Gene expression imaging using cell-surface expression of marker proteins

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Goal: To engineer novel marker fusion proteins that can be targeted for expression on the cell surface. These markers can be utilized for either radionuclide or optical non-invasive gene expression imaging.

Results. We suggest a system for the monitoring of gene expression utilizing green fluorescent fusion proteins that is based on the effect of in vivo transchelation of 99mTcO (oxotechnetate, OT). The system utilizes a novel family of membrane fusion proteins (FP) consisting of three integral parts: 1) membrane targeting/ spanning polypeptide domain; 2) marker protein (GFP) and 3) oxotechnetate-binding domain. The latter includes several triglycylcysteine repeats or a metallothionein (MT) -based domain. All integral parts of the FP were encoded into a contiguous cDNA and positioned under the control of a human cytomegalovirus promoter. Two different designs of FP have been developed differing in transmembrane orientation. Using GFP as a marker, we have shown that both FPs were expressed on the surface of transfected cells. Immunoblotting showed the presence of a single major protein product for both FP types. Cells transfected with FP bearing MT as the OT-binding domain show 10-15 fold higher OT binding than control cells. Cells transfected in vitro and in vivo were detectable by nuclear imaging.

Conclusion. The cell-surface marker approach in gene expression imaging will allow detection of transfected/transduced cells for monitoring of gene therapy.