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Differentiation of Therapeutic Stem Cells Using Micro RNAs
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Our project has two aims: (1) Identify micro RNAs that are regulated upon differentiation in culture, and (2) Restrict differentiation of stem cells by antisense inhibition or overexpression of stem cell-specific micro RNAs. We are making excellent progress. As outlined in our last progress report (February, 2006), we have completed the profiling of mRNAs and microRNAs from differentiating neural stem cells (NSC) in culture. Work is nearly complete to extend our bioinformatics analysis to compare specific interactions between microRNAs and mRNAs with both computational targeting predictions as well as direct biochemical assays of targeting. For example, the 3'UTR of the bHLH transcription factor Olig1 was cloned into a luciferase reporter plasmid (pMir-Report, Ambion). We constructed expression clones for many of the microRNAs we found to be regulated during NSC differentiation. After transfection into cells (Fig. 1), Olig1 3'UTR can be shown to be targeted by mir-9 and mir-187, but not mir-24 or mir-30d. This result, as well as several other, parallel assays (not shown), indicates that computational targeting predictions have a high degree of false positives, and that a negatively correlated expression pattern is not a good predictor of microRNA targeting. However, our analysis reveals a broad and very specific collection of regulatory mechanisms influenced by microRNAs during NSC differentiation, as we predicted. This work is heading towards preparation for the first two manuscripts in the next few months.

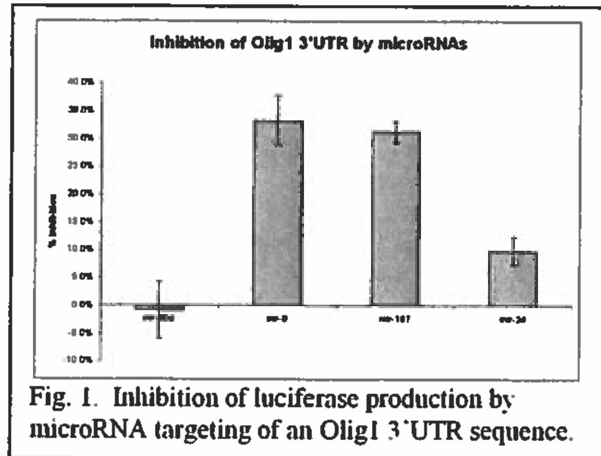

Fig. 1. Inhibition of luciferase production by microRNA targeting of an Olig1 3'UTR sequence.

For the second aim we now have validated targeting interactions with which to design, build and test overexpression plasmids and inhibitory microRNA inhibitors for use in transplanted cells. The collection of overexpression clones used in our luciferase validation assays (e.g. Fig. 1) are also useful for transfection into NSC prior to transplant. We recently ordered inhibitors for the neurogenic microRNAs, mir-9 and mir-124a, using a novel design from colleagues at the University of Iowa (Beverly Davidson's lab) and IDT, Inc. The design combines a locked nucleic acid approach with phosporothioate protection from degradation and has been shown to be effective in stem cells and in vivo. We expect delivery of these inhibitors shortly. They will be first tested in a targeting assay (like the one in Fig. 1) and then used to attempt to influence differentiation markers in NSC in culture and finally after transplant into injured spinal cord. We believe that our systems-level approach to studying a broad collection of regulated microRNAs and mRNAs will allow us to design appropriate target molecules intelligently to optimize the ability of transplanted stem cells to repair or ameliorate spinal cord damage.