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Table of Contents

Introduction.....	1
Body.....	1
Appendices.....	none

Erb2 trafficking and signaling in human vestibular schwannomas**Marlan R. Hansen**

During the first year of the award we have made substantial progress in achieving the aims of the proposal. I will discuss the progress for each aim of the proposal.

Specific aim 1. Determine the ability of merlin to regulate ErbB2 localization and activity in vestibular schwannoma (VS) cells.

Until recently we were unable to use human vestibular schwannoma (VS) specimens while we were working to obtain Human Subjects approval.

During this period, we focused on correlating the status of merlin phosphorylation with ErbB2 trafficking in normal Schwann cells

(SCs). First, we determined that the trafficking of ErbB2 into lipid rafts in SCs correlates with loss of axonal contact, phosphorylation of merlin on

Serine 518, and proliferation (Figs 1 and 2). Thus, phosphorylation of merlin on

Serine 518 (S518), which inhibits its growth suppressive function, is correlated with the

movement of ErbB2 into lipid rafts in the cell membrane. We have previously shown that ErbB2 constitutively resides in

lipid rafts in human vestibular schwannoma cells that lack functional merlin (Brown and Hansen, *Otology & Neurotology*, in press).

Since we did not initially have approval to work with human VS specimens, we worked on subcloning various merlin constructs, including those with

S518 mutations, into adenoviral vectors. This will allow us to directly test the role of merlin in regulating the trafficking of

ErbB2 in human vestibular schwannoma cells. Recently the Dept. of Defense and the Univ. of Iowa concurred that the

project did not require IRB approval since it did not qualify as Human Subjects research and in the coming year we will

be able to determine the extent to which replacement of merlin in human vestibular schwannoma cells regulates the

trafficking of ErbB2 within the cell membrane.

Specific aim 2. Determine whether phosphorylation of merlin on serine 518 (S518) by protein kinase A (PKA) inhibits merlin's ability to regulate ErbB2 trafficking and suppress VS cell proliferation.

Again here we were initially limited in the work we could do with human specimens.

However, we have been able to demonstrate that activation of protein kinase A (PKA) with forskolin (FSK 5 μ M) leads to

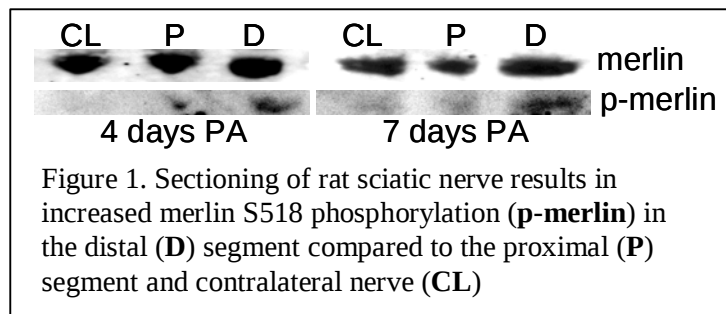


Figure 1. Sectioning of rat sciatic nerve results in increased merlin S518 phosphorylation (**p-merlin**) in the distal (**D**) segment compared to the proximal (**P**) segment and contralateral nerve (**CL**)

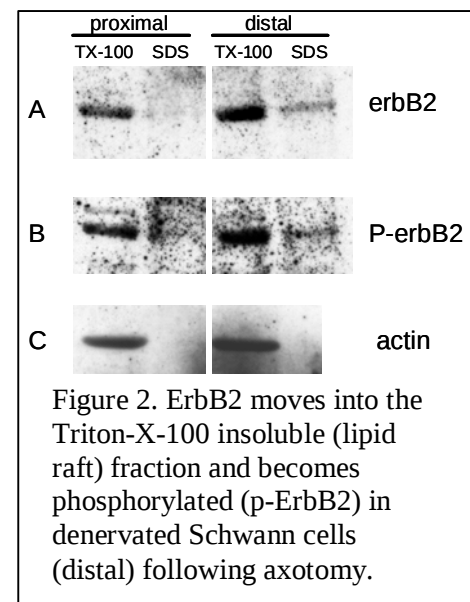


Figure 2. ErbB2 moves into the Triton-X-100 insoluble (lipid raft) fraction and becomes phosphorylated (p-ErbB2) in denervated Schwann cells (distal) following axotomy.

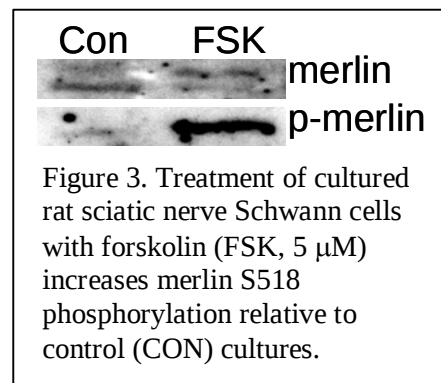


Figure 3. Treatment of cultured rat sciatic nerve Schwann cells with forskolin (FSK, 5 μ M) increases merlin S518 phosphorylation relative to control (CON) cultures.

Erb2 trafficking and signaling in human vestibular schwannomas**Marlan R. Hansen**

merlin S518 phosphorylation in rat SCs (Fig. 3) and that this correlates with proliferation and with movement of ErbB2 to the cell membrane. In the coming year, we will be able to directly test the role of S518 phosphorylation by PKA in regulating ErbB2 trafficking and VS proliferation.

Specific aim 3. Determine whether ErbB2 inhibitors potentiate the ability of radiation therapy (RT) to induce VS apoptosis and reduce proliferation. Following approval to proceed with the use of human tissue we have been able to perform radiation experiments on several cultured human VS specimens. We demonstrate that doses of ≥ 30 Gy are required to limit VS cell proliferation and that doses ≥ 40 Gy are required to induce apoptosis (Fig. 4). Furthermore, we show that inhibition of ErbB2, which reduces VS cell proliferation, protects VS cells against radiation induced cell death. Conversely, activation of ErbB2 by treatment with neuregulin, which promotes VS cell proliferation, increases the radiosensitivity of human VS cells. These results imply that (1) VS are radioresistant relative to most neoplasms, (2) the radiosensitivity of human VS cells depends on proliferative status, and (3) ErbB2 signaling sensitizes VS cells to

radiation by promoting proliferation. Thus, we have been able to make substantial progress on the proposed experiments in this aim and are now exploring the possibility that persistent activation of c-Jun N-terminal kinase signaling in protects human VS cells from radiation induced cell death by limiting the accumulation of reactive oxygen species.

