Molecular beacon – tool for real time studying gene activity in stem cells.

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Molecular beacon - tool for real time studying gene activity in stem cells

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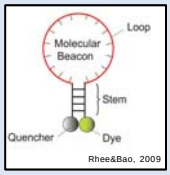
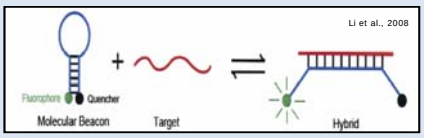
Cells respond to their internal genetic programs and external stimuli by modulating the synthesis of specific mRNAs. Direct observation of mRNA expression in living cells can provide valuable information with regards to understanding fundamental processes such cell differentiation, regeneration and cancerogenesis. Molecular beacon technology is based on fluorescence resonance energy transfer (FRET) and the complementary pairing principles. These fluorescent molecular probes are highly specific and sensitive and are one important tool in in vitro diagnostics. Here molecular beacons are used to follow expression of stem cell markers and neural markers in live differentiating cells.

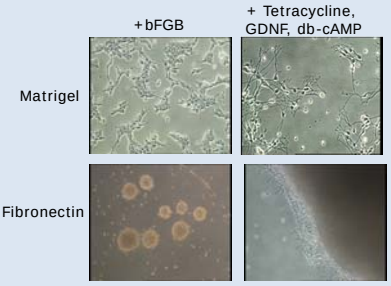


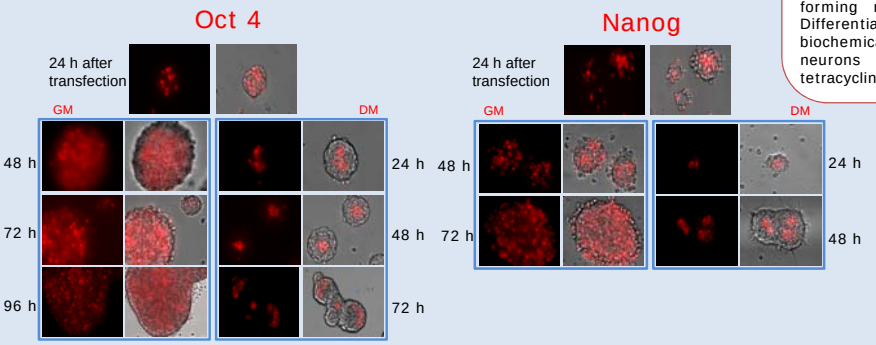


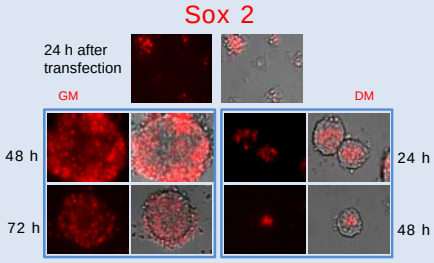
Classic structure and working principle of molecular beacon: A. loop portion, stem portion, fluorescence groups and quencher. B. sensing – in the closed state the molecular beacon is ready to measure mRNA; signaling – molecular beacon has hybridized to the complementary mRNA which displaces the quencher from fluorochrom which then can fluoresces; regeneration – displacement of the hybridized mRNA so that the sensor can be used again.

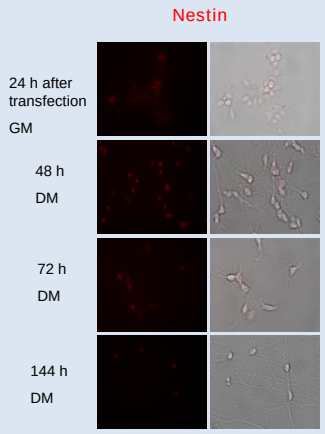
Neurospheres as an experimental model for studying stem cell differentiation. Neurospheres are heterogeneous cell clusters that consist of stem cells, various progenitor cells and more differentiated cells. Stem cells retain the capacity for proliferation, self-renewal and multipotency. Neurosphere forming (stemness) assay has been used not only to detect the presence of stem cells in embryonic and adult brain tissue, but also to study they diversity, phenotype and fate. It is a relevant model for neuronal development and neurogenesis. However, the techniques for real time identification of genes involved in stem cells proliferation, communication and differentiation as well as region-specific factors involved in these processes are limited.

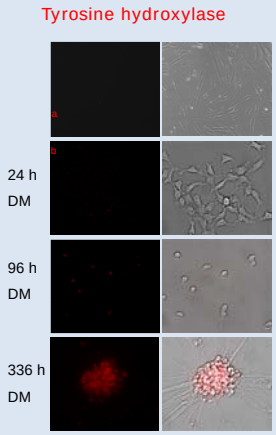
LUHMES – LUND HUMAN MESENCEPHALIC CELL LINE and derived neurospheres – Subclone of the tetracycline-conrolled, v-myc overexpressing human mesencephalic derived cell line MESC2.10 immortalized with LINX v-myc retroviral vector. Growth adherent on Matrigel and forming neurospheres on fibronectin. Differentiate into morphologically and biochemically mature dopamine-like neurons following exposure to tetracycline, GDNF, and db-cAMP











Detection of stem cells markers in neurospheres using molecular beacons. After 72 h growing in growth medium (GM) neurospheres were SLO transfected with molecular beacons for Oct4, Sox2 and Nanog. 24 h after transfection growth medium was switched to differentiation medium (DM). Gene expression was detected on different time points after transfection. Stem cells are localized in the center of the neurosphere. Molecular beacons – Texas red/BHQ2.

Detection of markers for neuronal progenitors (nestin) and highly specialized neurons (tyrosine hydroxylase). 24 h after transfection GM was switched to DM. Nestin expression decreases during differentiation of the progenitors. Molecular beacon Texas red/BHQ2.
TH expression in a. adipocytes and b. neurons. Signal is increasing and is detectable up to 14 days. Molecular beacon with 2'-O-methyl RNA backbone, Cy3, BHQ2.

Conclusions

- Using transfected Molecular beacons is easy, rapid and relatively cheap way to detect gene expression inside living cells.
- Cells do not need to be modified genetically which is a requirement for GFP/promoter construct technology.
- Signal can be detected as soon as one hour after transfection and is stable more than 14 days using modified backbone probes.
- Molecular beacons could track gain-of-expression but also loss-of-expression during differentiation which suggests that the probes could "regenerate" from a hybridized open state to a non-hybridized closed state inside living cells.