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13. ABSTRACT (Maximum 200 Words)
We have identified Hrs (**h**epatocyte growth factor-**r**egulated tyronsine kinase **s**ubstrate)as an NF2 binding protein using the yeast two-hybrid system. Hrs is also known to interact with STAM (signal transduction adapter molecules) Hrs appears to have growth suppressing functions at least in part mediated via binding to STAM with a resulting reduction in DNA synthesis. Progress is discussed in order of the three specific aims that were proposed originally.

1) Regulated overexpression of HRS in rat schwannoma cells results in similar effects as overexpression of schwannomin. This includes growth inhibition, decreased motility and abnormalities in cell spreading (Gutmann et al. 2001).

2) A recently emerging function for Hrs is the sorting of endosomes containing EGR-receptor. We have begun to examine this effect in RT4 cells overexpression Hrs or schwannomin.

3) We have begun to generate mouse embryonic fibroblast cell lines that express schwannomin or HRS under the control of the tet-regulatable promoter. These lines will be used to examine the effects of overexpression of either protein on proliferation and STAT signaling.

14. SUBJECT TERMS
Merlin, Schwannomin, yeast two-hybrid system, signal transduction, tumor suppressor proteins, Hrs

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PI Name	Stefan-M. Pulst
Key Words	Merlin, Schwannomin, Yeast two-hybrid system, Signal transduction, Tumor suppresser proteins, Hrs

Introduction

Germline mutations in the neurofibromatosis type 2 (NF2) gene predispose to tumors of multiple types. We have identified Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate) as an NF2 binding protein using the yeast two-hybrid system. In previous years we have used the yeast two-hybrid and in vitro binding assays to fine-map the binding domains in Hrs and schwannomin. This was followed by examining the functional interactions of the two proteins. The ultimate goal of this research is to dissect schwannomin-Hrs-STAM interaction and to understand the role of schwannomin in STAM-mediated signaling in Jak/STAT pathways. This will pave the way for the identification of novel drug targets for the treatment of patients with NF2 and for patients with surgically inaccessible meningiomas and ependymomas.

Body

Specific Aim 1: Further characterization of binding domains in Hrs, schwannomin, and STAM

Task 1: Months 1-12 Fine-mapping of interacting domains in Hrs and schwannomin. **Completed**
Task 2: Months 6-18 Analysis of STAM - schwannomin interaction. **Completed**
Task 3: Months Three hybrid assays for Hrs-STAM-schwannomin interaction.
Task 4: Months 18-24 Interaction analysis of Hrs isoforms. **Completed**
Task 5: Months 28-24 Interaction analysis of rat isoform Hrs-2. **Abandoned,** because it is now known that the previously reported cDNA by the Scheller group contained a sequence error. This isoform is identical to our isoform 1.
Task 6: Months 24-30 Isolation of human Hrs-2 isoforms **Completed**

Specific Aim 2: Distribution of Hrs, STAM and schwannomin in normal cells and NF2 tumors

Task 1: Months 6-12 Cellular distribution of schwannomin and Hrs in normal Schwann cells, schwannomas, meningiomas, and ependymomas. **Completed**
Task 2: Months 12-18 Determination of colocalization of schwannomin, Hrs, and STAM in cell lines. **Completed**
Task 3: Months 18-30 Overexpression of Hrs and/or schwannomin as GFP/BFP fusion proteins. **Modified**

Specific Aim 3: Schwannomin in Hrs-mediated signaling

Task 1: Months 1-12 Study of Hrs proliferative effects of Hrs in STS26T cells. **Completed**
Task 2: Months 12-24 Schwannomin and Hrs overexpression in mouse NF2 deficient cell lines. We are planning to finish these experiments in the last year of funding. We now have in hand human NF2-deficient schwannoma cells, *Hrs*-deficient mouse embryonic fibroblasts, and *Nf2*-deficient mouse embryonic fibroblasts. These resources will permit the execution of unique experiments that will define the roles of Hrs and NF2 expressed as single molecules or in combination.
Task 3: Months 12-24 Proliferative effects of schwannomin and Hrs fragments bearing

		deletions of their respective binding sites including STAM binding sites. **Completed**
Task 4:	Months 18-36	Analysis of Jak/STAT pathways after schwannomin and Hrs transfection. **Completed**
Task 5:	Months 24-36	Analysis of BAF-B03 cells after NF2 transfection including NF2 constructs bearing deletions or missense mutations. **Modified**

We decided to modify the indicated tasks as the role of HRS in trafficking of the EGF receptor (EGFR) has become an area of intense interest. A manuscript describing these results is in the final stages of preparation. The key findings in this forth-coming manuscript are that overexpression of HRS traffics EGFR to a pre-lysosomal compartment and that overexpression of HRS results in less phosphorylated (active) EGFR in the cell with the net result of reduced EGFR signaling. Once the manuscript is finalized, we will submit it to USAMRMC for inclusion in the final report.

Key Research Accomplishments

- We have now published our results analyzing how the NF2 tumor suppressor schwannomin and its interacting protein HRS regulate STAT signaling (Scoles et al., 2002, appended).
- In collaboration with Dr. D. Gutmann, we determined that NF2 acts upstream of Hrs (Sun et al., 2002, appended).
- We have generated novel cell lines that express NF2 and Hrs in an inducible fashion.
- We have determined the effect of overexpression of Hrs on the trafficking of the EGF receptor.

Reportable outcomes for entire project

Abstracts published:

Scoles, D.R., Gutmann, D.HG., Chen, M.S., Morrison, H., Huynh, D.P., and Pulst, S.M. Neurofibromatosis 2 (NF2) tumor suppressor schwannomin interaction with HRS regulates STAT signaling and Schwann cell proliferation. *Neurology* (Suppl 3) 54:A7 (2000).

Scoles, D.R., Chen, M.S., and Pulst, S.M. Effects of NF2 missense mutations on schwannomin interactions. *Biochemical Biophysical Research Communications* 290(1):366-374; 2002.

Scoles, D.R., Nguyen, V., Lam, S., and Pulst, S.M. The NF2 tumor suppressor schwannomin interacts with p110, a component of the eukaryotic initiation factor 3 (eIF3). *Neurology* (Suppl. 3) 58:A11 (2002).

Scoles, D.R.., Nguyen, V., Lam, S., Qin, Y., and Pulst, S.M. The NF2 tumor suppressor schwannomin interacts with the eukaryotic initiation factor 3 (eIF3) subunit p110. Mol. Biol. Cell 13 (Suppl):157a-158a (2002)

Scoles, D.R., Nguyen, V., Gutmann, D.H., and Pulst, S.M. The NF2 tumor suppressor interacting protein HRS regulates EGF receptor trafficking in schwannoma cells. Neurology (Suppl. 3) 2003

Scoles, D.R., Gutmann, D.H., and Pulst, S.M. The neurofibromatosis 2 (NF2) tumor suppressor schwannomin interacting protein HRS regulates EGF receptor signaling and trafficking in schwannoma cells. *Am. J. Hum. Genet.* Vol 73(5) (Suppl). Pg 197. (2004).

Scoles, D.R., Gutmann, D.H., and Pulst, S.M. HRS regulates EGF receptor signaling and trafficking in schwannoma cells. Mol. Biol. Cell (Suppl.): (2004) (In press).

POSTER PRESENTATIONS:

The NF2 tumor suppressor interacting protein HRS regulates EGF receptor trafficking in schwannoma cells. 55th American Academy of Neurology Annual Meeting. Honolulu, HI. 2003

HRS regulates EGF receptor signaling and trafficking in schwannoma cells. 43nd American Society for Cell Biology Annual Meeting. San Francisco, CA. (Upcoming). December 17, 2003

. PLATFORM PRESENTATIONS:

The neurofibromatosis 2 (NF2) tumor suppressor schwannomin interacting protein HRS regulates EGF receptor trafficking in schwannoma cells. 53rd Annual Meeting of The American Society of Human Genetics. Los Angeles, CA. November 7, 2003

Abstract presentations:

Neurofibromatosis 2 (NF2) tumor suppressor schwannomin interaction with HRS regulates STAT signaling and Schwann cell proliferation. 52nd American Academy of Neurology Annual Meeting, San Diego, California.(2000)

Scoles, D.R., Nguyen, V., Lam, S., and Pulst, S.M. The NF2 tumor suppressor schwannomin interacts with p110, a coponent of the eukaryotic initiation factor 3 (eIF3). *Neurology* (Suppl. 3) 58:A11 (2002).(Annual AAN meeting 2002)

Scoles, Vu D. Nguyen, Samuel Lam, Yun Qin, Stefan M. Pulst
Burns and Allen Research Institute and Division of Neurology, Cedars-Sinai Medical Center, and UCLA School of Medicine, Los Angeles, California (ASCB 42nd Annual Meeting, December 14-18, 2002, San Francisco.)

Scoles, D.R., Gutmann, D.HG., Chen, M.S., Morrison, H., Huynh, D.P., and Pulst, S.M. 2000.The neurofibromatosis 2 (NF2) tumor suppressor schwannomin interacting protein HRS regulates EGF receptor trafficking in schwannoma cells. 53rd Annual Meeting of The American Society of Human Genetics. Los Angeles, CA. November 7, 2003

Publications:

1: Scoles DR, Nguyen VD, Qin Y, Sun CX, Morrison H, Gutmann DH, Pulst SM. Related Articles, Links
Neurofibromatosis 2 (NF2) tumor suppressor schwannomin and its interacting protein HRS regulate STAT signaling.
Hum Mol Genet. 2002 Dec 1;11(25):3179-89.
PMID: 12444102 [PubMed - indexed for MEDLINE]

2: Sun CX, Haipek C, Scoles DR, Pulst SM, Giovannini M, Komada M, Gutmann DH. Related Articles, Links
Functional analysis of the relationship between the neurofibromatosis 2 tumor suppressor and its binding partner, hepatocyte growth factor-regulated tyrosine kinase substrate.
Hum Mol Genet. 2002 Dec 1;11(25):3167-78.
PMID: 12444101 [PubMed - indexed for MEDLINE]

3: Scoles DR, Chen M, Pulst SM. Related Articles, Links
Effects of Nf2 missense mutations on schwannomin interactions.
Biochem Biophys Res Commun. 2002 Jan 11;290(1):366-74.
PMID: 11779178 [PubMed - indexed for MEDLINE]

4: Gutmann DH, Haipek CA, Burke SP, Sun CX, Scoles DR, Pulst SM. Related Articles, Links
The NF2 interactor, hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), associates with merlin in the "open" conformation and suppresses cell growth and motility.
Hum Mol Genet. 2001 Apr 1;10(8):825-34.
PMID: 11285248 [PubMed - indexed for MEDLINE]

5: Scoles DR, Huynh DP, Chen MS, Burke SP, Gutmann DH, Pulst SM. Related Articles, Links
The neurofibromatosis 2 tumor suppressor protein interacts with hepatocyte growth factor-regulated tyrosine kinase substrate.
Hum Mol Genet. 2000 Jul 1;9(11):1567-74.
PMID: 10861283 [PubMed - indexed for MEDLINE]