Final Report

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It has been an exciting run for this project. We had initial success with transducing the human mesenchymal stem cells (MSCs) into immune-deficient NOD-SCID mice (SCID-17 to be exact, so that they would not reject human cells). With help from Dr. Koc's lab (who was the co-investigator on the grant), we were using the human Na^+/I^- Symporter (hNIS) lentiviral vector for transduction of human MSCs before transplant into the SCID-17 mice. The issues we ran into were:

1. The uptake of the imaging tracer (radioiodide or pertechnetate) also showed up in stomach and parotid grand due to endogenous mouse NIS gene expression, in addition to the hMSCs transduced with hNIS. We tried administering perchlorate by gavage to inhibit this endogenous NIS uptake, but the results were sub-optimal;
2. The signals from transduced hMSCs faded away quickly after transplant and initial imaging. We did not know what happened until the Korean group published their results on neural stem cells when they discovered that the CMV promoter was silenced due to epigenetic effects. We used the same CMV protmoter to drive the NIS expression.

So, we then turned to the alternative reporter by using the triple fusion reporter *Fluc-mRFP-ttk* from Sam Gambhir at Stanford, and we successfully put it into our second generation lentiviral vector under the promoter of myelo-prolific sarcoma virus (MPSV). The transduction using this new vector/reporter into human MSCs has been successful as verified by luminometer and enzyme assays. We conducted a new round of imaging experiments for imaging *fluc* expression using D-luciferin on BLI and *tk* expression using [F-18]-FHBG on microPET. Dr. Omer Koc left Case to join another institute in 2005, which slowed down the project a bit (especially for manuscript writing). However, Dr. Gerson's lab (who is also a co-investigator) step in for the new triple fusion reporter and lenti-vector with the MPSV promoter. Yunhui Kim form the Korean group mentioned above join the group in late 2006. We performed a new line validation: osteogenic, adipogenic and chondragenic assays to compare wild type and reporter gene transduced hMSCs to determine if the transduction of the (triple) reporter gene by way of lentiviral vector has changed any of the key features or potentials of hMSCs. The results are very re-assuring: transduction of the reporter did not alter the important potentials of human MSCs. On the transcriptional level, we are conducting DNA microarray study on human MSCs transduced with a reporter gene via viral vector. It is also very important to know whether the stem cells alter the profile of gene expression after viral vector transduction. This last portion of gene chip study was not in the original proposal, but the issue was raised by the Stanford group and we feel compelled to do it. Finally, we started a series of very exciting new development to imaging the differentiation of stem cells after transplant. This is to track their function in addition to tracking their transplant. We have got very encouraging results, and are seeking funding from NIH to continue this line of important research.

Many of the works reported here have been presented at various meetings including a annual meetings of Society of Nuclear Medicine, Society of Molecular Imaging, and Academy of Molecular imaging, etc. Several manuscripts are either in press, under the review or in preparation.

Publications & Presentations

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