Chemokine receptor 4 expression in the little skate, *Leucoraja erinacea*

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Understanding the mechanisms of stem cell activity and blood vessel growth has important implications in cellular transplantation strategies and cancer research. The elasmobranchs (sharks, skates and rays) offer a unique perspective on these physiological processes. This study supports previous data indicating that secreted factors produced during the period of reproductive activity in *Leucoraja erinacea* may be responsible for regulating mobilization of stem cells and angiogenesis via endocrine, paracrine, and chemotactic mechanisms.

Previous research has established that the hematopoietic stem cell niches of the little skate (*Leucoraja erinacea*) are similar to mammalian bone marrow, but that fundamental differences exist as well. For instance, elasmobranchs have two primary hematopoietic niches known as the Leydig organ and the epigonal organ.[1] These supply blood cells of the various lymphoid and myeloid lineages to the body, which appears to be regulated by some of the same transcription factors as in mammals.[1] However, elasmobranchs do not possess the endosteal (*i.e.,* bone) component of the mammalian niche. Moreover, erythropoiesis occurs in the spleen of *L. erinacea* rather than in the bone marrow, which serves as the primary erythropoietic tissue in adult mammals.

Hematopoietic stem and progenitor cells (HSPC) allow for immune surveillance under steady-state conditions in the bloodstream. These cells are regulated by specific cellular and molecular cues, but the mechanisms that regulate their trafficking are only partially understood. The hematopoietic environment serves as a three-dimensional "facility" wherein cellular and molecular crosstalk regulates the balance between self-renewal and differentiation of HSPC. Although there have been great successes in understanding the mechanisms of stem cell activity, limitations remain in part due to the physical environment of the endosteal niche (Fig 1). Recent studies have shown that mammalian hematopoiesis involves another portion of the bone marrow environment that is independent of bone cells, referred to as the vascular niche.[2,3,4]

In bone marrow, many molecules are responsible for homing, mobilization, and adhesion of HSPC. The most centrally important of these are Chemokine Receptor (CXCR) 4 and its ligand, CXCL12. One elegant recent study in *Nature Medicine* established that CXCR4 is responsible for both mobilization and angiogenesis in mice.[5] Thus, we investigated CXCR4 expression in *L. erinacea* as it relates to increased proliferation and angiogenesis during the reproductive period.[6,7]

In this study, we used expressed sequence tag (EST) data provided by the Mount Desert Island Biological Laboratory (MDIBL) and semi-quantitative polymerase chain reaction to demonstrate CXCR4 is expressed in *L. erinacea*. Specifically, CXCR4 was not expressed in the heart, which served as a control tissue, while the Leydig organ demonstrated constitutive expression, and CXCR4 was expressed in the spleen until animals became reproductively active. Interestingly, during the period of reproductive activity there appeared to be a switch, with CXCR4 expression being turned on in the epigonal organ and turned off in the spleen.

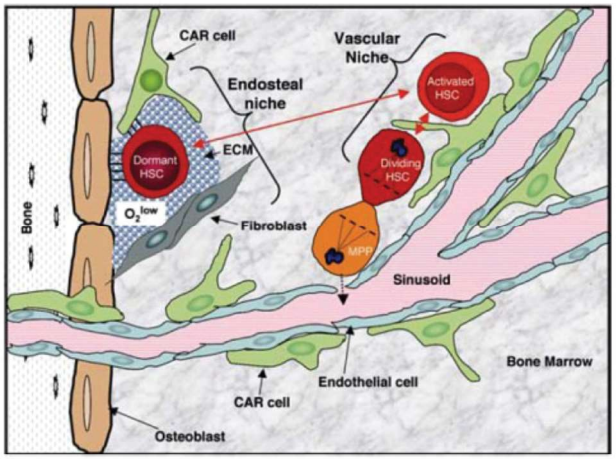

Figure 1. Schematic overview of mammalian bone marrow endosteal and vascular niches. ECM, extracellular matrix; HSC, hematopoietic stem cell; MPP, multipotent progenitor cell; CAR, CXCL12-abundant reticular cell.[8] Copyright permission granted (License number 2484901366494) by Wiley and Sons Publishing.

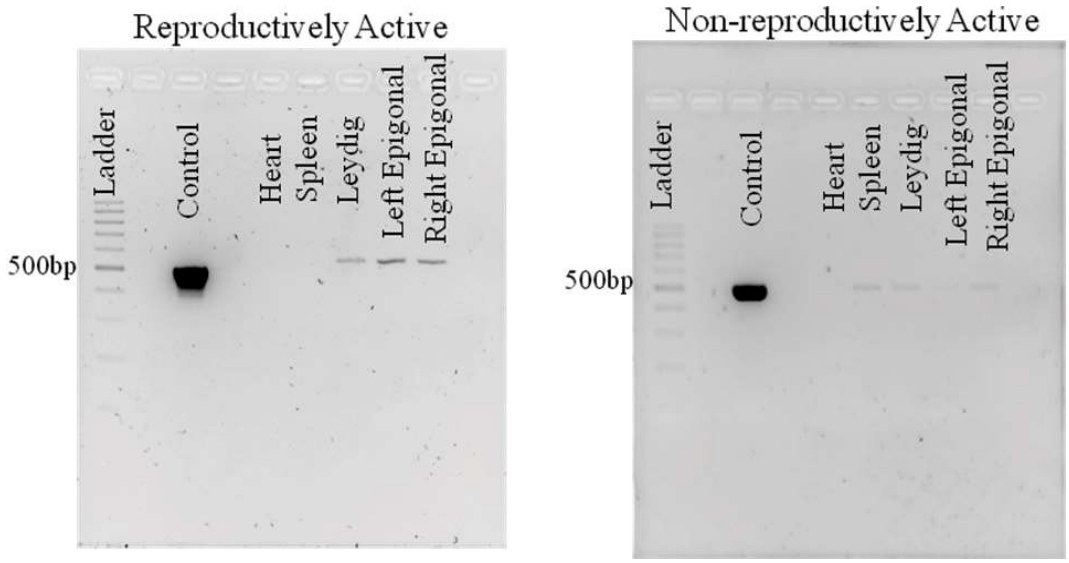

Figure 2: Representative gels comparing tissue CXCR4 expression in reproductively active *versus* non-reproductively active *L. erinacea.* PCR assay control lane is a low molecular weight bacteriophage lamda DNA fragments from PCR kit manufacturer (Qiagen, Valencia, CA).

Until now, CXCR4 expression had not been identified in elasmobranchs. With sequencing of the *L. erinacea* genome and EST database available via MDIBL, ongoing studies will characterize expression and function of key cellular and molecular factors regulating hematopoiesis and angiogenesis, and shed light on the evolution of these physiological processes. Epigonal organ and gonads of elasmobranchs represent a unique morphological association where these tissues are directly connected in one complex. Cellular and vascular relationships in this complex have not been documented in any other animal; they allow functional interactions crucial for normal reproductive-endocrine and immune activities. That is, during the period of reproductive activity there is dynamic bidirectional cross-talk between these two primary physiological tissues[6,7,8]. Thus, ongoing investigations of vascular hematopoietic niches in *L. erinacea* may provide novel insight into reproductive-immune interactions and complex cellular and molecular activities within mammalian hematopoietic niches.

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