Epigenetic editing in the promoter of *CXCL11* gene

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Statement of the Problem: Epigenetic engineering (editing) is an exciting path to novel therapeutics. Custom-designed demethylases allow gene-specific reactivation of epigenetically silenced genes. Progress in this area depends in great part on the choice of enzymatic effectors and their targeted binding to promoters. Here we report a successful use of a combination of TDG and Tet1 to enhance transcriptional responsiveness of *CXCL11* gene in a murine fibroblast line.

Methodology & Theoretical Orientation: We designed multiple fusion constructs (n=6) aimed to bind in relative vicinity of each other in the key regulatory areas of mCXCL11. Zinc-finger protein arrays served as DNA-binding domains; murine TDG isotype 2 and human Tet1CD were the enzymatic effectors. Constructs with catalytically inactive single aminoacid mutant enzymes served as controls. The constructs were delivered into 3T3 fibroblasts via lentiviral transduction.

Findings: After 2 weeks of constitutive expression the pyrosequencing analysis demonstrated a decrease in CpG methylation by up to 40 percentage points in several loci in the targeted area. This was associated with a nearly 5-fold increase in transcriptional responsiveness of *CXCL11* after stimulation with a combination of IFNγ and LPS. The maximum transcriptional responsiveness measured ~X 2000 times over baseline, vs. ~X 400 times in the control.

Conclusion & Significance: We conclude that a combination of multiple TDG and Tet1 complexes with zinc-finger arrays is a promising approach in targeted demethylation and that *CXCL11* is a rewarding target for future experimentation.

Figure 1: Example of a fusion demethylase complex bound to the DNA target.

Recent Publications:

1. Gregory D J, Zhang Y, Kobzik L and Fedulov A V (2013) Specific transcriptional enhancement of inducible nitric oxide synthase by targeted promoter demethylation. Epigenetics 8(11):1205-12.
2. Gregory D J, Mikhaylova L and Fedulov A V (2012) Selective DNA demethylation by fusion of TDG with a sequence-specific DNA-binding domain. Epigenetics 7(4):344-9.
3. Gregory D J, Kobzik L, Zhang Y, McGuire C C and Fedulov A V (2017) Transgenerational transmission of asthma risk after exposure to environmental particles during pregnancy. Am J Physiol Lung Cell Mol Physiol. 313(2):L395-L405.
4. Mikhaylova L, Zhang Y, Kobzik L and Fedulov A V (2013) Link between epigenomic alterations and genome-wide aberrant transcriptional response to allergen in dendritic cells conveying maternal asthma risk. PLoS One 8(8):e70387.
5. Fedulov A V and Kobzik L (2011) Allergy risk is mediated by dendritic cells with congenital epigenetic changes. Am J Respir Cell Mol Biol. 44(3):285-92.

Biography

Alexey V Fedulov has his expertise in Epigenetic Engineering and Transgenerational Epigenetic Studies. His lab has built a model of targeted reactivation of epigenetically silenced genes by fusion complexes comprised of DNA demethylase enzymes and sequence-specific DNA binding domains. His laboratory studies are in immune and epigenetic mechanisms of lung disease including early life asthma origins. The ultimate goal of the studies is to find novel therapeutic approaches by modulating epigenetic control of gene expression.