Nucleolar Protein 1 (Nol1) Expression in the Mouse Brain

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ABSTRACT

Nucleolar protein 1 (Nol1) is a cell cycle dependent gene highly expressed in proliferating tissues. In order to test whether Nol1 could be used as a marker of dividing neural stem cells within mouse brain, Nol1 expression was analyzed using mouse carrying a gene trap modification of Nol1 gene. High Nol1 expression was found within the hippocampus, olfactory bulb, cerebral and cerebellar cortex. Nol1 was expressed not only in the dividing cells within the brain, but as well in the postmitotic neurons. This suggested a general role of Nol1 in assembling of ribosomes in cells with high protein production.

Key words: Nol1, nucleolus, neuron, brain, mouse

Introduction

Nucleolar protein 1 (Nol1, proliferation-associated nuclear antigen 120, p120) is an evolutionary highly conserved gene coding 120-kDa, RNA-binding, nucleolar-specific protein\(^1,2\). Its expression in the majority of malignant tissues, but also in the non-malignant, highly proliferative tissues correlates with the rapidity of cell cycle\(^3\). Therefore, Nol1 was firstly implicated as a tumor cell marker. It is expressed early in G1 phase of the cell cycle and peaks during S phase\(^1,2\). In clinical praxis Nol1 expression was shown as a useful prognostic marker of poor tumor prognosis\(^4,5\). The expression of Nol1 is induced rapidly following growth stimulation\(^6\), and overexpression of human Nol1 gene produces tumors in the nude mice\(^7\).

Neurogenesis in mammalian brain is completed at the mature stage\(^8\) and vast majority of neurons escape cell cycle and remain as postmitotic cells. However, some regions of the adult mammalian brain, subventricular zone (SVZ) adjacent to the lateral ventricle, subgranular zone (SGZ) of the hippocampal dentate gyrus, and olfactory bulb\(^9,10\) are able to generate neurons continuously throughout life.

To address the question whether Nol1 could be used as a marker of dividing neural stem cells within mouse nervous system, mouse carrying a gene trap modification of Nol1 gene was used. The obtained results, showing high expression of Nol1 in hippocampus, olfactory bulb, cerebral and cerebellar cortex, suggested that Nol1 expression in the mouse brain was not specific to the stem cell populations, but mirrored intensive processes of ribosome assembly and high levels of the protein synthesis in the neurons.

Materials and Methods

Nol1\(^{gt1\text{Gaj}}\) mouse line

Embryonic stem cell genome was modified by gene trap method. This included the insertion of nonhomologous DNA construct carrying selector (neoR) and marker (lacZ)\(^11\). From selected embryonic stem cells, animal carrying the corresponding gene modification was generated. Modified Nol1 gene was tagged and its expression was monitored by lacZ activity\(^14\). While Nol1 heterozygotes mate normally, Nol1 homozygotes are not viable.

Tissue preparation and expression analysis

Nol1 heterozygotes and their wild type controls were sacrificed and fixed by perfusion of 2% paraformaldehyde and 0.2% glutaraldehyde in phosphate-buffered saline.

Received for publication June 1, 2007.
Brain was removed from the skull and subsequently immersed in the same fixative for one hour. 300 μm thick sagittal, frontal and horizontal sections of the brain were obtained using vibratome. Sections were rinsed in PBS and incubated at 37 °C overnight in the staining solution containing X-gal as a substrate for β-galactosidase (0.5 mg/ml X-gal, 2mM MgCl2, 10mM K4Fe(CN)6, 10mM K3Fe(CN)6 in PBS). Sections were mounted and photographed using microscope Olympus AX70 and camera Nikon DXM1200.

Results

In order to analyze expression of Nol1 gene in the central nervous system of adult mouse, the brains of Nol1gt1Gaj adult mice and their wild type controls were cut in sagittal, frontal and horizontal directions. The activity of beta-galactosidase was detected by histochernical staining with X-gal, which mirrored Nol1 expression. In the brain, Nol1 expression was found within hippocampal and olfactory region and cerebral and cerebellar cortex.

Morphological analysis of all described Nol1 expressing regions using higher magnifications showed that the signal was visible within nucleoli of lacZ positive cells (Figure 1a). This confirmed that beta-galactosidase distribution corresponded to that of Nol1, and allowed to distinguish the expressing cells from the rarely observed background, which was represented as diffuse blue staining. In addition, the staining enabled to recognize the typical morphologic features of neurons, and therefore to identify cells expressing Nol1 as neurons (Figure 1a).

In the hippocampus, expression of Nol1 was present in the CA1, CA2 and CA3 of Ammon’s horn and in the dentate gyrus (Figure 1b and 1d). As Nol1 expression was confined to the cell nucleolus, Nol1 presence within Ammon’s horn could be assigned to the pyramidal cells of that region. Moreover, Nol1 was strongly expressed by the granular cells of the dentate gyrus.

Another region highly expressing Nol1 was cerebral cortex. Single scattered positive cells were visible in all layers, but the main areas of the expression were the external granular layer and the pyramidal layers (layers 2 and 3) (Figure 1c). The presence of staining was confined to the granular and pyramidal cells in those regions. Although expression was found along the whole cortex, the strongest expression was present within temporal caudal lobe in the entorhinal cortex.

Analysis of Nol1 expression within olfactory bulb revealed Nol1 expression in all regions where neuronal cell bodies were located. Thus, expression was present in the glomerular cell layer containing small periglomerular neurons, mitral cell layer containing much bigger cell bodies and granular cell layer with numerous small cell bodies (Figure 1e).

**Fig. 1.** Expression pattern of Nol1 within mouse brain. Nol1 expression was localised within a nucleolus of a nerve cell (1a, arrow). The strongest Nol1 expressing regions were cortical layers 2 and 3 (1b, uppermost arrow; 1c) and dentate gyrus (DG, black arrow) and Ammon’s horn (AH, white arrow) of hippocampus (1b,1d). Within olfactory region, expression of Nol1 was visible in glomerular (GL), mitral (M) and granular (GR) cell layers (1e). N – nerve cell nucleus, C – cortex, 2 and 3 correspond to cortical layers 2 and 3, CA1 – CA1 region of hippocampus.
Nol1 expression in the cerebellum was visible only within granular layer and in Purkinje cell layer, but not in the molecular layer.

**Discussion**

The main aim of this work was to verify whether Nol1 could be used as a marker of proliferating cells within mouse brain, in particular dividing neural stem cells. Following current knowledge about Nol1 and regions of neurogenesis within rodent nervous system, it was expected that Nol1 expression would be found in hippocampus, subventricular zone and olfactory bulb. Although expression in the hippocampus and the olfactory bulb was confirmed, it was present in virtually all cells in these regions. Therefore the fact that Nol1 was found in the Ammon’s horn and the glomerular and the mitral cell layer of the olfactory bulb could not be explained with reports which state that neurogenesis could occur in these regions, as neurogenesis in these regions is of limited extent\(^\text{12,15}\). Fact that Nol1 was highly expressed in hippocampal, olfactory and cerebral and cerebellar cortex regions, and not in the subventricular zone, suggested that Nol1 expression in the brain was not specific to the proliferating cells but it was present in the wide population of postmitotic neurons.

Various markers of cellular proliferation in the central nervous system have already been tested\(^\text{16,17}\). Schmetsdorf et al. showed that postmitotic neurons express the cell cycle related proteins previously related only to the mitotic events. Moreover, Migaldi et al.\(^\text{18}\) reported presence of Nol1 in all postmitotic cells 10 years ago. However, high Nol1 expression in the proliferating cells regularly blurs this fact in the praxis. Our finding of Nol1 in the postmitotic neurons was in line with these reports. The facts that Nol1 is cell cycle regulated, expressed in highly proliferative cells, and correlates with tumor grade, led to the widely accepted conclusion that Nol1 is specific to cellular proliferation. Our finding of Nol1 in the postmitotic cells implies a more general function of Nol1 in the process of ribosome assembly. Nol1 is a mammalian homologue of a yeast protein known as Nop2p, which is required for the methylation of 27S RNA and processing of mature 25S ribosomal RNA (rRNA) during ribosome biogenesis\(^\text{19}\). Mutations in yeast Nop2p protein disrupt rRNA processing by abolishing methylation, therefore suggesting Nol1 is rRNA methyltransferase. Recent data about factors involved in proliferation and differentiation of the bone marrow cells indicate that these processes require Nol1\(^\text{20}\). On the other hand, apoptosis of HeLa cells in the presence of antisense Nol1 suggests that Nol1 is required for growth of mammalian cells and therefore the use of antisense Nol1 in anticancer therapy was proposed\(^\text{21}\). The effects on cellular proliferation are believed to be result of Nol1 involvement in ribosome assembly. Subcellular localization of Nol1 within nucleolus supports this hypothesis\(^\text{22}\).

Since a cell-cycle dependent function of Nol1 could not be envisaged in the postmitotic neurons, the additional more general Nol1 function(s) could be suggested. Location within nucleolus and binding of rRNA indicate the role of Nol1 in assembling of ribosomes. However, cell cycle dependence should not be strictly tied to the mitotic events, because of several reasons: firstly, genesis of ribosomes, which is extensive in neurons, is in another cell types cell cycle dependent process and this does not necessary imply that Nol1 is directly connected to cell cycle events. Moreover, arrest of cell cycle in neurons is not a definite state. Neurons can express cell cycle related proteins in normal\(^\text{17}\) or in pathological conditions\(^\text{23}\), and neurogenesis can be induced by hypoxia\(^\text{4}\). Although final explanation of Nol1 expression in postmitotic neurons is still a matter of debate, the known facts about NOL1 protein and its herein reported presence in the mouse brain, implicate that presence of NOL1 is not only required in the dividing cells, but as well in the cells with high protein synthesis rate, e.g. the brain neurons.

**Acknowledgements**

We thank Sandra Mavríc for help in vibratome sectioning. This research was supported by grants 108-1018170-1902 from Ministry of Science, Education and Sports of the Republic of Croatia and CRP/CRO06-02 from International Center for Genetic Engineering and Biotechnology.

**REFERENCES**


IZRAŽAJ BJELANČEVINE JEZGRICE 1 (Nol1) U MOZGU MIŠA

SAŽETAK

Bjelančevina jezgrice 1 (Nucleolar protein 1, Nol1) je gen izražen u stanicama koje se umnažaju, te mu izražaj ovisi o fazi staničnog ciklusa. Kako bismo testirali hipotezu da je Nol1 potencijalni biljeg stanica u diobi u mozgu miša (živčane matične stancica), analizirali smo izražaj Nol1 u mozgu miša kojem je Nol1 preinačen postupkom genske zamke. Ekspresija Nol1 je nađena u hipokampusu, njušnom bulbusu, te u kori velikog i malog mozga. Ovaj nalaz je ukazao da Nol1 nije izražen samo u stanicama koje se dijele, već i u diferenciranim živčanim stanicama. Pretpostavljeno je kako Nol1 ima ulogu u sintezi ribosoma i bjelančevina u stanicama živčanog sustava.