Exploring Apoptosis as a Barrier to Gene Expression in Therapeutic Gene Delivery

Low transgene expression is a major obstacle facing the implementation of gene therapy; however, the causes of this problem are not well understood. One observation recorded in the literature is the induction of apoptosis following viral vector gene delivery. An apoptotic response to gene delivery would be consistent with evidence in many cell systems that cellular stress (e.g., UV radiation, heat shock, and withdrawal of growth factors) stimulates intracellular signaling molecules that ultimately turn on the cell death machinery if nothing acts to redirect this pathway. If viral vector infection is causing an analogous stress response, it may be possible to redirect the signaling pathway via molecular intervention, leading to improved viability of the transformed cells and higher levels of the therapeutic transgene product.

Adenoviral vectors are widely used for therapeutic gene delivery to the liver and display suboptimal transgene expression, particularly in vivo. There is evidence that infection of hepatocarcinoma cells by a replication-deficient adenoviral vector leads to apoptosis of the target cells at high multiplicities of infection (MOI). It has also been shown that intracellular signaling molecules involved in regulation of cell death and survival, including ERK, Akt, and NF-kB, are stimulated as a result of adenoviral vector infection. Using the Ad-hepatocyte system, our objective will be to determine if transgene expression in hepatocytes (both primary and transformed) is diminished by apoptotic signaling caused by adenoviral vector uptake and if expression can be enhanced by a corresponding molecular intervention.

Initial results indicate that primary hepatocytes are more sensitive to replication-deficient adenoviral infection than hepatocarcinoma cells as indicated by loss of cell viability. The next goal is to determine if death is occurring via an apoptotic pathway and if so, to quantify the level of apoptosis as a function of MOI, time, and protein expression. In addition, differences in the induction of signaling pathways following adenoviral vector infection will be explored for primary and hepatocarcinoma cell lines. The final goal will be to manipulate one or more of the signaling pathways concurrent with adenoviral infection for the purpose of improving therapeutic gene expression.