

## SOX2 and OCT4 mRNA-Expressing Cells, Detected by Molecular Beacons, Localize to the Center of Neurospheres during Differentiation - DTU Orbit (09/11/2017)

### SOX2 and OCT4 mRNA-Expressing Cells, Detected by Molecular Beacons, Localize to the Center of Neurospheres during Differentiation

Neurospheres are used as in vitro assay to measure the properties of neural stem cells. To investigate the molecular and phenotypic heterogeneity of neurospheres, molecular beacons (MBs) targeted against the stem cell markers OCT4 and SOX2 were designed, and synthesized with a 2'-O-methyl RNA backbone. OCT4 and SOX2 MBs were transfected into human embryonic mesencephalon derived cells, which spontaneously form neurospheres when grown on poly-L-ornithine/fibronectin matrix and medium complemented with bFGF. OCT4 and SOX2 gene expression were tracked in individual cell using the MBs. Quantitative image analysis every day for seven days showed that the OCT4 and SOX2 mRNA-expressing cells clustered in the centre of the neurospheres cultured in differentiation medium. By contrast, cells at the periphery of the differentiating spheres developed neurite outgrowths and expressed the tyrosine hydroxylase protein, indicating terminal differentiation. Neurospheres cultured in growth medium contained OCT4 and SOX2-positive cells distributed throughout the entire sphere, and no differentiating neurones. Gene expression of SOX2 and OCT4 mRNA detected by MBs correlated well with gene and protein expression measured by qRT-PCR and immunostaining, respectively. These experimental data support the theoretical model that stem cells cluster in the centre of neurospheres, and demonstrate the use of MBs for the spatial localization of specific gene-expressing cells within heterogeneous cell populations.

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