CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

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Project Number

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Project Title

Selective Expression of Ligands Slit2, SlitL2, and their Putative Receptors Robo1 and Robo4 in Hematopoietic Stem Cells

Abstract

Objectives/Goals

The goal of the project is to investigate novel factors in hematopoietic, or blood-forming, stem cell (HSC) migration by testing for Slit or Roundabout (Robo) expression in HSC and performing chemotaxis assays to assess the effects on HSC migration.

Methods/Materials

Experiments involved the secreted proteins Slit1, Slit2, and Slit3, the membrane-bound protein Slit-like2 (SlitL2), and the receptors Robo1 and Robo4. Gene expression analysis consisted of isolating RNA from mouse bone marrow, reverse transcribing it into DNA, and testing that with Slit and Robo primers in quantitative real-time PCR.

After amplifying Slit DNA and transfecting the vector into cell cultures, the supernatant from the cells, which produce Slit from the DNA, was collected and used in chemotaxis assays. In transwell plates for migration assays, live HSC were placed in the upper chamber and allowed to migrate through the membrane and into the lower chamber containing the protein of interest. Cells were collected from the bottom chambers and analyzed on a FACS machine to determine the percentage of migrated HSC.

Results

Quantitative real-time PCR runs detected selective expression of Slit2, SlitL2, Robo1, and Robo4 in HSC. Most notably, Robo1 and Robo4 were differentially expressed at several-fold greater levels in HSC than in other hematopoietic populations, indicating that Slits may play a larger role in HSC migration. Chemotaxis assay data suggested that while Slits alone had no noticeable effect on hematopoietic cell migration, Slit2 did inhibit HSC migration towards stromal cell-derived factor-1ALPHA (SDF-1ALPHA) by 50%.

Conclusions/Discussion

For continual blood cell replacement in adults, a small number of HSC migrate between the bone marrow and circulating blood. Although a vital process, little is known about these movements and only a few factors have been found. Recent research has shown that the Slit protein family and their receptors Robo have similar effects as repellants or inhibitors of migration in various cell types. This study discovered that there was differential expression of Robos in HSC, highly suggestive of Slit and Robo's important role in HSC migration. Indeed, preliminary migration assay data identified Slit2-induced inhibition of HSC migration towards SDF-1ALPHA, a clinically important finding for bone marrow transplantations because SDF-1ALPHA is a chemoattractant in HSC mobilization and homing.

Summary Statement

Selective HSC expression of Slit2, SlitL2, Robo1, and Robo4, in addition to Slit2 inhibition of HSC migration towards SDF-1ALPHA, indicated that Slit/Robo interplay had a role in HSC migration, a valuable finding with clinical applications.

Help Received

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