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Establish an in vitro model for the study of NF2 gene function

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13. ABSTRACT (Maximum 200 Words)

Mutation analyses of the NF2 gene from NF2 patients suggest that the inactivation of the NF2 gene, and the consequent lack of gene product (protein known as Merlin/Schwannomin) is the primary cause of this disease. Within the three years period, we plan to develop a laboratory protocol to establish Schwann cell culture using surgical specimens from NF2 patients, and compare the tumor cells from patients with different NF2 gene mutations. In addition, we want to extend the life span of primary culture cells by immortalizing them using a retrovirus which we engineered. This will not only ensure the reproducibility of results within the NF2 community, but also provide scientists greater access to certain materials for the study of biologic function of Schwann cell and also important for testing therapeutic approaches.

During the past 12 months period, PI, Dr. Gene Hung has relocated himself from House Ear Institute to Arena Pharmaceuticals on March, 2002. An application for grant sponsorship transfer and additional request for a no cost extension (12 months) to this grant have been submitted.

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Establish an in vitro model for the study of NF2 gene function.

P.I. Hung, G

Introduction

In the Eight years since the NF2 gene was identified, NF2 research has been divided into four main areas: 1) Natural history; 2) NF2 gene function; 3) In vitro and in vivo models; and 4) Therapeutic intervention. Although there have been some important discoveries, we are still unable to answer key questions about what factors predict the tumor growth rate in patients, the pathogenesis of NF2, whether the current NF2 mouse knock-out model can be used to represent human NF2, and whether gene therapy is the future therapy for NF2. Most researchers feel that the lack of an in vitro model system has limited their research progress and development of such a model should be a high priority. The purpose of this study is to develop a NF2 gene deficient in vitro model that can be used to further understand NF2 gene function and to facilitate development of new treatments. In this research study, we propose to develop a primary and permanent human Schwannoma cell culture system, and with this in vitro model, to test the hypothesis that different NF2 gene mutations result in different degrees of loss in NF2 gene function and the loss in NF2 gene function directly controls the tumor growth rate."

To test the hypothesis, we propose the following Specific Aims:

1) Establish a reproducible protocol for the primary culture of human Schwann and schwannoma cells and characterize NF2 gene function by studying cytoskeleton organization and tumor cell growth rate in culture.

2) Immortalize one normal Schwann cell culture and two schwannoma cell cultures with different mutations and phenotypes and characterize the cell lines.

Successful completion of these aims will allow us to: 1) better understand NF2 gene function in Schwann cells at the cell and molecular level and 2) obtain a useful in vitro tool for screening of new therapeutic agents for NF2.
Body

STATEMENT OF WORK

Establish an in vitro model for the study of NF2 gene function.

Specific Aim 1: Establish a reproducible protocol for the primary culture of human Schwann and schwannoma cells and characterize NF2 gene function by studying cytoskeleton organization and tumor cell growth rate in culture.

Task 1: Months 1-20 Establish primary culture protocol by collecting normal vestibular nerve tissues and schwannoma tissues and store all cultures in liquid nitrogen at their passage 3.

Report:
Total of 32 human vestibular schwannoma tumors were banked and of these 32 tumors, 16 tumors were cultured and stored for further analysis. In addition, eighteen normal human sciatic nerves and their cultures were banked.

Specific Aim 2: Immortalize one normal Schwann cell culture and two schwannoma cell cultures with different mutations and phenotypes and characterize the cell lines

Task 1: Primary culture cell immobilization by retrovirus.

Total of 3 schwannoma cultures derived from different NF2 patients and one normal human Schwann cell culture were immortalized.

Task 2. Characterization of the stable long term cell line.
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The characterization of one immortalized human schwannoma cell line has been completed. We expect to finish the characterization of 3 other lines in next 12 months period.

Key Research Accomplishments

- Establishment of NF2 specific vestibular schwannoma primary culture bank.
- Establishment of human normal Schwann culture bank
- Partially immortalized a normal Schwann cell culture.
- Overexpression of NF2 gene able to inhibit NF2 gene mutated schwannoma cell proliferation under serum free condition

Reportable Outcomes

- Society for Neuroscience Meeting Nov. 2001 San Diego, California

Abstracts for "Overexpression of the NF2 gene inhibits schwannoma cell proliferation through promoting PDGFR degradation" will be submitted for presentation.

- Establishment and characterization of a schdwannoma cell line from a patient with NF2 Manuscript
  Published on J of international Oncology.

- Overexpression of the NF2 gene inhibits schwannoma cell proliferation through promoting PDGFR degradation. Manuscript submitted to international J of Cancer

- Abnormal Schwann cell biology in neurofibromatosis 2-related neuropathy Manuscript submitted to Glia

-Human primary vestibular schwannoma tissue and culture bank

-Establish a retroviral vector transduced potential human Schwann cell line.
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Conclusions

The goals of this project are to develop a methodology to establish a reliable in vitro system and test its credibility for the study of NF2 gene function in Schwann cells. Over the past thirty-six months of the project, we have established a standard method for culturing primary schwannoma cells and normal Schwann cells. In addition, total of 3 schwannoma cultures derived from different NF2 patients and one normal human Schwann cell culture were immortalized. Of those stable lines, one immortalized human schwannoma cell line has been completely characterized. Due to the relocation of PI and transferring grant sponsorship, a no cost extension (12 months) has been applied to Army Medical Research and Materiel Command.