc. 120 (1998) 6548–6555.
[19] L.S. Campos, D.P. Leone, J.B. Relvas, C. Brakebusch, R. Fassler, U. Suter, C. ffrench-Constant, Beta1 integrins activate a MAPK signalling pathway in neural stem cells that contributes to their maintenance, Development 131 (2004) 3433–3444.
[20] D.W. Huang, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, Nat. Protoc. 4 (2009) 44–57.
[21] D.W. Huang, B.T. Sherman, R.A. Lempicki, Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists, Nucleic Acids Res. 37 (2009) 1–13.
[22] M. Durbeej, Laminins, Cell Tissue Res. 339 (2010) 259–268.
[23] C. Popov, T. Radic, F. Haasters, W.C. Prall, A. Aszodi, D. Gullberg, M. Schieker, D. Docheva, Integrins α2β1 and α11β1 regulate the survival of mesenchymal stem cells on collagen I, Cell Death Dis. 2 (2011) e186.
[24] R.A. Ihrie, A. Alvarez-Buylla, Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain, Neuron 70 (2011) 674–686.
[25] J.A. Siegenthaler, A.M. Ashique, K. Zarbalis, K.P. Patterson, J.H. Hecht, M.A. Kane, A.E. Folias, Y. Choe, S.R. May, T. Kume, J.L. Napoli, A.S. Peterson, S.J. Pleasure, Retinoic acid from the meninges regulates cortical neuron generation, Cell 139 (2009) 597–609.
[26] B.M. Nadin, P.J. Pfaffinger, Dipeptidyl peptidase-like protein 6 is required for normal electrophysiological properties of cerebellar granule cells, J. Neurosci. Off. J. Soc. Neurosci. 30 (2010) 8551–8565.
[27] P. Bornstein, T.R. Kyriakides, Z. Yang, L.C. Armstrong, D.E. Birk, Thrombospondin 2 modulates collagen fibrillogenesis and angiogenesis, The Journal of Investigative Dermatology, 5, Symposium proceedings/the Society for Investigative Dermatology, Inc. [and] European Society for Dermatological Research, 2000, pp. 61–66.
[28] J. Masjkur, M.A. Rueger, S.R. Bornstein, R. McKay, A. Androutsellis-Theotokis, Neurovascular signals suggest a propagation mechanism for endogenous stem cell activation along blood vessels, Curr. Drug Targets CNS Neurol. Disord. 11 (2012) 805–817.
[29] A. Androutsellis-Theotokis, M.A. Rueger, D.M. Park, J.D. Boyd, R. Padmanabhan, L. Campanati, C.V. Stewart, Y. LeFranc, D. Plenz, S. Walbridge, R.R. Lonser, R.D. McKay, Angiogenic factors stimulate growth of adult neural stem cells, PloS One 5 (2010) e9414.
[30] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(−delta delta C(T)) method, Methods 25 (2001) 402–408.
[31] A. Sturn, J. Quackenbush, Z. Trajanoski, Genesis: cluster analysis of microarray data, Bioinformatics (Oxford, England) 18 (2002) 207–208.