Antisense oligonucleotide inhibition of CTGF in Chronic Myeloid Leukemia

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BACKGROUND
Chronic Myeloid Leukemia (CML) is a progressive hematopoietic malignancy where the abnormal Bcr-Abl fusion protein skews hematopoietic stem cell differentiation toward the myeloid lineage, creating an aberrantly self-renewing progenitor population that gives rise to leukemia stem cells (LSC). Though Tyrosine Kinase Inhibitors (TKI) have greatly increased life expectancy for Chronic Myeloid Leukemia (CML) patients, they have difficulty targeting leukemia stem cells (LSC) that drive disease progression, relapse and Blast Crisis (BC) CML transformation.

PCR Microarray Results: A PCR microarray analysis of ECM and adhesion protein expression in different myeloproliferative neoplasms identified a significant overexpression of Connective Tissue Growth Factor (CTGF) in BC CML.

Connective Tissue Growth Factor (CTGF) identified a significant increase in BC vs CP CML expression of CTGF.

Figure 1: CTGF expression in different myeloproliferative neoplasms.

METHODS
Cloning and design of CTGF overexpression plasmid:

- We inserted the full-length CTGF gene into a lentiviral plasmid.

Figure 3: Cloning the pCDH-CTGF lentiviral overexpression plasmid.

RESULTS

pPCR Results of ASO Optimizing Experiments

Experiment 1:
- Conditions: Cells were treated with the drug for 24hrs, 48hrs, 72hrs. Concentrations of both the control ASO and CTGF ASO included 25 uM, 50 uM, 100 uM and 200 uM.
- Results: The pPCR results of the CTGF expression showed that as time of incubation increased and as concentration increased, the knockdown in CTGF expression increased. This could be due to the concentration of ASO being so high that it is toxic.

Conclusion: The CTGF ASO inhibits CTGF well, but more tests need to be made with reasonable dosage and longer time periods.

Figure 5: In antisense gene therapy, short single-stranded pieces of chemically modified nucleotides, known as oligonucleotides are inserted into cells. These short strands are chemically engineered to be complimentary to specific mRNA in the cell, physically blocking translation or recruiting an enzyme known as RNase H to degrade the mRNA.

Figure 6: The qPCR results of the CTGF expression was normalized against a housekeeping gene HPRT. In this bar graph, the 2^-delta Ct value was taken and used as the y-axis values, whereas the x-axis shows the conditions the cells were treated with.

CONCLUSIONS
Implications of Results: Taken together, this data improves our understanding of the mechanisms responsible for CML growth and progression, and also indicate that CTGF represents a viable therapeutic target for the treatment of CML when used in combination with TKI's.

Next Steps: Further testing needs to be performed in order to optimize conditions for the ASO treatment, more experiments will need to be designed preferably with longer time periods and smaller concentrations.

We would need to determine if the decrease in CTGF has any negative effects on the cell and assess for toxicity.

The ASO’s should also be tested with TKI’s to treat Chronic Phase CML, in order to investigate if this combination stops blast crisis transformation.

- performed in vivo (on mice)

Figure 7: Schema of the drug discovery and development pipeline.

REFERENCES

Figure 4: Transfection of K562 cells. After 72 hours, fluorescent images were taken of K562 transfected with (a) pCDH-Empty control and (b) pCDH-CTGF plasmids. (c) Fold change in CTGF mRNA expression by qRT-PCR of RNA extracted from whole cellular lysates of transfected cells versus pCDH-Empty control. This was used to to establish stable cell lines for in vitro characterization of CTGF expression.

SPECIFIC AIMS
Objectives: The lack of curative results from TKI monotherapy underscores the urgent need to map disease progression of chronic phase (CP) CML to its acute stage, blast crisis. This underlying population of leukemia stem cells are believed to be the cause of the blast crisis progression and relapse.

Hypothesis: We targeted CTGF in hopes of improving our understanding of the mechanisms responsible for CML growth and progression, and also indicate that CTGF represents a viable therapeutic target for the treatment of CML when used in combination with TKI's.