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ScienceDirect
Experimental Neurology 204 (2007) 828–831
Experimental Neurology
www.elsevier.com/locate/yexnr

Short Communication

Sox-2 is expressed by glial and progenitor cells and Pax-6 is expressed by neuroblasts in the human subventricular zone

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Received 16 October 2006; accepted 12 December 2006
Available online 20 December 2007

Abstract

Transcription factors (TFs) are responsible for the specification and fate determination of cells as they develop from progenitor cells into specific types of cells in the brain. Sox-2 and Pax-6 are TFs with key functional roles in the developing brain, although less is known about TFs in the rudimentary germinal zones in the adult human brain. In this study we have investigated the distribution and characterization of Sox-2 and Pax-6 in the human subventricular zone (SVZ). Sox-2 immunoreactivity showed a nuclear labeling pattern and colocalised on GFAP immunoreactive cells as well as on bromodeoxyuridine (BrdU)-positive cells, whereas Pax-6 immunoreactivity was detectable in the nucleus and the cytoplasm of SVZ cells and colocalised with PSA-NCAM-positive progenitor cells. Thus, our data surprisingly reveal that these TFs are differentially expressed in the adult human SVZ where Sox-2 and Pax-6 specify a glial and neuronal fate, respectively.

Keywords: Human brain; Neurogenesis; Transcription factor; Progenitor cell; Subventricular zone; Bromodeoxyuridine

Transcription factors (TF) are responsible for the fate determination of progenitor cells. Although little is known about TF in the human subventricular zone (SVZ), in the rodent brain, previous studies have shown the importance of Pax-6 as a specification cue for migrating progenitor cells to become periglomerular and granular interneurons upon reaching the olfactory bulb (Hack et al., 2005). On the other hand, in the adult rodent brain, Sox-2 is preferentially expressed by glial cells and/or progenitor cells in the SVZ (Komitova and Eriksson, 2004). Thus, the combination of Pax-6 and Sox-2 represent important components in the specification of the adult progenitor cells into neurons and glia in the adult rodent brain. More specifically, Pax-6 is a member of the paired box gene family and is expressed in developing and adult brain (Stoykova and Gruss, 1994). Pax-6 is one of the key factors for central nervous system patterning and plays an essential role in the differentiation of cortical radial glia during development (Goetz, 1998; Simpson and Price, 2002). The TF Sox-2 belongs to the highly conserved Sox (sex determining region of Y-chromosome, SRY-related HMG-box) gene family (Wegner, 1999). Sox-2 is essential for neural induction of the ectoderm and is expressed in neuroepithelial stems cells during embryonic development (Cai et al., 2002; Zappone et al., 2000) and retains neural progenitor identity by interacting with pro-neural basic helix-loop-helix factors thus counteracting neuronal differentiation (Bylund et al., 2003; Graham et al., 2003), a point to note in this study. The localisation of these TFs has never been demonstrated in the human brain.

In the adult human brain, endogenous progenitor cells and neurogenesis originates in the SVZ of the basal ganglia and the subgranular layer (SGZ) of the hippocampus (Curtis et al., 2003; Eriksson et al., 1998). In response to injury or disease the number of progenitor cells, and subsequently the number of new neurons, is increased in the rodent and human brain (Arvidsson et al., 2002; Curtis et al., 2003; Curtis et al., 2005). Although little data are available from human brain material, high levels of activating transcription factor 2 (ATF2) have been associated with neurogenic cell fate decisions in the human

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0014-4886/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2006.12.008

SVZ in Huntington's disease (HD), while low levels were seen in the human hippocampus where increased neurogenesis is not observed in animal models of HD (Pearson et al., 2005).

This study was focused at evaluating the presence of two TFs, Sox-2 and Pax-6, in the adult human SVZ. The basal ganglia from seven normal untreated brains and two cases that had been treated with 5-Bromo 2′-deoxyuridine (BrdU; 250 mg; 2.5 mg/ml, 100 ml in a single intravenous injection) with at least 3 months survival after BrdU administration – for diagnostic purposes – as previously described (Eriksson et al., 1998), were obtained post mortem (Neurological Foundation of New Zealand Human Brain Bank, Department of Anatomy with Radiology, the University of Auckland; the Göteborg University human brain collection, respectively). These studies were approved by the respective institutional Human Subjects Ethics Committees. Normal brains were received from cases with no history of neurological disease and on pathological examination showed no pathological abnormalities.

For antibody staining, we used the following antibodies: Rabbit anti-Sox-2 (dilution 1:200; Chemicon); goat anti-Sox-2 (dilution 1:200; SDS); mouse anti-glial fibrillary acidic protein (GFAP; dilution 1:2,000; Chemicon); guinea pig anti-GFAP (dilution 1:2,000; Advanced Immunochemicals); mouse anti-Pax-6 (dilution 1:1,000; BABCO); rabbit anti-Pax-6 (dilution 1:1,000; Covance); rabbit anti-Pax-6 (dilution 1:1,000; Chemicon); rat anti-BrdU (dilution 1:100; Biosite); and mouse-anti polysialylated neural cell adhesion molecule (PSA-NCAM; dilution 1:1,000; Chemicon). Fluorescent secondary antibodies were used in a species specific manner and were: goat anti-mouse AlexaFluor 488 or 555 and goat anti-rat AlexaFluor 488/555 or 594 (1:200; Molecular Probes) and goat anti-guinea pig AlexaFluor 488 or 647 (1:200; Molecular Probes). For revealing staining with 3′3′ Diaminobenzidine (DAB), secondary goat anti-mouse (Sigma, St. Louis, Missouri; diluted 1:500) and goat anti-rabbit (Sigma, St. Louis, Missouri, diluted 1:500) secondary antibodies were applied to the sections in a species-specific manner for 12 h; the sections were then incubated ABC solution (Vector kit) and then DAB (in 0.1 M phosphate buffer and 0.01% H_2O_2, applied for 20 min) chromogen was used to produce latent staining. Sections were washed in PBS and 0.2% Triton X-100 (3 × 10 min) between each blocking and antibody step.

All primary antibodies were diluted in a solution of PBS, 0.2% Triton X, 1% normal goat serum and 0.04% merthiolate (incubation buffer). Floating sections were incubated in primary antibody on a shaker for 48 h at 4 °C, washed (3 × 10 min washes) and secondary antibodies in incubation buffer were applied and incubated overnight at room temperature. Sections were washed and mounted onto chrome alum dipped slides, air dried, dehydrated, cleared in xylene and coverslipped.

The fluorescent-labeled sections were mounted and coverslipped with fluorescent mounting medium (DakoCytomation) and imaged by using a confocal laser scanning microscope. Each fluorescent label was imaged serially to eliminate detection of bleed-through and other artificial fluorescence. The confocal images were captured in a *Z*-series with an interslice gap of 1 μm.

To analyze TF expression in the human SVZ, brain homogenates from subventricular zone were prepared and analyzed using Western blotting. Homogenates from unfixed, fresh frozen human brain tissue were used for Western blot analysis according to the previously published methods for TFs by Pearson et al. (2005). For immunoblotting we used rabbit anti-Sox-2 (1:1,000; Chemicon), anti-Pax-6 (1:1,000; Covance, Berkeley, USA) or anti-beta actin (1:5,000; Abcam Ltd.) antibody. Chemiluminescence detection reagents (ECL; Amersham Pharmacia) enabled visualization of peroxidase reaction products.

Using confocal laser scanning and conventional light microscopy, we determined the localisation of the TF Sox-2 and Pax-6 in the human SVZ. Sox-2 labeling revealed very discrete nuclear labeling on cells in both the SVZ and ependymal layer (EPL). In particular, the EPL contained tightly packed cells that were aligned along the ventricular edge. In the SVZ, the cells were scattered throughout, including some labeled nuclei in the gap region, but the most prominent Sox-2 labeling was beneath the gap region close to the CN (Fig. 1A). In contrast, there was very little Sox-2 labeling in the CN. Triple labeling experiments revealed that Sox-2 staining colabeled with BrdU-positive cells close to the gap region of the SVZ (Figs. 1B–E). Also, Sox-2 colocalised with glial fibrillary acidic protein (GFAP)-positive cells in the SVZ both close to both the gap region and close to the CN (Figs. 1F–H) and within each 20× field contained 4–10 double positive cells. The Sox-2/BrdU-positive cells were however never GFAP positive. Sox-2-positive cells were never colabeled with neuronal markers such as neuronal nuclei (NeuN) or microtubule associated protein 2 (MAP2). Thus, in this study on the human SVZ, Sox-2 is predominantly expressed by a subset of glial-like cells and in a subset of progenitors that had incorporated BrdU; it is difficult to determine using human brains whether all glial cells have a Sox-2 expressing stage. However, previous studies have demonstrated that some GFAP-positive cells have a large regenerative potential and since, in the rodent brain, Sox-2 is expressed by progenitor cells and glial cells, our results confirm that this is the case in humans (Komitova and Eriksson, 2004). We also performed Western blotting analysis for Sox-2 in isolated human SVZ samples that revealed a single prominent band at approximately 34 kDa, which is the expected weight of the Sox-2 protein (Fig. 1I).

Bands of 45 and 52 kDa appeared at higher antibody concentrations; they were presumably a result of post-translational modifications.

Interestingly, the labeling in the human brain for Pax-6 was located predominantly in the nuclear component of cells in the SVZ and less frequently in cell processes (Fig. 1J). The labeling was homogenous throughout the SVZ except in the gap region immediately beneath the ependymal layer. In the CN, the Pax-6 labeling was not nuclear but was evident in the cytoplasm and processes of cells with a neuronal phenotype, and these were predominantly located in the CN region just outside of the SVZ (Fig. 1J); it is likely that Pax-6 plays a dual role and is perhaps involved in cell signaling when localised in the cytoplasm. Double labeling studies of Pax-6 with internexin, vimentin,