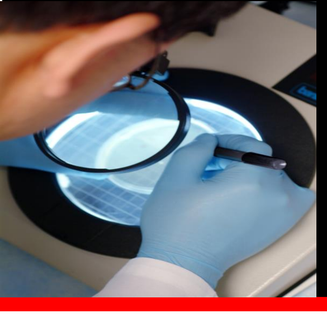

The Auditions For Kruppel Like Factor 5 Interacting Proteins

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ABSTRACT

Kruppel Like Factor 5, KLF5, is a critical transcription factor. KLF5 is known as a regulator of tissue development in the gut(McConnell, B.B., 2011), heart(Drosatos, K., 2016), lungs(Wan, H., 2008), eyes(Kenchegowda, D., 2011), bladder(Bell, S.M., 2011), muscles, skin, etc. In the skin, KLF5 has been noted in tumorigenesis and wound repair (Ge et al., 2017). Interested in the molecular mechanisms regulating stem cell plasticity and how it goes awry in skin diseases such as chronic wounds or skin cancer, the Ge Lab set out to find KLF5 targets and how they regulate skin function.

RESULTS

We tested around 40 genes, most of them were nuclear proteins including epigenetic regulators, transcription factors, and regulators of RNA stability. Among all 40, we found that 30 were successfully expressed, and 24 interacted with KLF5 in the Co-IP assay. We repeated this assay twice, and 15 consistently interacted with KLF5.

Creating a putative list of KLF5 interacting proteins

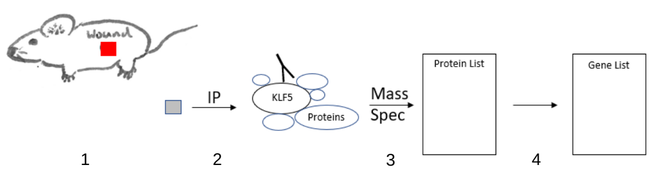

Figure 1

Wounded tissues and cells are harvested[1], which most likely have proteins significant to the wound repair process. Then, through immunoprecipitation with KLF5 specific binding antibodies[2], KLF5 is separated from the sample along with proteins that are bounded to its surface. Next, this slurry of KLF5 and other bounded proteins are inserted into a mass spectrometer, which counts peptide fragments to search a database for proteins[3]. With this list we can also determine the genes[4] associated with the protein expression.

Preparing Lysate For Testing

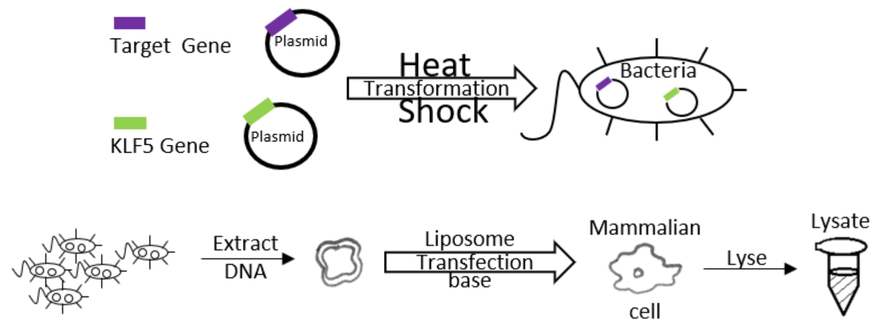

Figure 2

Taking the gene list, gene-containing plasmids were created and through the process of heat shock, we transformed bacteria with the desired plasmids. After the bacterial colony has replicated, we extracted its DNA and with a liposome, as a vector, we transfected mammalian cells with the DNA. Once the gene had time to be expressed, the cells were lysed giving us our lysate.

Co-Immunoprecipitation and Western Blots

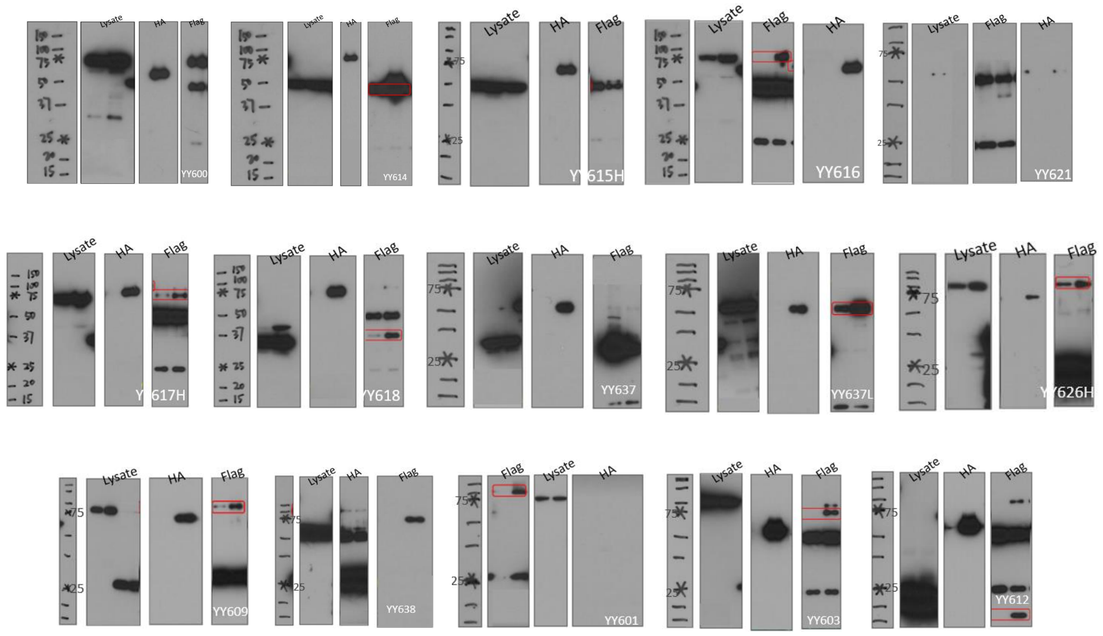

Figure 3

Key:
HA- No KLF5
HA&F- Has both KLF5 and candidate protein
Western- Western Blot

Plasmid	YY 600	YY 601	YY 603	YY 609	YY 612	YY 614	YY 615 H	YY 616	YY 617H	YY 618	YY 621	YY 626 H	YY 637	YY 637 L	YY 638
Gene	dNp6 3a	Pabp c1	Ddx 3x	Fxr1	Mcri p1	Trim 21	SSB	Dhx1 5	DDX5	Elavl 1	Fbl	Xrcc 5	Prmt 7S	Prmt 7L	Junb
MW(kD)	65	71	73.5	76	11	52	45	88	68	36	61	81	42	77	38

Figure 4

Aside from the lysate in figure 2, a second lysate containing only KLF5 is made. This second lysate will act as a negative control. Once both lysates are prepared, a KLF5 specific binding antibody is added to the lysates. During this time, the antibody will bind to KLF5, which may have the candidate protein bound to it. Following the addition of the antibodies, we add in beads with an affinity that will bind to the KLF5 antibodies. Due to the magnetic nature of the beads, a magnetic stand can be used to pull down the beads, which has the KLF5 protein complex bound to it. With the complex now separated from the lysate, we resuspend the supernatant in Phosphate-Buffered Saline Tween(PBST), so that the proteins denature. Once denatured, glycerol and sample buffer is added so that we can place this supernatant into gel wells. When the gel electrophoresis finishes, we will then have a protein covered gel ready to be transferred to a membrane. This membrane will be covered in substrate to be visualized with a film. Once the Co-IP is finished, we ran western blots of the substrate to double check that the candidate protein, was fully expressed in the mammalian cell. Since, both lysates should contain the candidate protein, two bands should be seen with the same molecular weight.

Conclusions

KLF5 potentially interacts with 15 proteins to regulate wound repair.

References

1) Ge et al. Cell 2017; 169: 636-650

2) McConnell et al. 2011

3) Drosatos et al. 2016

4) Wan et al. 2008

4) Kenchegowda et al. 2011

5) Bell et al. 2011