**Measurement of fetal γ-globin expression due to KLF1 being a negative or positive regulator**

**I. INTRODUCTION**

In the human body, the most common cell type is the red blood cells. The regulation of these cells has many different factors that aids in their transcription. Krüppel-like transcription factors (KLFs) are factors that play a role in many functions such as cell proliferation, apoptosis, tissue, and erythrocyte development. The KLFs family, there are about 17 members identified in mammals. KLF1 was the first factor of the KLF family to be identified.

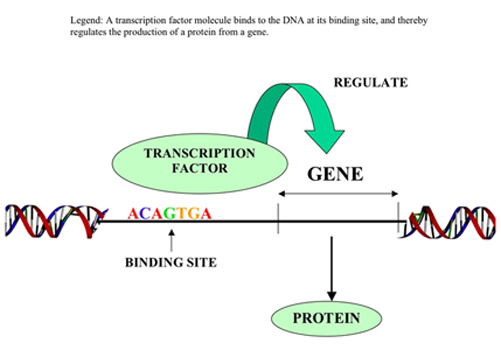
 Red blood cells are composed types of globin genes.  KLFs are very important in fetal γ- globin genes in terms of regulation. KLFs either bind to the CACCC promoter element which can promote or repress the activity of the globin gene, as shown in Fig. 1.There are two phases of development in the production of red blood cells (erythropoiesis), primitive which is embryonic and definitive which is adult. Described by Pang et al (2012), primitive production of red blood cells is an ongoing research because there is not much understanding as opposed to definitive production of red blood cells. The suppression of β- globin genes is necessary in early development, therefore there is more γ-globin genes expressed than β-globin genes. In adult cells, there is more expression of β-globin genes as opposed to γ-globin genes.

Figure 1. This is an image to show how a transcription factor binds to a promoter sequence and can regulate the activity of a certain protein or gene. The transcription factor would be KLF1 and the binding site would be CACCC (promoter element).

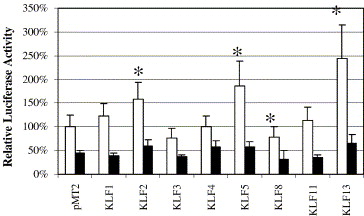
KLF1 plays a role in β-globin genes, and it’s known to be a positive regulator. KLF1 binds to the β-globin CACCC promoter element and β-globin locus control region which turns on the transcription of β-globin genes. The CACCC promoter region is also present in γ-globin genes.  Using transfection assays, four of the KLFs resulted in regulating γ-globin genes according to Ping et al (2005). Those are said to be KLF2, KLF5, KLF8, and KLF13 which also dependent on if there is a CACCC promoter present as well (Ping et al, 2005). KLF1 in relation to fetal γ-globin genes has always been topic where researchers did not have strong evidence of it being a positive or negative regulator. To determine if the expression of KLF1 is a positive or negative regulator of fetal γ-globin genes by using a cell line that does not contain KLF1 or γ-globin genes present in it already. K562 cells used in Ping’s research (2005), has γ-globin present in them due to them being human cells.

Figure 2. The black bars represent the expression of KLF1 and compared to the control in Ping, 2005.

**II. EXPERIMENT**

To determine if KLF1 is a positive or negative regulator of -globin genes, a cell line or system has to be chosen. The cell line being proposed to use is from drosophilae. S2 cells lack both globin genes and the KLF1 factors therefore, can be transfected by both. There will be a control to have another form of measurement (explained further on). Luciferase is an enzyme or protein that produces bioluminescent light, and are found in fireflies. Fireflies contain this protein, and that is what we see when they light up.

*Plasmid construct*

Two constructs, γLuc, and KLF1 will be generated. The γLuc construct will be a fusion of the γ-globin promoter (CACCC element), and luciferase encoded into the gene. Luciferase will be used to measure the expression of γ-globin genes. Since luciferase is encoded in the gene, every time γ-globin is expressed, so will luciferase. This means that the production of the γ-globin gene can be measured through the expression of luciferase. The expression construct, will be KLF1 cDNA created with the SV40 early promoter. SV40, simian virus 40, is polyomavirus that is used to as a promoter for general expression in the cDNA created as the expression construct.

Plasmids carry their own origin of replication, therefore they are good at cloning and replicating independently within a cell. This will occur after we generate our constructs through electroporation and luciferase assay.

*Electroporation and Luciferase Assay*

Ten micrograms of γLuc fusion plasmid construct, and forty micrograms of the KLF1 cDNA are co-transfected into S2 cells through electroporation. Pulse is applied which causes the pores of the cell membrane to open during just the pulse, and the constructs are transfected into the S2 cells. Empty vector for the expression construct will be used for the control. Some cells with have the reporter construct and the expression construct and some cells, which are the control, will have the reporter construct and a empty vector. The cells are given some time to harvest and grow.

To measure an expression of a gene, the luciferase assay will then be used to see the expression of the protein, luciferase. Luciferase corresponds with the expression of γ-globin genes, so the luciferase reporter gene aids in tracking the expression of the γ-globin genes. If there is transcription of γ-globin genes, then light will be emitted. This is also done with the cells with the empty vector, which does not contain any of the KLF1 construct to see if there is more or less of luciferase enzyme activity.

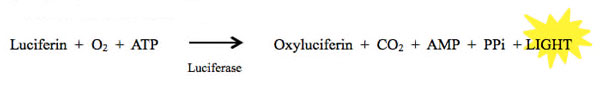


Fig 3. The mechanism/reaction of the luciferase assay. ATP and Luciferin are put into a test tube, when luciferase is added, it emits light, along with the breaking of ATP.

*Western Blotting*

To insure the expression of KLF1s as well, western blotting will be used. Western blotting has a protein of interest and antibody that can detect the protein of interest. Our protein of interest would be KLF1 and anti-EKLF, the antibody. It would recognize KLF1 and polyacrylamide gel electrophoresis. This separates the proteins involved. Then, the separated proteins are transferred onto a more durable material, like a membrane. An electric field aids in the migration of the proteins out of the gel and onto the membrane. Then KLF1 is probed with the antibody. The results will be recorded to see the expression of KLF1 in the S2 cells.

**III. DISCUSSION**

The results of this experiment will lead to one of a few possible outcomes. If the assay shows that fetal γ-globin genes and luciferase is transcribed, therefore it will show a good amount of light is emitted in the cells that were co-transfected with the KLF1 cDNA, which means less light emitted in with the empty vector. This would mean the KLF1 is a positive regulator of γ-globin genes. Another outcome would be the opposite and good amount of light is emitted with the empty vector. Which also means, less luciferase enzyme activity and less light emitted for the co-transfection with the reporter and expression construct. This would indicate that KLF1 is a negative regulator of γ-globin genes.

If the light is emitted if at the same levels for both the empty vector and expression construct then KLF1 does not impact the regulation of fetal γ-globin genes.

The basis of this experiment was to conduct another experiment with a cell line that does not compose of KLF1 already. The drosophila cell line, S2, does not have KLF1 or γ-globin genes so it helps in starting off with a cell that does not have a chance of swaying the results due to the presence of the potential regulator of the gene. Although, I had only checked for KLF1 in drosophila, S2, cells, not the other KLFs. If there was another Krüppel-like transcription factor, then it could affect the results.

**REFERENCES**

Hu, Jie Hong et al. “SYSTEMATIC RNAi STUDIES ON THE ROLE OF Sp/KLF FACTORS IN GLOBIN GENE EXPRESSION AND ERYTHROID DIFFERENTIATION.” *Journal of molecular biology* 366.4 (2007): 1064–1073. *PMC*. Web. 20 Nov. 2017.

Zhang, Ping, Priyadarshi Basu, Latasha C. Redmond, Pamela E. Morris, Jeremy W. Rupon, Gordon D. Ginder, and Joyce A. Lloyd. "A Functional Screen for KrÃ¼ppel-like Factors That Regulate the Human Î³-globin Gene through the CACCC Promoter Element." *Blood Cells, Molecules, and Diseases* 35.2 (2005): 227-35. Web.

Pang, C. J., W. Lemsaddek, Y. N. Alhashem, C. Bondzi, L. C. Redmond, N. Ah-Son, C. I. Dumur, K. J. Archer, J. L. Haar, J. A. Lloyd, and M. Trudel. "Kruppel-Like Factor 1 (KLF1), KLF2, and Myc Control a Regulatory Network Essential for Embryonic Erythropoiesis."*Molecular and Cellular Biology* 32.13 (2012): 2628-644. Web.

Vinjamur, Divya S. et al. “Krüppel-Like Transcription Factor KLF1 Is Required for Optimal Γ- and Β-Globin Expression in Human Fetal Erythroblasts.” Ed. Andrew C. Wilber. *PLoS ONE* 11.2 (2016): e0146802. *PMC*. Web. 28 Oct. 2017.

Cherbas, Lucy et al. “The Transcriptional Diversity of 25 *Drosophila* Cell Lines.” *Genome Research* 21.2 (2011): 301–314. *PMC*. Web. 10 Sept. 2017.

Vinjamur, Divya S. et al. “Krüppel-like Transcription Factors KLF1 and KLF2 Have Unique and Coordinate Roles in Regulating Embryonic Erythroid Precursor Maturation.” *Haematologica* 99.10 (2014): 1565–1573. *PMC*. Web. 10 Sept. 2017.

Chiplunkar, Aditi R et al. “The Krüppel-like Factor 2 and Krüppel-like Factor 4 Genes Interact to Maintain Endothelial Integrity in Mouse Embryonic Vasculogenesis.” *BMC Developmental Biology* 13 (2013): 40. *PMC*. Web. 30 Apr. 2017.

Chiplunkar, Aditi R. et al. “Krüppel-Like Factor 2 Is Required for Normal Mouse Cardiac Development.” Ed. Elena Aikawa. *PLoS ONE* 8.2 (2013): e54891. *PMC*. Web. 30 Apr. 2017.