CREATING AN INDUCIBLE P-57 LENTIVIRUS VECTOR

UNDERGRADUATE RESEARCH OPPORTUNITIES PROGRAM

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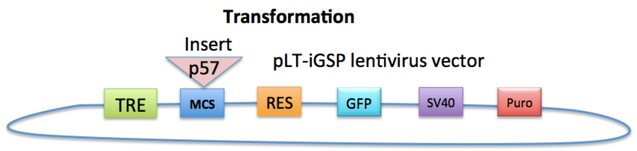
Introduction

- **P-57 is a Cyclin-dependent Kinase Inhibitor (CKI)**
- **CKIs act to inhibit the cell cycle**
- **Activation of P-57 induces cell cycle arrest in G_0 phase**
- **P-57 can be inserted into a Lentivirus vector**
- **Tetracyclin rresponse element (TRE) of Lentivirus vector allows for in-vitro control of p57 expression via. exposure to Doxycycline (Dox)[1]**
- **Goal of this research project was to create an inducible p57 lentivirus vector and confirm its fuctions via Dox activation in human mammary epithelial cell line (MCF10A)**

Methods

- P-57 DNA was obtained from cDNA.
- Blunt-end restriction digest into pLT-igsp plasmid backbone
- The Plasmid was then transformed into E.coli



- The pLT-p57-iGSP transfection was then conducted in 2P3T cells, from which the virus was generated.
- Viral particles were transduced into MCF10A cells along with empty pLT-igsp vectors, which served as a control.
- pLT-iGSP and pLT-p57-iGSP cells were selected for via puromycin resistance to generate cell lines.
- Both the empty vector and and the p-57 inserted cells were then visualized under varying Doxycyclin doses varying from low dose (0.2 µg/ml) to high dose (5µg/ml).
- Both bright field and GFP pictures were obtained.
- Cells were then lysed and prepared for Western Blotting.

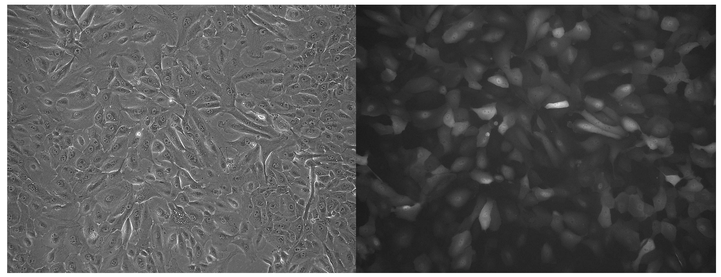
Results

Expression Test

Figure 1.

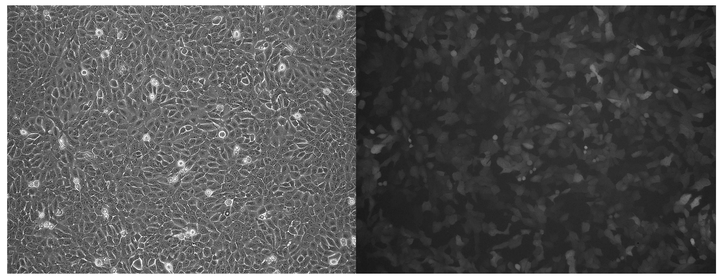
pLT-p57-igsp MCF10A 1.0 µg/ml DOX

Bright Field **GFP**



pLT-iGSP MCF10A 1.0 µg/ml DOX (Control)

Bright Field **GFP**

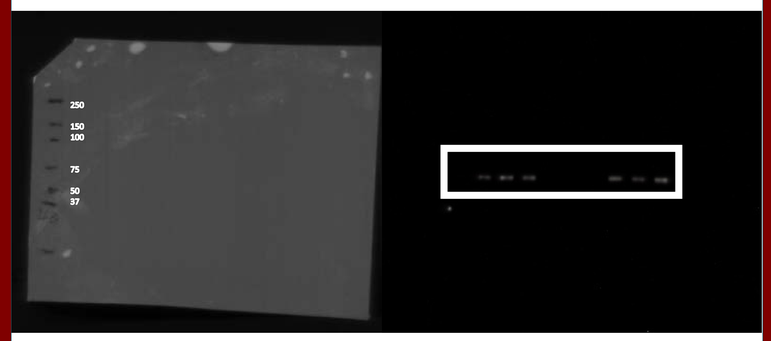


Western Blot

Ladder **UV**

Expected size: ~50 KDa

Ladder p57 1.0 igsp 1.0 p57 5.0 igsp 5.0



Discussion

- Presence of GFP in Figure 1A demonstrates that p-57 was expressed in MCF10A cells with 1.0 µg/ml of Doxycyclin
- Cells transduced with the p-57 insert demonstrated both a lower cell count and an increase in GFP expression. Data not shown here.
- Western blot confirmed the expression of p-57 protein in pLT-p57-iGSP cells via the bands visible in the UV image. (Figure 2)
- Bands were not visible in the UV Western Bltot gel for pLT-iGSP (Figure 2).
- The ladder in Figure 2 was used as a reference to confirm that the antibody staining was of a size specific to the p57 protein.

References

1. Leung CT, Brugge JS (2012) Outgrowth of single oncogene-expressing cells from suppressive epithelial environments. Nature 482:410–413 Available at: http://dx.doi.org/10.1038/nature10826.

Acknowledgments

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