CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Sudeep Banerjee

Project Number
S0402

Project Title
Ras Activated Tumor Suppressor Genes Nore1A and RASSF1A Have Separate Proapoptotic Effector Mechanisms

Abstract

Objectives/Goals
Induction of apoptosis by Ras may be mediated by its effector RASSF1, which appears to function as a tumor suppressor. Analysis of another Ras effector Nore1, which is structurally related to RASSF1 showed that it is frequently down-regulated in tumor cell lines. Like RASSF1, this correlates with inactivating methylation of the Nore1 promoter rather than gene deletion. Immunofluorescence microscopy indicates that the RASSF1 protein is localized in the microtubules of the cell # specifically Actin, Alpha and Beta tubulin. I predict that Nore1A is colocalized to the same microtubule structures.

Methods/Materials
Cell fixation was conducted on H23 cell culture. The fixed cells were then transfected and allowed to incubate. Then immunofluorescent antibodies were administered to tag the cytoskeleton and proteins of interest, thereby producing observable cell slides, which were viewed under an immunofluorescent microscope.

Results
Localization is based on the degree of diffusion of the layers. The immunofluorescent microscopy results were analyzed for both RassF1A and Nore1. The red layer, representative of Beta Tubulin, shows clearly defined microtubules. The green layer, representative of RassF1A, also is defined along the same distribution. Therefore we can deduce that RassF1A is localized to the microtubule. Then there is the representation of Nore1A and Beta tubulin. The Beta tubulin is still punctate or clearly defined while the Nore1A is diffused throughout the cell indicating that no localization can be determined. The same pattern was determined with all three microtubular structures: beta tubulin as well as alpha tubulin and actin filaments.

Conclusions/Discussion
The immunofluorescent images of Nore1A suggest that localization with actin filaments, alpha or beta tubulin does not exist. Although RASSF1 and Nore1 are structurally similar and the Ras gene activates both, my study shows that they do not localize to the same area in the microtubular structure of the cell. Hence if Nore1 is not localized with RassF1 then its effector mechanism for growth inhibition must be different.

Summary Statement
This project is about the intracellular localization of tumor supressor gene proteins in an effort to explain their mechanism of action

Help Received
This project was carried out at the molecular biology laboratory of Massachussetts General Hospital under the supervision of Dr. Ramnik Xavier. The compilation and presentation of the project was supervised by Mr. Wayne Garabedian