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Monitoring and visualizing microRNA dynamics during live cell differentiation using microRNA-responsive non-viral reporter vectors

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Abstract

MicroRNA (miRNA) activity differs with cell type, suggesting it can be used as a cell marker. In this study, we developed novel miRNA–responsive non-viral reporter vectors to continuously monitor and visualize miRNA dynamics during differentiation and to efficiently purify target living cells. Each vector codes miRNA-responsive and reference reporter genes in a single mRNA. These two genes are independent modules but transcribed by a single promoter, which enables us to distinguish miRNA-mediated post-transcriptional repression from transcriptional repression. We generated stable, miRNA-responsive vector-containing human induced pluripotent stem cells (hiPSCs) using the *piggyBac* transposon or episomal vectors. We could continuously monitor the differentiation status of living hiPSCs by detecting the activity of hiPSC-specific miRNA (miR-302a*). In addition, we could selectively sort hiPSC-derived cardiomyocytes using cardiomyocyte-specific miRNA (miR-208a or miR-1)-reporter vectors. Our miRNA reporter system provides a simple way to quantitatively and continuously monitor and visualize changes in the cellular state and should facilitate a broad range of studies that depend on cellular changes including drug discovery and cell-fate conversion.

Keywords

Pluripotent stem cell; microRNA; Differentiation; Live cell imaging; Non-viral vector; Cell sorting