Identification and quantification of gene expression during angiogenesis in the skate, *Leucoraja erinacea*

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The skate, *Leucoraja erinacea*, provides a unique model for studies of hematopoiesis (blood cell production and differentiation) and angiogenesis (formation of new blood vessels). These animals lack bone, allowing easier access to unique and less complex hematopoietic stem cell niches; moreover, they undergo extensive angiogenesis during their reproductive cycles, thereby potentially providing novel insight into the importance of vascular niches. This study further demonstrates the comparability between mammalian and elasmobranch hematopoietic stem cell activity and begins to quantify changes in gene expression during periods of increased angiogenesis and cellular mobilization.

Bone marrow, the tissue responsible for hemopoiesis in mammals, is comprised of vascular and endosteal niches[5]. *L. erinacea* and other elasmobranchs (sharks, skates and rays) have cartilaginous skeletons, *i.e.,* no endosteum, and thus, house hematopoietic stem cells (HSCs) within only a vascular niche. The epigonal organ (EO) of the skate, a homolog of mammalian bone marrow in direct cellular and vascular contact with the gonads, shows a significant increase in cellular turnover (proliferation and apoptosis), as well as angiogenesis, when females transition into reproductive activity[2,3,4]. These interactions between primary immune tissue and primary reproductive organs in *L. erinacea* suggests further study of these neuroendocrine-immune relationships may provide novel insight into the cellular and genetic mechanisms of hematopoiesis and angiogenesis.

In 2012, our lab observed chemokine receptor 4 (CXCR4) expression in the EO of *L. erinacea*, suggesting homologous molecules are involved in chemokine signaling between elasmobranchs and mammals. While they are known to be pleiotropic molecules, CXCR4 and CXCL12 are key regulators in the retention of HSCs in the bone marrow of mammals[5,6]. In 2013, *L. erinacea*-specific primers for a number of genes with hematopoietic and angiogenic effects were designed using newly developed databases with genomic and transcriptomic information, and the expression of CXCR4's cognate ligand, CXCL12, was observed in the EO[1].

The goals of the current experiment were to demonstrate additional genes, as well as to assess the expression levels of genes in *L. erinacea* that are homologous to genes with known hematopoietic and angiogenic functions in mammalian bone marrow. Once identified, we aimed to quantify the change in expression of these genes between non-reproductively active (NRA) and reproductively active (RA) states. Standard PCR was used to demonstrate the expression of VEGFR1, VEGFR2, c-kit and SCF, which are receptor/ligand pairs known to play key roles in hematopoiesis and angiogenesis in mammals.[7] qPCR was then used to quantify the expression of CXCR4 and CXCL12 in ten female skates (five NRA and five RA)[1].

PCR Primers were developed for CXCR4, CXCL12, c-kit, SCF, VEGFR1, VEGFR2 genes, and β-actin (the qPCR baseline standard) using *L. erinacea* transcriptome data. The NCBI database was used to get the FASTA code for the human sequence of each gene, which was compared to the *L. erinacea* transcriptome to find a contig (an area of sequence similarity) with the best alignment score. A reciprocal cross was performed to compare with the human genome and ensure that the correct gene had been found. The PrimerQuest tool from Integrated DNA Technologies was then used to design and order primers from the contig.

In RA *L. erinacea* we observed a trend of increased CXCR4 expression and decreased CXCL12 expression compared to the NRA group, which indicates the possibility that reproductive activity plays a role in mobilization. However, possibly due to our limited sample size of ten EO tissues (from 5 animals) for each group, the data did not indicate a statistically significant difference of increasing CXCR4 expression of decreasing CXCL12 expression as hypothesized. Data for SCF, c-kit, VEGFA, VEGFR1 and VEGFR2 genes currently show no trends or significant changes in levels of expression in RA compared to NRA individuals. The interaction between CXCR4 and CXCL12 and a panel of additional genes are currently under investigation to further elucidate the role of stem cell mobilization in angiogenesis and revascularization. In continuing this work, we will increase our sample size to reduce the impact of variation among individual specimens.

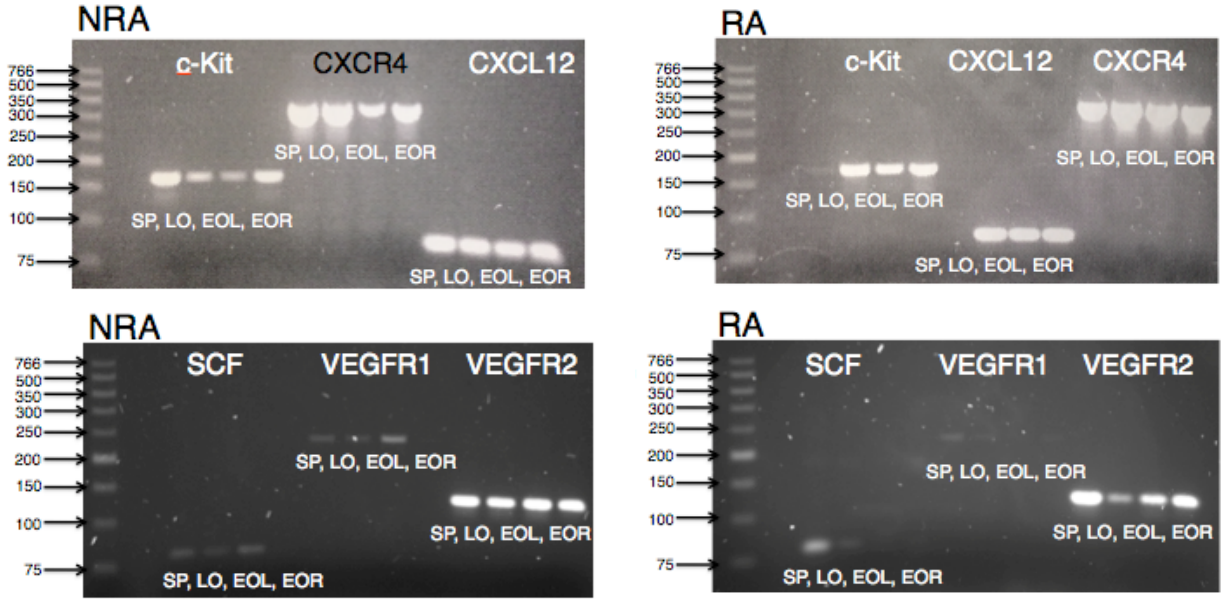

Figure 1. Representative PCR amplicons demonstrating gene expression in hematopoietic tissues of *L. erinacea*. Tissues from left (EOL) and right (EOR) epigonal organs as well as the spleen (SP) and Leydig organ (LO).

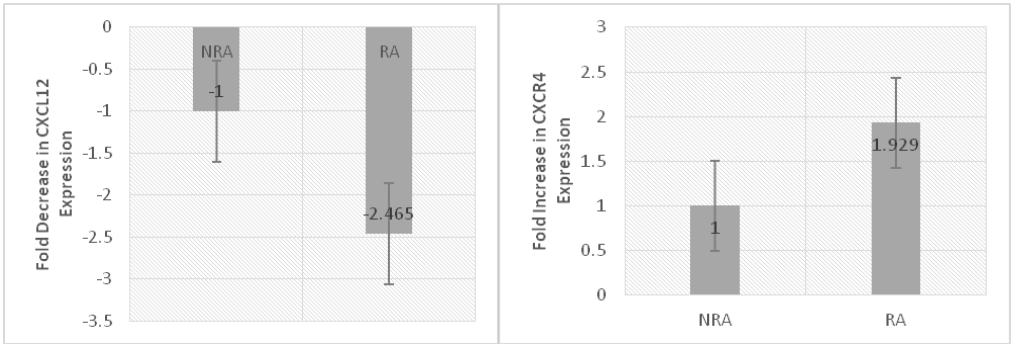

Figure 2. qPCR histogram plots depicting fold change (ratio of the initial value *versus* the final value) of gene expression from NRA to RA; positive or negative one-fold change for NRA is baseline by which RA up- or down-regulation is scaled.

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