A specific factor from DU-145 prostate carcinoma cells that binds a cis-acting element in the 5'UTR of IGF-I receptor

Yi-Shun Lin, Janet Gervais, Michael Woods, Gretchen Temeles, Tony Giordano, Vladimir Karginov, Paul Eder
Message Pharmaceuticals, Inc., 30 Spring Mill Drive, Malvern, PA 19355, USA

Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer death for men in the United States. There is currently no cure for prostate cancer that has metastasized to bone marrow. Prostate tumor cells express high levels of IGF-I receptor (IGF-IR) and because bone marrow is rich in the ligands IGF-I and IGF-II, it has been proposed that a paracrine loop exists between osteoblasts and prostate tumor cells that favors bone metastases. Published studies indicate that ablation of IGF-IR expression in tumor cells by antisense RNA results in the loss of transformed phenotype.

Message Pharmaceuticals is developing a technology that exploits RNA/RNA binding protein interactions as a target for modulation of protein expression. We have applied this technology to the IGF-IR mRNA in order to develop novel therapies for metastatic prostate cancer. We have used this technology to map sites in the mRNA that interact with proteins both in vitro and in a cell-based reporter assay system. For *in vitro* assays, the UTR was divided into a series of overlapping 200 nucleotide regions that were used as transcription templates for probes in RNA mobility shift assays. Further subdivision of regions with RBP activity resulted in the identification of a 60 nucleotide segment that specifically bound proteins in extracts from DU-145 prostate carcinoma cells. The functional activity of these same regions was analyzed using luciferase reporter assays in transiently transfected DU-145 cells. The largest fragment, containing 90% of the 5'UTR, had a repressive effect on luciferase activity, while the 60 nucleotide region that was active *in vitro* showed a stimulatory effect relative to the empty vector control. The molecular basis for these observations is currently under investigation.