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Shedding of NG2 proteoglycan during human brain development and vascularization

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NG2 is a membrane spanning proteoglycan with a large extracellular domain showing binding sites for several molecules, including extracellular matrix components and growth factors. In the developing CNS, NG2 is expressed by subpopulations of oligodendroglia precursors and additionally by microvascular pericytes. The expression of NG2 is downregulated in mature oligodendrocytes and also pericytes of stable brain vessels in healthy brains do not express the proteoglycan. Our previous studies have demonstrated that during human cerebral cortex development, 'activated' NG2-pericytes form vascular cord that allow endothelial cell migration and elongation thus contributing to vascular sprouting. Braced by these first results, we have concentrated our attention on the possible involvement of NG2 and NG2-expressing cells on cell/cell and cell/matrix relationships during vessel growth, in developing human brain at 9 and 22 weeks of gestation. Double immunolabelling has been carried out utilizing anti-NG2 antibodies directed against different antigenic sites of the core protein; namely, a rabbit anti-NG2 D2, mouse monoclonal antibodies from two different clones, F9 and D7, and a commercially available rabbit anti NG2 (CSPG4, Sigma). At the earlier developmental stage, $NG2^+$ pericytes are already an integral part of the vessel wall and are involved, together with $Glut^+$ endothelial cells, in basal lamina collagen type IV and VI deposition. In double immunolabelling with two different NG2 antibodies (D2/F9), the staining patterns almost completely overlap and also coincide with collagen type IV/VI distributions. Later on, NG2 D2 and NG2 F9 staining patterns are largely unrelated, D2 appearing associated with both pericytes and vessel basal membrane, whereas NG2 F9 is primarily restricted to pericytes. It is known that in 'in vitro' assay almost the entire ectodomain of NG2 can be released by proteolitic shedding. These observations support the concept that both membrane-bound and unbound NG2 forms are involved in vessel growth, and that the released proteoglycan may contribute as an ECM substrate to endothelial cell migration and sprouting.

Key words
NG2 proteoglycan, pericytes, microvessel growth, human brain, confocal microscopy