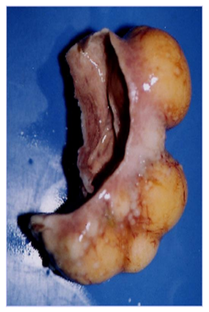
Novel tools for analysis of gene expression in the skate, *Leucoraja erinacea*

Taylor Hersh , Carnegie Mellon University, and Dr. Bram Lutton, Endicott College

BACKGROUND

- Chemokine Receptor 4 (CXCR4) activation induces mobilization of hematopoietic precursor cells during angiogenesis and revascularization[1]
- CXCR4 expression was found in the epigonal organ of reproductively active (RA) and non-reproductively active (NRA) *L. erinacea*[2]
  - Primers were designed using Expressed Sequence Tags (ESTs)



The epigonal ovary complex

- Limitations of ESTs:
  - An EST is a short sub-sequence of a cDNA sequence
  - End up with a short, low-quality fragment
  - Only produces a portion of a gene
  - "Small" library (~33,000 ESTs for *L. erinacea)*
- Ben King and MDIBL collaborators designed new genomic (SOAP *de novo*) and transcriptomic (assembly of new paired-end RNA-seq data) databases with updated information for *L. erinacea.* Benefits of these new databases include:
  - Paired-end RNA-sequencing produces reads from both 3' and 5' ends
  - Can overlap large segments to get a continuous gene sequence
  - Much larger library
- **Research goal: use the novel databases to once again look for CXCR4 expression in *L. erinacea* epigonal organ tissue**

METHODS

The methodology of this experiment consisted of two parts: primer design and gene expression analysis.

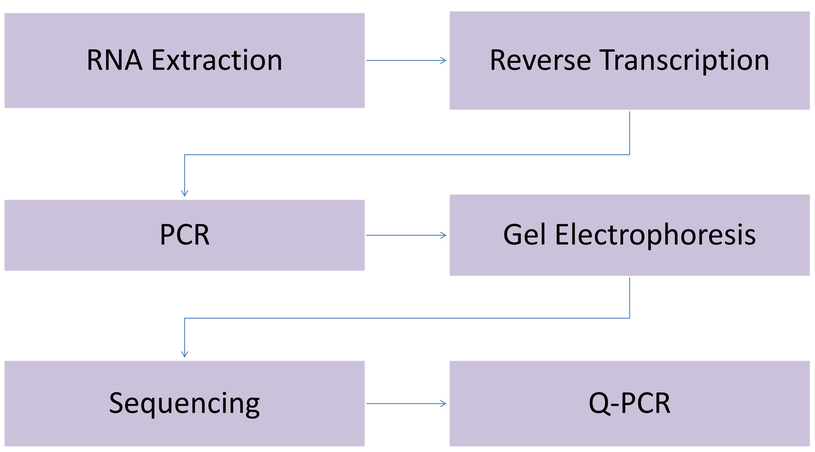
NCBI → generate FASTA sequences of genes (*i.e.* CXCR4)

↓

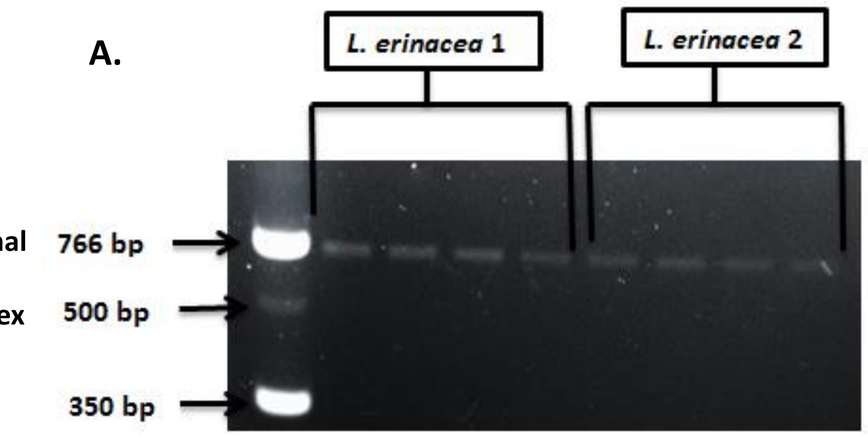
BLAST the sequence against the new skate transcriptome → produces a contig

↓

BLAST the contig against the human genome to ensure that the correct gene has been found (*i.e.* do a "reciprocal check").



RESULTS



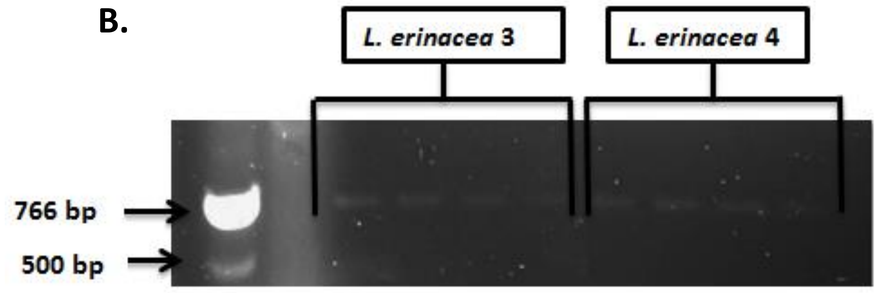


Figure 2: Agarose gel electrophoresis reveals that CXCR4 expression is present in the epigonal organ of both NRA (A.) and RA (B.) *L. erinacea.*

Using the same paired-end RNA-seq and SOAP *de novo* data, primers have been designed for other genes of interest. Our next step will be to look for expression of these different genes in hematopoietic tissue of *L. erinacea*.

Table 1: Genes of interest with potential hematopoietic and angiogenic functions and/or interactions.

Gene	Function
CXCL12	Hematopoietic stem cell (HSC) homing and quiescence, binds to CXCR4
MMIF	Innate immunity regulator, binds to CXCR4
UBA52	Produces ubiquitin, binds to CXCR4
UBB	Produces ubiquitin, binds to CXCR4
VEGFA	Angiogenesis, binds to VEGR1 and VEGFR2
VEGFR1	Contributes cells to vasculature
VEGFR2	Regulates endothelial cell proliferation
c-Kit	Cell survival, differentiation, and proliferation
SCF	Directs HSCs to stem cell niches, binds to c-Kit
LNK	Negative regulator of HSC self-renewal, interacts with c-Kit
LYN	Innate immune response, interacts with c-Kit
PTPN6	Regulates cell signaling pathways in hematopoietic cells, interacts with c-Kit
SOCS1	Suppresses cytokine signaling, interacts with c-Kit
SOCS6	Suppresses cytokine signaling, interacts with c-Kit
SRC	Angiogenesis, interacts with c-Kit
TEC	Regulates immune functions, interacts with c-Kit
ANGPT2	Agonist to angiogenesis
HIF-1α	Embryonic vascularization and hematopoiesis

CONCLUSIONS

- The new paired-end RNA seq and SOAP *de novo* databases for *L. erinacea* provide a viable and easy way to search for genes.
- CXCR4 expression is present in epigonal tissue of both RA and NRA *L. erinacea*.[2]

FUTURE DIRECTIONS

- Sequence and clone the CXCR4 gene
- Use quantitative PCR to compare levels of CXCR4 expression in NRA and RA *L. erinacea*
- Carry out the same process of primer design and gene expression analysis for the genes mentioned in Table 1 because literature shows that those genes potentially play angiogenic and hematopoietic roles in mammals.[1]

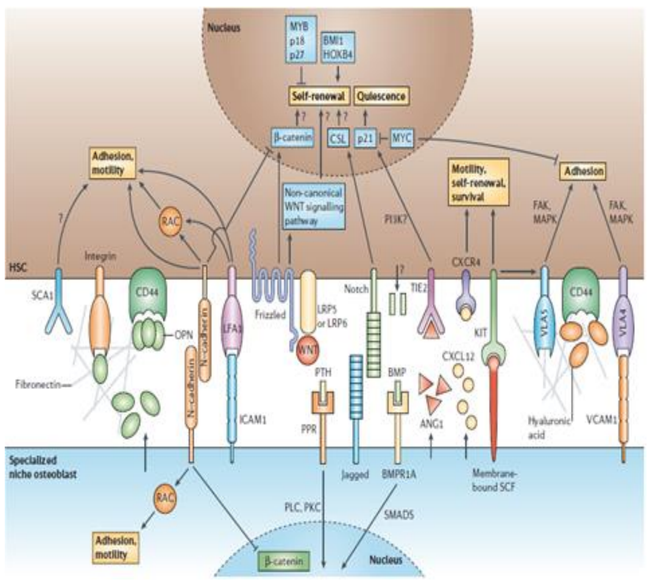


Figure 3:A schematic detailing the plethora of receptor-ligand interactions that occur at the endosteal niche and hematopoietic stem cell junction in mammals. Data like this inspired our decisions regarding what genes to investigate next.[3]

ACKNOWLEDGEMENTS

I would like to sincerely thank the following: Dr. Bram Lutton, for his guidance throughout this project; Ben King, for his valuable collaboration; the Forrest Lab, for their moral support and materials; the National Science Foundation, for funding this research; and the Mount Desert Island Biological Laboratory, for hosting me this summer.

REFERENCES

[1] Jin, D.K. *et. al.* Cytokine-mediated deployment of SDF-1 induces revascularization through recruitment of CXCR4+ hemangiocytes. *Nature Medicine* **12**, 557-567 (2006).

[2]Lupica, N., Cacciatore, C., and Lutton, B. Chemokine receptor 4 expression in the little skate, *Leucoraja erinacea*. *The Bulletin (MDI Biological Laboratory)* **51,** 47-48 (2012).

[3]Wilson, A., and Trumpp, A. Bone-marrow hematopoietic -stem -cell niches. *Nature Reviews* **6**, 93-106 (2006).