The role of the intermediate filament (nanofilament) protein nestin in neural progenitor cell and astrocyte differentiation

Akademisk avhandling

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I. Lebkuechner I, Andersson D, Möllerström E, Wilhelmsson U, Pekna M, Pekny M. (2014) Heterogeneity of Notch signaling in astrocytes and the effects of GFAP and vimentin deficiency. *Manuscript*

II. Wilhelmsson U, Lebkuechner I, Yang X, Nagy A, Pekny M. (2014) Increased hippocampal neurogenesis in mice deficient for intermediate filament (nanofilament) protein nestin. *Manuscript*

III. Puschmann TB, Zanden C*, Lebkuechner I*, Philippot C, de Pablo Y, Liu J, Pekny M. (2014) HB-EGF affects astrocyte morphology, proliferation, differentiation, and the expression of intermediate filament proteins. *Journal of Neurochemistry, 128(6):878-89*

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ABSTRACT

Nestin, a class VI intermediate filament (nanofilament) protein, is commonly used as a marker for neural stem/progenitor cells (NSPCs), but its role in neurogenesis remains elusive. Nestin is also expressed in immature and reactive astrocytes. The up-regulation of intermediate filament proteins glial fibrillary acidic protein (GFAP), vimentin and nestin is a characteristic feature of reactive astrocytes, accompanied by alterations in the expression of many other genes. We found that transgenic mice deficient for nestin have increased number of newly born hippocampal neurons 6 weeks after BrdU *in vivo* labeling, suggesting increased generation and/or survival of newly born neurons. We also showed that *in vitro* nestin deficient astrocytes provide a more pro-neurogenic environment that results in a 2-fold increase in neuronal differentiation of NSPCs.

Astrocytes are highly heterogeneous cells and fulfill a variety of important functions in healthy as well as diseased brain. In addition, astrocytes can exhibit features characteristic of NSPCs and modulate neurogenesis by inhibiting neuronal differentiation of NSPCs through Jagged1-mediated Notch signaling. Given the vast array of astrocyte functions, classification of astrocyte subpopulations on a molecular level is highly desirable. We used single cell quantitative real-time PCR to investigate the heterogeneity of astrocytes with respect to their Notch signaling competence. Our results show that the Notch signal sending but not Notch signal receiving competence of astrocytes depends on GFAP and vimentin. Further, we showed that nestin and heparin binding EGF-like growth factor (HB-EGF) may serve as classifiers of astrocyte subpopulations *in vitro*.

Utilizing our newly developed Bioactive3D and standard 2D cell culture systems, we showed that HB-EGF alters astrocyte morphology towards a more radial glia-like phenotype. HB-EGF affects proliferation, differentiation and expression of Notch signal pathway related genes and leads to the up-regulation of nestin expression. HB-EGF in cell culture media results in partial de-differentiation of astrocytes and therefore should be used with caution.

Keywords: intermediate filament (nanofilament) proteins, nestin, neural stem/progenitor cells, astrocytes, GFAP, vimentin, HB-EGF

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