Developing an mRNA-Mediated Gene Expression Strategy in Mouse Embryonic Stem Cells

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The treatment of many injuries and diseases requires engineered tissues. However, there is a severe lack of specific cells for tissue engineering. Embryonic stem cells (ESCs) are a promising source, but current differentiation methods cannot generate clinically usable specific cells. The long term goal of this research is to develop an mRNA-based differentiation strategy to generate specific cells from ESCs for tissue engineering. Our working hypothesis is that if biochemically synthesized an mRNA that determines cell fate is expressed in ESCs, it will promote ESCs to differentiate into a desired cell type. In order to test this hypothesis, I used synthetic mRNA that coded for Green Fluorescence Protein (GFP). GFP mRNA was introduced into ESCs and the cells were cultured for 24 and 48 hours then analyzed using flow cytometry and fluorescence microscopy. Flow cytometry showed that ESCs with synthetic GFP mRNA had significantly higher fluorescence than the control cells and cell growth was unaffected, indicating that ESCs were capable of synthesizing GFP. Fluorescence of GFP in transfected cells was directly observed with a fluorescence microscope. The results showed that biochemically synthesized mRNA can be successfully expressed in ESCs. The results from this project proved the concept of mRNA-mediated gene expression and this method could be used to generate specific cell types from ESCs for regenerative medicine.

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