CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

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Project Number

S0509

Project Title

Angiopoietin-2 Induces Myeloid Cell Adhesion via G Protein-Coupled Receptor X

Abstract

Objectives/Goals

Angiopoietin-2 (Ang-2) is an angiogenic factor secreted by activated endothelium that has been shown to play a role in inflammatory diseases. Ang-2 signals on endothelial cells via the receptor tyrosine kinase Tie2, but the myeloid receptor mediating Ang-2's paracrine effects remains unknown. Ang-2 shares high sequence homology with a C-type lectin, which binds to a myeloid cell-expressed G-protein-coupled receptor X (GPRX). Hence, I hypothesized that Ang-2-mediated myeloid cell adhesion, anti-inflammatory activity, and tumor infiltration proceed through GPRX.

Methods/Materials

I evaluated myeloid cell adhesion by performing adhesion strengthening assays with whole bone marrow (BM) cells from GPRX knockout (GPRX-/-) and wild type (WT) control mice. Free radical production was quantified through reactive oxygen species (ROS) assays. Finally, leukocyte subsets were characterized in GPRX mixed BM chimeric mice, which had been subcutaneously injected with B16 melanoma tumor cells.

Results

This study is the first to describe a G protein-coupled receptor, GPRX, as the myeloid receptor for Ang-2 involved cell adhesion and anti-inflammatory signaling. Ang-2 triggers strong adhesion of mouse BM cells to Intracellular Adhesion Molecule-1 and does not trigger ROS. Adhesion strengthening is dependent on GPRX and reduced with GPRX-/-. As Ang-2 expression is characteristic of tumor vasculature, I predicted that GPRX deficiency would reduce leukocyte recruitment to tumors. However, in studies of implanted B16 tumors in BM chimeric mice reconstituted with a mix of WT and knockout cells, I observed the preferential accumulation of GPRX-/- myeloid cells in tumors. This suggests a more complex role for GPRX in the tumor environment and may reflect reduced apoptosis, as GPRX enhances neutrophil apoptosis.

Conclusions/Discussion

Based on the model from this study, Ang-2/GPRX signaling would recruit phagocytes to sites of angiogenesis, where they would remove debris and erythrocytes. Ang-2 would not induce inflammatory activation, preventing excessive tissue damage. Identifying the GPRX-Ang-2 axis as an important signaling pathway for myeloid cell recruitment enables us to specifically target either the angiogenic or myeloid cell effects of Ang-2. In the future, GPRX monoclonal antibodies or small molecule inhibitors may be developed to directly target the GPRX-Ang-2 signaling axis as a form of treatment for inflammatory diseases.

Summary Statement

I evaluated adhesion strengthening and inflammation in vitro, as well as leukocyte infiltration into tumors in vivo, to identify G protein-coupled receptor X as the receptor mediating Angiopoietin-2#s paracrine effects on myeloid cells.

Help Received

Qualified Scientists guided and provided laboratory resources