Is the overexpression of KLF1 a positive or negative regulator of fetal γ-globin genes?

**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) have 3 zinc Cys/His2 zinc fingers. KLF1 being 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. 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.

KLF1 plays a role in β-globin genes, and its known to be a positive regulator. KLF1 binds to the the β-globin CACCC promoter element and β-globin locus control region which turns on the transcription of β-globin genes. CACCC promoter region is also present in γ-globin genes, but KLF1 regulates BCL11A, known repressor of γ-globin expression. 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, 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. Therefore, there has been a lot of debate. It is inferred to be a negative regulator, but we wish to determine if the overexpression of KLF1 is a positive or negative regulator of fetal γ-globin genes.

**EXPERIMENT**

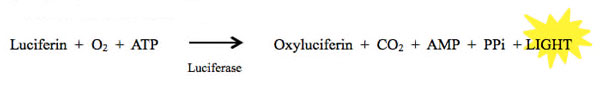
To test our research question, a cell line or system has to be chosen. Human erythroid cell lines will be used for this particular experiment, as well as zebrafish cell line for the control.

*Plasmid construct*

Three reporter constructs, KLF1, LUC, and γCACCCLuc will be generated. Human cell lines contain KLF1 but the generation of KF1 is necessary to test the overexpression. Plasmids carry their own origin of replication, therefore they are good at cloning and replicating independently within a cell. Plasmids have a sequences of base pairs that is recognized by the restriction enzyme. It is sliced, and foreign DNA can be incorporated (recombinant DNA). After the generating the expression constructs, a luciferase assay is used.

*Luciferase Assay*

To measure an expression of a gene, it allows emission of light to represent the amount of expression. 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. Same is done with the zebrafish cell line, which does not contain any of the KLF1 construct, or KLF1 at all.



http://bitesizebio.s3.amazonaws.com/content/uploads/2013/06/luciferase-reporter-assay.jpg

**DISCUSSION**

The results of this experiment will prove one of two outcomes. The control (zebrafish cell line) will show that fetal γ-globin genes are transcribed at normal levels, therefore the transfection assay will show a good amount of light is emitted. If the light is emitted if exceptionally high or the same as the control than the overexpression of KLF1 positively regulates the transcription of γ-globin genes. I would believe that the overexpression overpowered the repressor protein BCL11A and γ-globin is synthesized. If there is dim or no light at all emitted then the the overexpression of KLF1 negatively regulates the transcription of γ-globin genes. Previous studies/research has showed that KLF1 at normal levels is a negative regulator, but we wanted to test if the overexpression of KLF1 would make a difference. There has always been debate as to if KLF1 turns off transcription for γ-globin genes, so the results can go either direction.

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