Development of Fluorescent Probes for Gene Expression Analysis and Purification of Heterogeneous Stem Cell Populations

Indu S. Voruganti[1], Hetal V. Desai[1], Eric M. Darling[1,2,3,4]

Brown University

[1]Department of Molecular Pharmacology, Physiology and Biotechnology, [2]Department of Orthopaedics, [3]School of Engineering, [4]Center for Biomedical Engineering

Introduction

Adipose-derived stem cells (ASCs) from lipoaspirate are a widely investigated type of stem cell with potential applications in the area of musculoskeletal tissue regeneration (Fig. 1). However, studies using ASCs are complicated by heterogeneity, which exists at both the population and cellular levels. This heterogeneity is problematic for both basic science experiments and translational applications because individual cells can only respond to stimuli according to their individual capabilities. Current attempts to identify cells with high differentiation potential in heterogeneous populations using surface markers have had limited success. However, several early pre-differentiation genes have been identified that have implications in osteogenesis. Visualizing the mRNA for these "early marker" genes would enable investigators to pinpoint the cells in a mixed stem cell population that actually possess the potential to become viable tissues.

Molecular beacons serve as an excellent visualization tool for detecting early, reliable, and traceable mRNA differentiation biomarkers. Molecular beacons are fluorescently functionalized nucleic acid hybridization probes capable of imaging the mRNA of various genes in live cells without altering gene function.[1] (Fig. 2).

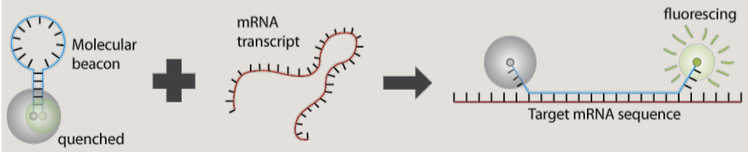

Fig. 2: Schematic of molecular beacon binding. The loop region of the hybridization probe encounters the target mRNA transcript and binds the complementary sequence, forcing the ends apart and affording fluorescence.

Objective

- Develop **molecular beacons** for early osteogenic genes: HOXA10[2]
- Use them to analyze real-time gene expression in live cells
- Use them to identify stem cells with the highest differentiation potential in a heterogeneous population
- Sort these heterogeneous stem cells using **fluorescence activated cell sorting (FACS)**[3] (Fig. 3)
- Facilitate more successful and uniform stem cell differentiation and tissue formation

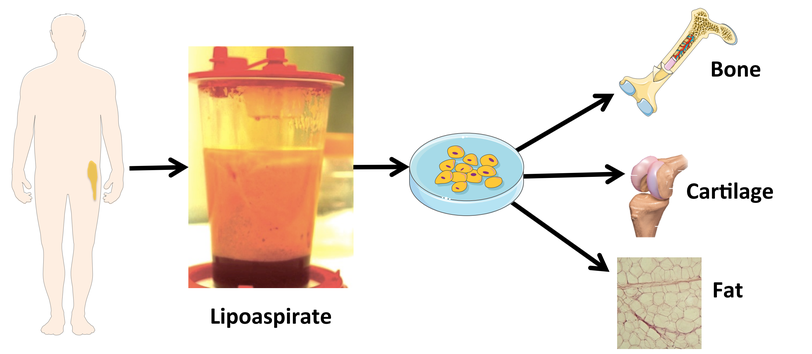

Fig. 1: Stem cells are undifferentiated cells which in time change into various cells, tissues and organs of the body. Lipoaspirate obtained from fat tissue contains an abundance of stem cells useful for various therapies.

Materials and Methods

Beacon treatment and FACS of ASCs – Adipose-derived adult stem cells (ASCs) were treated with custom-designed molecular beacons for an early osteogenic gene (HOXA10) using using an Amaxa Nucleofector® electroporation machine (Lonza AG, USA) according to manufacturer's instructions. FACS analysis using FACSAria (BD Biosciences, USA) was carried out on one million cells with HOXA10 beacon. The sample was excited with a 488 nm laser for detection of the 6FAM fluorophore on the HOXA10 beacon (Ex: 492 nm/Em: 517 nm). Cells positive for signal and negative for signal were collected in respective tubes containing base medium.

Osteogenic differentiation of ASCs – ASCs sorted for positive and negative beacon signal, as well as unsorted cells, were plated in a 96-well plate at a density of 8,000 cells/well. 12 wells were given osteogenic differentiation medium, and 12 wells were given control medium lacking growth factors[4]. After 7 days of differentiation, cells were lysed and assessed for their osteogenic capabilities using an ALP activity assay.

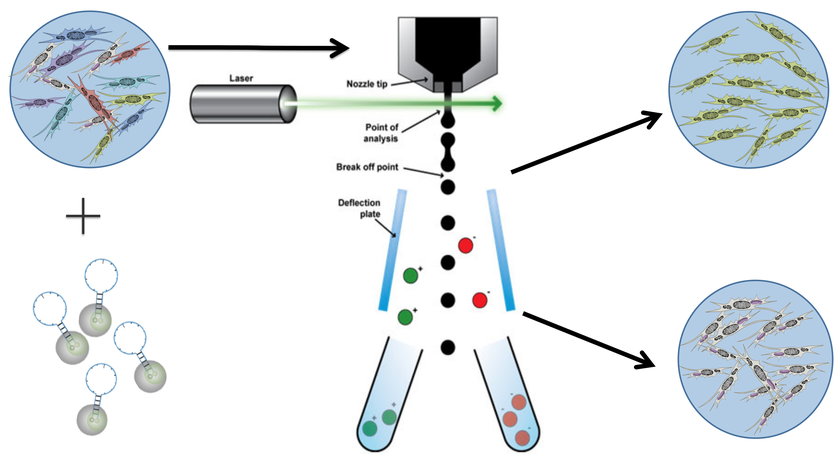

Fig. 3: Fluorescence-activated cell sorting (FACS) provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. (http://www.abcam.com/ps/CMS/Images/FACS%20live%20cells1.jpg)

Results & Discussion

FACS and Differentiation of ASCs – ASCs treated with molecular beacons for HOXA10 via electroporation displayed heterogeneous signal throughout the cell population 1 hour after beacon treatment in all experimental iterations. Thus, HOXA10 seems to be a suitable early marker of osteogenesis, displaying heterogeneous signal in the population.

In this study, we demonstrated electroporation as a useful method of beacon administration for cells in suspension, with low cytotoxicity, achieving cell viabilities similar to those reported by the manufacturer (Lonza AG, USA). Following electroporation, a sample of cells treated with the HOXA10 beacon was sorted using FACS. However, FACS did not yield the expected number of cells positive and negative for the signal. This was likely due to the presence of phenol red, a pH indicator, in the cell medium causing false positive fluorescence. Thus, future experiments conducted in colorless solution may yield cleaner distinctions between positive and negative beacon signal, and FACS may prove to be a useful technology for stem cell sorting.

Conclusions & Future Directions

- Molecular beacons allow for acquisition of live-cell gene expression data to clarify aspects of heterogeneity in stem cell populations
- Molecular beacons and FACS can be useful technologies for sorting heterogeneous stem cells
- HOXA10 is a useful early marker of stem cell heterogeneity and can be detected with molecular beacons
- Enriching stem cell populations holds promise for improving regenerative therapies
- Future directions: optimizing FACS for stem cell sorting, identifying positive and negative control cell types for our genes, and identifying other pre-differentiation markers that may serve as useful internal early markers of cellular differentiation

References

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