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Cerebral Angiogenesis Shows Nestin Expression in Endothelial Cells

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Abstract. The class VI intermediate filament protein nestin has been generally considered as a specific marker for neural precursor cells or developing muscles In the prenatal developing rat central nervous system (CNS) we localized immunoreactivity for the nestin in blood vessels Although the widespread nestin expression in cerebral blood vessels persisted in early postnatal periods, it was down-regulated in the adulthood However, when the adult rat brains were subjected to procedures that trigger neovascularization, e g grafting fetal nervous tissue or C6 glioma, the abundant immunoreactivity was detected in all newly formed vessels and adjacent host vasculature Our results demonstrate that nestin expression in endothelial cells lining cerebral vessels accompanies the process of angiogenesis

Key words: Angiogenesis — Endothelium — Nestin — Immunohistochemistry — Rat

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Introduction

Expression of the intermediate filament (IF) nestin in endothelial cells of the intact nervous system and brain tumors has been noticed by several investigators (Dahlstrand *et al* 1992, Tohyama *et al* 1992, Frisén *et al* 1995) Recently, we demonstrated that expression of the nestin in vasculature was not restricted exclusively to cerebral blood vessels but was ubiquitous The most abundant immunoreactivity in endothelial cells was observed during embryonic development in both extra- and intraembryonic tissues (Mokrý and Němeček 1998) The aim of present study was to characterize events that are associated with expression of the nestin in the cerebral vasculature

Materials and Methods

We screened paraffin-embedded sections of whole rat fetuses and brains of postnatal Wistar rats (including pregnant rats) for the presence of IF nestin using anti-Rat-401 monoclonal antibody and peroxidase immunohistochemistry (labelled streptavidin-biotin method) Rat-401 antibody was obtained from the Developmental Studies Hybridoma Bank, maintained by the Department of Biological Sciences, University of Iowa, IA, USA The brain tissue was obtained from animals of the following ages E14 (embryonic day 14, $n = 3$), E15 ($n = 5$), E16 ($n = 2$), E19 ($n = 2$), postanatal day 0 (P0, $n = 2$), P7 ($n = 2$), P14 ($n = 2$), three-month old adult (pregnant) rats ($n = 6$) In addition to normal tissues, we also examined the expression of the nestin in sections of rat brains that had been previously transplanted with fetal neural tissue or C6 glioma ($n = 7$) Transplantation procedure has been thoroughly described elsewhere (Mokrý *et al* 1993 Mokrý and Němeček 1995)

Results

In the CNS of E14-E16 rat fetuses, immunohistochemistry using Rat-401 antibody revealed the strongest positive signal in the neuroepithelium and radial fibres This overall immunoreactivity for the nestin nearly masked immunostaining of blood vessels vascularizing the neuroepithelium In blood vessels of choroid plexus primordia, nestin expression was identified in endothelial and perivascular cells In the prenatal (E19) and neonatal (P0) rat brain, nestin-immunoreactivity ceased in postmitotic neural elements of other areas Therefore, e g in the cortical parenchyma, blood vessels were responsible for major immunoreactivity of the nestin Walls of the most vessels were ascertained by nestin-immunostaining of varying intensity that was confined to perivascular lining and endothelia In the CNS of P7 (postnatal day 7) rats, almost all cerebral vessels of different calibres (incl vessels supplying the choroid plexus) became visible after peroxidase immunodetection of the nestin (Fig 1) In P14 brains, endothelia of most capillaries expressed nestin, however, intensity of the immunostaining was lower when compared with P7 brains and few vessels were devoid of any specific signal Completely different staining pattern was specific for brains of adult pregnant rats The brain surface was lined with nestin$^+$ endfeet of the marginal glia Similar positivity was observed in astroglial perivascular endfeet accompanying underlying blood vessels in the cortical molecular layer (I) Deep cortical layers (II-VI) were devoid of any immunoreactivity for nestin Most endothelial cells were nestin-negative, positive immunostaining being confined only to occasional endothelial cells

Figure 1. Immunohistochemical detection of the nestin in the rat brain Nestin-immunoreactivity of neural cells ceased in the cortex of P7 rats, but remained in blood vessels (Magnification ×60)

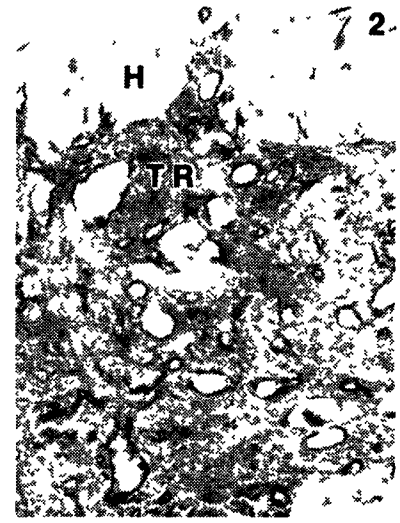

Figure 2. Immunohistochemical localization of the nestin in vasculature of neural grafts Dilated lumina of blood vessels nourishing the 21d intracerebral transplant (TR) became clearly apparent due to the expression of nestin In the host parenchyma (H), only adjacent capillaries were apparent (Magnification ×110)

One week after transplantation of isogeneic embryonic neural tissue, the nestin was identified in activated astrocytes throughout the host brain, of the positive astrocytes only elements adjacent to a needle tract area adopted morphology of reactive astrocytes (RAs) Overall strong expression of the nestin in astrocytic perivascular endfoot processes masked immunostaining of other vascular cells Two and three weeks postgrafting, immunoreactivity for nestin became clearly visible in endothelia of capillaries nourishing intracerebral transplant due to the ceased expression in astrocytes Thus, anti-nestin immunohistochemistry enabled to visualize capillaries as well as large vessels that vascularized solid grafts (Fig 2) This reactivity for nestin was intense, it was mainly confined to endothelia, although the positive perivascular sheath of astrocytic endfeet was also present Nestin$^+$ capillaries were also observed in the host parenchyma in the close vicinity to intracerebral transplants In animals that were left to survive for 1, 2 or 3 months, only few nestin$^+$ capillaries were found in peripheral portions of large intracerebral grafts In intraventricular grafts, intense immunoreactivity was observed in blood vessels that apposed to a wall of cerebral ventricles and became vascularized In these solid intraventricular grafts, immunohistochemical detection of the nestin in endothelial cells allowed to depict their complete capillarization

In rat brains inoculated intracerebrally with C6 glioma cells 6 days previously, neoplastic cells filled in the whole needle-track area and began to form a rapidly increasing

tumour mass in the right hemisphere At the implantation site, blood vessels of different calibres including large paralytic vessels became visualized with nestin immunostaining Perivascular spaces of these cerebral vessels were infiltrated with nestin$^+$ C6 cells By 10 days a solid tumour composed of nestin$^+$ C6 cells and capillaries was formed Anti-nestin immunohistochemistry visualized also peritumoral vessels which enabled perivascular propagation of neoplastic cells (Fig 3) One month after the transplantation of C6 cells, tumor mass reached its largest size In areas rich in glioma cells, immunostaining for nestin revealed positive vascular endothelia surrounded by positive perivascular C6 cells or host RAs that were included in the tumour Perivascular processes of RAs lined adjacent cerebral vessels and permitted to identify also blood vessels in the close vicinity to tumor

Discussion

In E14–19 rat fetuses, we observed more distinct immunoreactivity of endothelial cells for IF nestin in non neural tissues (Mokrý and Němeček 1998) than in the CNS Nestin-positivity of CNS vessels became quite clearly apparent in the first postnatal week when the nestin expression ceased in surrounding neural cells As the number of cerebral vessels identified with anti-nestin immunohistochemistry declined in the adulthood, it was apparent that expression of nestin gene in a vasculature was temporally restricted The descrease of nestin expression in the course of nervous tissue maturation has been thoroughly described in previous studies (Frederiksen and McKay 1988, Lendahl *et al* 1990, Tohyama *et al* 1992, Zimmerman *et al* 1994) In adult brains, immunohistochemistry utilizing Rat-401 monoclonal antibody as primary antibody labelled only sporadic cerebral vessels reflecting the low endothelial cell turnover

Figure 3. Peroxidase immunohistochemistry identified the nestin expression in vascular cells in the periphery of 10d C6 glioma C6 cells migrate along perivascular spaces of adjacent blood vessels (arrowheads) in the recipient brain tissue (Magnification ×130)

In the experimental part of our study, we investigated the nestin immunoreactivity in adult rat brains that were intracerebrally injected with cells that induce local neovascularization of the grafted tissue In the first group of experimental animals, solid pieces of E14 fetal rat brain were grafted into the brain parenchyma of adult animals Following transplantation, the grafted tissue suffering from hypoxia obviously induces a release of signals that initiate the process of vascularization This process consists of several steps which include ingrowth of host capillaries into the transplant (angiogenesis) and their inoculation to pre-existing and collapsed capillaries derived from the donor's brain tissue (Krum and Rosenstein 1987) Our finding of nestin expression in vessels in the close vicinity to the graft confirmed that also adjacent host cerebral vessels are really activated during angiogenesis

Within the graft, we observed the nestin immunoreactivity in endothelia of all vessels as well as in immature neural cells, immunoreactivity confined to vasculature lasted for long time (more than 3 months) whereas it rapidly ceased in neural cells that matured After 1 month postgrafting, all capillary endothelia in intraventricular grafts expressed the nestin, whereas only peripheral blood vessels of intracerebral grafts remained nestin$^+$ The second group of experimental animals was intracerebrally injected with the C6 glioma cells We found that in a tumor mass of a solid C6 glioma, endothelia of all blood vessels nourishing the tumor expressed nestin The finding of nestin$^+$ endothelial cells in human astrocytomas and glioblastomas has been previously described by Tohyama et al (1992) who used anti-nestin 129 polyclonal antiserum that binds the human nestin

Conclusions

Our results demonstrate that in the CNS, anti-nestin immunohistochemistry identifies not only immature neural cells but also cerebral blood vessels Widespread vascular immunoreactivity for the nestin is restricted to prenatal and early postnatal periods, whereas it is greatly reduced in the endothelium of blood vessels supplying the adult intact brain Neovascularization that occurs e g in growing neural grafts or brain tumors, is accompanied with specific expression of IF nestin in all endothelial cells Our findings indicate that the nestin expression in endothelium of rat cerebral vessels is closely associated with cerebral angiogenesis

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