Developing an Anti-Tumor Therapy:

Expression of mLIGHT Cytokine in *Escherichia coli & Salmonella enterica* Typhimurium

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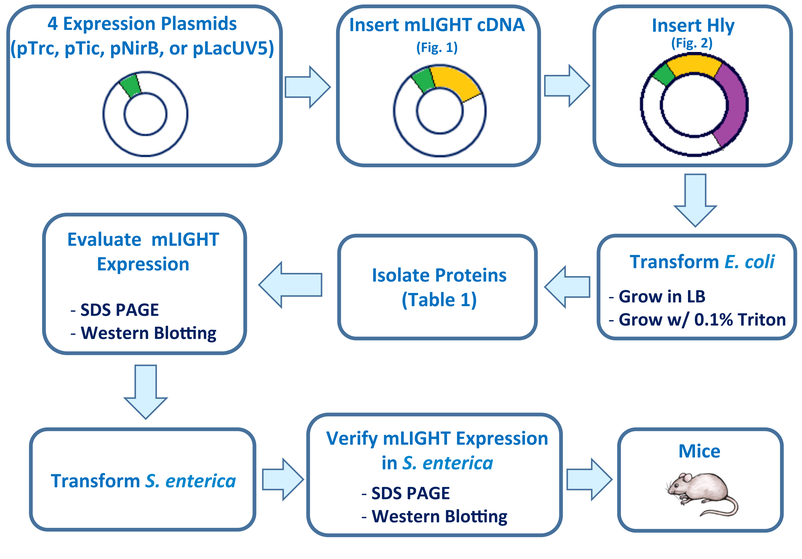
I. BACKGROUND

- Conventional cancer treatments have limitations & adverse side effects.
- *Salmonella enterica* Typhimurium colonizes tumors & metastases, including hypoxic regions resistant to chemotherapy and radiation. [1]
- *S. enterica* can be used to deliver cytokines promoting tumor rejection. [1]
- LIGHT cytokine, a TNF superfamilly member, initiates apoptosis of tumor cells. [2]
  - LIGHT binds the HVEM receptor on T cells, costimulating CTL activation.
  - LIGHT also binds LTβR on stromal cells, upregulating chemokines to recruit activated T cells into the tumor tissue.
- Secreted cytokines are more effective at signaling the immune system. [3]

II. SPECIFIC AIMS

- Construct expression plasmids containing mLIGHT and the hemolysin secretion sequence.
- Evaluate mLIGHT expression in *E. coli*.
- Transform *S. enterica* and evaluate mLIGHT expression.

III. METHODS



VI. RESULTS: Western blotting

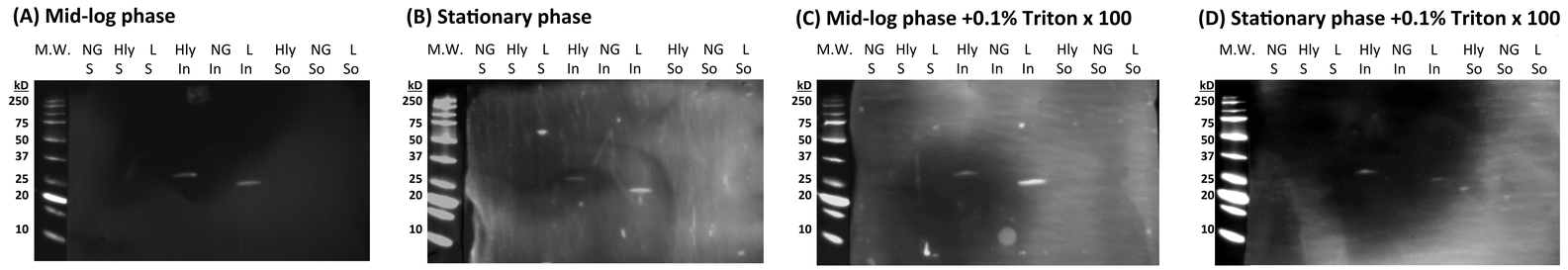


Figure 3. Western immunoblotting of proteins from mid-log and stationary phase cultures: secreted proteins (S), insoluble proteins (In), and soluble proteins (So) isolated from *E. coli* containing the pTRC-mLIGHT-Hly (Hly), pTRCmLIGHT (L), or no gene (NG) expression vectors. mLIGHT expression was observed in membrane or inclusion bodies of cells containing the mLIGHT expression vectors. Molecular weight of mLIGHT is 26 kDa and 32 kDa with the Hly secretion signal. **(A)** Mid-log phase culture. **(B)** Stationary phase culture. **(C)** Mid-log phase culture treated with 0.1% Triton x 100. **(D)** Stationary phase culture treated with 0.1% Triton x 100.

V. Proteins Isolated

Table 1: **Protein samples isolated from *E. coli*.** Whole cell, soluble, insoluble, and secreted proteins isolated from *E. coli* containing no gene control, pTRCmLIGHT, or pTrc-mLIGHT-Hly expression vector during mid-log phase (MID) and stationary phase (STA). Soluble proteins include cytoplasmic proteins. Insoluble proteins include membrane associated proteins and inclusion bodies.

	No Gene		pTrcmLIGHT		pTrcmLIGHT-Hly	
	MID	STA	MID	STA	MID	STA
Whole Cell	☑	☑	☑	☑	☑	☑
Soluble	☑	☑	☑	☑	☑	☑
Insoluble	☑	☑	☑	☑	☑	☑
Secreted	☑	☑	☑	☑	☑	☑

IV. mLIGHT Plasmid

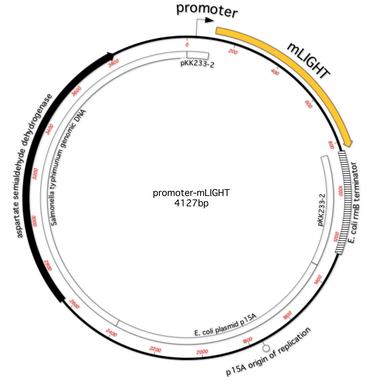


Figure 1: Promoter-mLIGHT plasmid construct. Four expression plasmids were constructed with either the *trc*, *tic*, *nirB*, or *lacUV5* promoter.

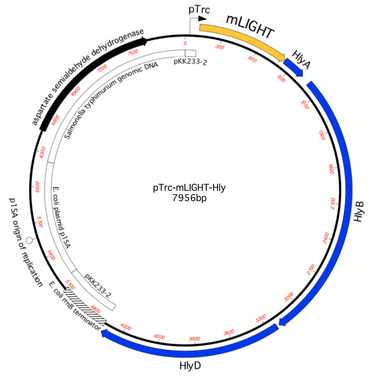


Figure 2: pTrc-mLIGHT-Hly plasmid construct.

VII. CONCLUSIONS

- Sequencing verified the pTrc-mLIGHT-Hly expression vector was made.
- Western blotting indicated mLIGHT is being expressed, but not secreted.

  E. coli containing the pTrc-mLIGHT-Hly expression vector expressed mLIGHT. The protein was found in the membranes or inclusion bodies, but not as cytoplasmic or secreted proteins in both mid-log and stationary phase cultures.
- Growing cells in 0.1% Triton x 100 did not induce secretion of mLIGHT.
- Lack of mLIGHT in the secreted protein fraction may be due to low expression or a non-functional secretion system. [4]

VIII. FUTURE DIRECTIONS

- Construct new expression vectors with different secretion sequence and/or promoter.
- Optimize expression by using ribosome binding site of higher affinity.

IX. REFERENCES

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3. Morishige T, Yoshioka Y, Tanabe A, Yao X, Mizuguchi H, Tsunoda S, Tsutsumi Y, Mukai Y, Okada N, and Nakagawa S. 2010. Comparison of the anti-tumor activity of native, secreted, and membrane-bound LIGHT in mouse tumor models. *International Immunopharmacology.* 10:26-33.

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