CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)
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Project Number
S0429

Project Title
Effects of MMP-Carrying Inflammatory Leukocytes on Tumor Cell Intravasation

Abstract

Objectives/Goals
To determine the role of inflammatory leukocytes, specifically chicken heterophils carrying MMP-9 and monocytes/macrophages carrying MMP-13, on tumor cell intravasation (entrance of blood vessels) and metastasis.

Methods/Materials
Western blotting determined if chMMP-9 and chMMP-13 were the proteins aiding tumor cell lines 45a and 47a in remodeling and intravasation. Proteins from tumors were separated by gel electrophoresis. Proteins transferred onto PVDF membranes and incubated separately with chMMP-9 and chMMP-13 antibodies. Membranes developed in a dark room after the addition of a chemiluminescent substrate. Proteins from tumor environment run through zymogram gel by electrophoresis. Gelatin ingrained in zymogram gel reveals which proteins are gelatinases (implies they are also collagenases). Gelatin stained with blue dye and gelatinase MMPs shown as white bands in blue gel.
DNA samples collected from the lower chorioallantoic (CAM) membrane and liver. Samples underwent real-time alu PCR analysis for alu sequences unique to primates (45a and 47a came from a human tumor). PCR machine records the number of cycles needed to detect alu sequences. Cycle number is converted into cell numbers using a mathematical function. High number of cells equates superb ability to intravasate and metastasize.
Same numbers of tumor cells from each cell line were seeded on the upper CAM. Six days after seeding, tumors were excised and stained with antibodies specific for chMMP-9 or chMMP-13 to visualize heterophils or monocytes/macrophages, respectively. Images of the tissue were taken and stained inflammatory leukocytes were quantified using Adobe Photoshop.

Results
47a has more chicken MMP-9, MMP-13, and higher ability in intravasation and metastasis than 45a. More inflammatory leukocytes are found near 47a tumors than 45a. This means that the heterophils and monocytes/macrophages carrying MMP-9 and MMP-13 help 47a intravasate (and thus metastasize) at higher frequencies than 45a.

Conclusions/Discussion
The hypothesis of this study was that MMP-9-carrying heterophils and MMP-13-carrying monocytes/macrophages directly contribute to tumor cell intravasation. The results obtained during the experiments verify this hypothesis.
Future studies may include the effects of anti-inflammatory drugs on tumor intravasation and metastasis.

Summary Statement
This research focuses on the effects of inflammatory leukocytes--specifically chicken neutrophils and monocytes/macrophages--on tumor cell intravasation (entrance of vasculature) and metastasis.

Help Received
Dr. Elena Deryugina for guidance, suggestions, trainings; used Dr. James P. Quigley's lab equipment at The Scripps Research Institute