Vestibular Schwannoma: Molecular Pathogenesis and Clinical Treatment

D. Bradley Welling, M.D., Ph.D.
Department of Otolaryngology-Head and Neck Surgery
The Ohio State University Columbus, OH U.S.A.

Key words: vestibular schwannoma, molecular biology, mouse models, treatment

Clinical Relevance

Vestibular schwannomas are benign tumors of the vestibular portion of the 8th cranial nerve which are usually slow growing within the internal auditory canal or cerebellopontine angle. Tinnitus, hearing loss, facial nerve paralysis, facial paresthesias, loss of vision, dysequilibrium, brainstem compression, hydrocephalus, cerebrovascular compromise and death are known complications arising from these tumors. The location of the origin of the tumor is likely to be important in the time course of the onset of symptoms. Tumors which arise within the internal auditory canal are likely to cause tinnitus and hearing loss at an early stage. Tumors which arise in the cerebellopontine angle may grow to a larger size without becoming symptomatic. Vestibular schwannomas grow within the Schwann Cells of the vestibular nerve, most often in the inferior vestibular nerve, compressing the facial and auditory nerves peripherally. The morbidity from these tumors is significant. Tinnitus, hearing loss and facial nerve paralysis are frequent and very difficult for patients. Catastrophic events such as stroke or death are far less common, but none-the-less of great importance as well.

Molecular Biology Overview

One of the areas of focus in our laboratory has been to better understand and characterize the promoter of the NF2 gene. By characterization of the promoter of the NF2 gene, we hope to improve diagnostic tests which may effect clinical outcomes, and to improved tissue specificity for mouse models. We have also studied the role of merlin in embryogenesis. Eventually we hope to more completely understand why vestibular nerves are prone to get schwannomas when the cochlear, facial and other cranial nerves are far less likely to develop tumors. Last, and most importantly we hope to understand molecular mechanisms which may lead to specific new drug treatments not currently available.

From an historical viewpoint (and from a review of only the English literature), it is of interest to note the following landmarks in the study of vestibular schwannoma.

1777 Sandifort first described acoustic neuroma (vestibular schwannoma)
1882 von Recklinghausen described multiple peripheral neurofibromas (NF1)
1882 Wishart also described bilateral acoustic neuromas (NF2)
1891 Starr and McBurney, a neurologist and surgeon, first attempted resection
1893 Beevor and Balance performed the first successful resection of vestibular schwannoma
1917 Cushing report an 80% mortality rate in tumor removal
1925 Dandy reported a 25% mortality rate reduction
1960 House & Hitselberger described the middle fossa approach and revived the translabyrinthine approach to tumor removal
1993 Trofatter et al. and Rouleau et al. working in independent laboratories cloned and identified the NF2 gene, a tumor suppressor which required inactivation of both alleles for tumor formation (Rouleau et al., 1993). Trofatter et al., 1993)

It took over 200 years from the time of tumor discovery to the time of gene discovery. Trofatter's group named the protein product "Merlin" because it resembled other members of the protein 4.1 family, a moesin, gizrin, radixin, like gene. Rouleau's group named it "Schwannomin" for the obvious reason that its mutation resulted in the formation of schwannomas. Since 1993, neither group has been willing to concede to the other and so in 2000, Dr. Chang and I suggested that to unify the name of the protein and to honor both groups, we should call the protein "schwannomerlin". Finally the two groups were united. They concluded together that neither group liked our suggestion (Welling and Chang, 2000).

Although merlin is homologous to other 4.1-related family proteins, with a globular N-terminal domain, an a-helical domain and a charged carboxy terminus, it was the only tumor suppressor known in the family at the time. (Since that time, another tumor suppressor, DAL-1 has been identified in the same 4.1 superfamily. It has been shown by other laboratories that merlin modulates Schwann cell proliferation and inhibits actin-cytoskeleton mediated processes including cell motility, cell spreading, and cell attachment (Gonzalez et al., 1996, Deguen et al., 1998, Koga et al., 1998, Gutman et al., 1999). Control of Schwann cell proliferation is lost by inactivation of both NF2 alleles. Additionally, merlin has been shown to be essential for embryonic development, as homozygous knockout mouse models are embryonic lethal (McClatchey et al., 1997). Heterozygous knockout models showed malignant tumors which will be discussed later (McClatchey et al., 1998). Conditional homozygous knockout mice have developed some schwannomas, but no vestibular schwannomas have been shown (Giovannini et al., 1999, 2000).

It appears that growth inhibition by merlin depends on interaction with CD44 (Herrlich et al., 2000, Morrison et al., 2001). Merlin is regulated by phosphorylation and at high cell density, merlin is hypophosphorylated which inhibits cell growth. At low cell density, merlin is phosphorylated, inactive and growth permissive. This is believed to be Rac1 mediated phosphorylation by a Pak2 interaction (Shaw et al., 2001, Kissil et al., 2002, 2003, Xiao et al., 2002).

We published some years ago our mutational analysis which showed a wide distribution of mutations within the 17 coding exons of the NF2 gene except for exon 17, in both NF2 related vestibular schwannoma and spontaneous unilateral vestibular schwannoma. We noted that in unilateral vestibular schwannoma small deletions represented 76% of the mutations identified whereas in the NF2 related vestibular schwannoma, point mutations were identified in 58%. Additionally, we identified a CpG hot spot, where CGA (arg) was mutated to a TGA (Stop) codon frequently. We were unable to suggest a strong phenotype – genotype relationship (Welling, 1998). It has been noted that although phenotypes are often similar within families, there can be great variation within NF2 families as well. This would suggest that mutation type alone is not the sole determining factor in the severity of the individuals clinical course. Not all vestibular schwannomas we studied had identifiable NF2 mutations within the coding regions (Welling et al., 1996, Jacoby et al., 1996).

We then set out to characterize the NF2 promoter
by identifying a BAC clone containing the genomic NF2 DNA. We were able to identify further upstream in the 5'-flanking regions, both positive and negative regulatory elements regulating the transcription of the NF2 gene. The presence of GC rich sequences which were regulated by the Sp1 transcription factor were identified as positive regulatory elements (Welling et al., Otol HN Surg 2000, Chang et al., Genomics 2002).

We then examined ten vestibular schwannomas where a mutation within the coding regions of exons 1-17 could not be identified in collaboration with MacCollin (MacCollin et al., 1994). We hypothesized that mutations in the promoter region may contribute to the formation of vestibular schwannoma, but no mutations were identified in promoter regions (unpublished data). Even this negative finding may be useful however as we might suggest that for clinical screening purposes, it does not appear to be necessary to include screening the promoter regions. This finding suggests that other epigenetic regulators such as gene methylation are potential influencing factors (Kino et al., 2001).

(For a more detailed review of the molecular biology see Neff et al., 2006).

Mouse Models

As shown by McClatchey et al. in 1998, NF-2 homozygous knockout mice die at the time of gastrulation in embryogenesis. NF-2 heterozygous knockout mice develop malignant tumors, but no benign tumors as shown:

- osteosarcoma (63%)
- lymphoma (15%)
- lung adenocarcinoma (10%)
- hepatocellular carcinoma (9%)
- fibrosarcoma (9% )
- schwannomas (0%)

Giovannini et al. further improved the mouse model by introducing a mutant NF2 gene into the mouse germline that retained its function but could be inactivated by Cre-mediated recombination. Both benign and malignant Schwann cell tumors in soft tissues, peripheral nerves, and trigeminal schwannomas were found beginning at 10 months of age in up to 35% of mice.

Thus far, no murine model has developed vestibular schwannoma which leads us to ask if vestibular schwannoma is a distinctly human disease. It has been shown that the 8th cranial nerve is different in mice and men. Human vestibular bipolar ganglion cells have no myelin sheaths, but mice are myelinated (Giovannini et al., 2000). In a study by Stemmer-Rachaminov et al., 2004 human vestibular schwannoma were compared with murine schwannomas by ten blinded pathologists. There were clear differences in that human schwannomas were encapsulated whereas murine schwannomas were unencapsulated and more infiltrative. We are currently working on improving the genetically engineered mouse model by using the NF2 promoter to target tissues influenced by NF2 specificity. We are also interested in optimizing the time of mutation expression.

We have recently published a paper showing the importance of nf2 in its expression in the neural ectoderm as early as embryonic day 5.5. Mutation in the nf2 gene causes failure of closure of the neural tube. The NF2 promoter directs strong transgene expression in the spinal ganglia and retinal epithelium as well which may relate clinically to retinal abnormalities detected in patients with NF2 (Chang et al., 2006).

Another option we have been exploring is the human vestibular schwannoma tissue xenografts in the SCID mouse model. Lee et al. previously published a xenograft model with human schwannomas implanted in the subrenal capsule of mice and adjacent to the sciatic nerve. In the latter he reported a 89% survival rate. Our experiment with both malignant rat schwannoma xenografts and human vestibular schwannoma xenografts was recently published (Chang et al., 2006). We concluded that MRI reliably visualizes rat and human schwannoma xenografts in SCID mice and that Rat KE-F11 xenografts demonstrate a consistent solid phenotype while RT-4
xenografts result in cystic tumors. Multi-planar tumor volumes were calculated for human vestibular schwannoma xenografts using MRI. Vestibular schwannoma xenografts demonstrated biologic variability. Vestibular schwannoma xenografts may prove to be a reliable animal model for testing novel imaging modalities and chemotherapeutic interventions, but the lack of a native immune system complicates the model somewhat as well (Chang et al., 2006).

**Treatment Development**

The development of new treatment options and the testing of new treatment options depends upon two advances. First, understanding the basic molecular mechanisms in vestibular schwannoma formation is critical to drug development. Further *in vitro* and *in vivo* testing of proposed pharmacologic interventions is necessary. Secondly, the outcomes of current treatment options including observation, stereotactic radiation and surgical intervention must be clearly defined. Current literature is replete with retrospective studies with a very strong inherent bias and weak agreement.

In order to assist in delineating the molecular pathways involved in vestibular schwannoma formation, we have investigated the microarray expression differences between vestibular schwannoma and vestibular nerves. (Welling et al., 2002, Chang et al., 2005) The goal was to seek patterns of gene expression consistently elevated or decreased across all tumors. Of 25,920 genes screened, 42 genes were significantly up-regulated (by a factor of three or more) consistently across at least 6 of the 8 tumors examined. Additionally, multiple genes were found to be significantly down-regulated in the majority of vestibular schwannomas examined. Of these genes, eight genes involved with cell signaling and division were down-regulated, including an apoptosis-related, putative tumor suppressor gene LUCA-15 which was down-regulated in 7 of 8 schwannomas studied. Interesting and potentially important pathways for tumorigenesis are suggested by the deregulated genes. Two mediators of angiogenesis, endoglin and osteonectin, were highly elevated in selected tumors examined. Endoglin is a transforming growth factor-receptor that is known to be an endothelial marker for angiogenesis in solid tumors, and osteonectin is a secreted glycoprotein that interacts with extracellular matrix proteins to decrease adhesion of cells from the matrix, thereby inducing a biological state conducive to cell migration. Endoglin was found to be significantly up-regulated in all of the solid tumors but not in any of the cystic tumors examined. The difference in endoglin gene expression may be a key to unlocking why some schwannomas develop into the aggressive cystic phenotype (Charabi et al., 2000). Osteonectin was elevated in all of the tumors studied and may be a target for potential therapies including angiogenesis inhibitors (Vajkoczy et al., 2000).

Another example of a deregulated signaling pathway suggested by the microarray data is the retinoblastoma protein (pRb)-cyclin-dependent kinase pathway. Among genes involved in G1-S progression, CDK2 was found to be down-regulated in 7 of 8 tumors. In addition, up-regulation of transforming factor RhoB was found in all of the schwannomas examined (Lasak et al., 2002).

We also evaluated the role of cyclin D1 and D3 in vestibular schwannoma formation (Neff et al. 2006). G1 regulators of the cell cycle, cyclin D1 and D3, have been implicated in the regulation of Schwann cell proliferation and differentiation. We evaluated 15 sporadic vestibular schwannomas. None of the 15 vestibular schwannomas showed detectable cyclin D1 staining, however, five of ten vestibular schwannomas stained positive for the cyclin D3 protein. Cyclin D3 staining was taken up in the nucleus of schwannoma tumor cells in greater proportion than Schwann cells of adjacent vestibular nerve. We concluded that the cyclin D1 protein does not appear to play a prominent role in promoting cell cycle progression in benign vestibular schwannomas. In contrast, cyclin D3 expression was seen in half of the tumors examined, suggesting that it may have a growth-promoting role in some schwannomas.
Our microarray data generated far more questions than answers. We have been able to further investigate several additional pathways with immunohistochemistry and real-time PCR. Much is still needed to be done.

Current Treatment Options

The treatment of vestibular schwannomas remains controversial. Observation, microsurgical removal and stereotactic radiation are the current treatment options. The optimal timing and method of treatment for schwannomas is difficult to discern because the current literature is largely retrospective and the results seemingly quite disparate. For example, the percentage of tumor which will grow if followed over time as reported in the literature is variable.

<table>
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<th>% Growth</th>
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<tr>
<td>Fucci et al. 30%</td>
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<td>Selesnick and Johnson 50%</td>
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<td>Massick et al. 66%</td>
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<td>Charabi et al. 85%</td>
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We are currently without a good way to predict which tumors will grow rapidly or regress. The average growth rate is 1-2 mm per year, but growth rates as high as 25 mm per have been reported as has spontaneous regression of tumor. The primary drawback to observation is that 75% of patients who may have been hearing preservation surgery candidates lose that opportunity over time (Charabi et al.). Facial nerve preservation and brainstem compression are also concerning if the tumor grows rapidly or the patient fails to follow up as directed. Even following growth patterns with serial imaging may not be as reliable as patients and clinicians would hope. Bedersen reported that vestibular schwannoma do follow a linear growth pattern, but Charabi et al. disagreed. In their series, tumors could lay dormant for some time and then without explanation accelerate growth rapidly. Other potential predictors of growth such as patient age, tumor size, and the type of tumor (NF2-associated, solid, cystic) have not been predictive. In fact, even measures of nuclear proliferation such as Ki-67 and MIB-1 antigens have not correlated well with growth rates (Antinheimo et al., 1995).

Our current indications for observation of the vestibular schwannoma are if the patient is medically infirm or if the patient is greater than 65 years of age. Also, the patient must not be in eminent danger of hydrocephalus from brainstem compression. If the patient’s tumor is in their only hearing ear or if they refuse other treatments, we will recommend an initial follow-up MRI in 6 months. If the tumor is stable, we will obtain MRI’s yearly thereafter. Additionally, for tumors within the internal auditory canal with good hearing, we will offer the option to remove the tumor through a hearing conservation approach or to wait and watch for growth and monitor hearing. Our hearing preservation rate with surgery is only 55% for tumors less than 1.5 cm in diameter. This is taken into account when counseling the patient. If observation is selected and if no growth is seen on follow up MRI’s, and if useful hearing is preserved, we will continue to monitor tumor growth and hearing function. If hearing declines or if the tumor grows, we will offer tumor removal or stereotactic radiation.

Surgical morbidity must be considered in the treatment recommendations as well. The experience of the surgical team is an important consideration (Welling et al., 1999). In over 500 cases of microsurgical tumor removal, our mortality rate is 0.4%, with severe central nervous system impairment in 0.5%. The rate of facial nerve function preservation is 90% House-Brackmann grade I or II. The advantages and disadvantages of microsurgical tumor removal are as follows:

Advantages
- The risks have been well characterized
- Total tumor removal (99% unilateral tumors, 88% bilateral tumors)
- Single follow up MRI @ 5years
- Low recurrence rate of 0.4%
- Subtotal resection, only 5% regrowth
- Hearing preservation option in approximately 1/3 of patients
- Facial nerve House I-II >90%

**Disadvantages**
- Craniotomy
- Hospital stay of 3-7 days
- Off work for 3-6 weeks
- Known risks:
  - CSF leak - 8%
  - Meningitis - 1%
  - Headache, especially with suboccipital approach
  - Facial nerve injury <10%
  - Hearing loss - 45%
  - Stroke - 0.5%
  - Death - 0.4%

Neurofibromatosis type 2 (NF2) presents special challenges for the patient and treating physician alike. One area of uncertainty pertains to the difficulty of successfully removing the NF2-associated vestibular schwannoma. Samii (Samii et al., 1997) reported that of 120 NF2 associated tumors removed from 83 patients through the suboccipital approach, that the facial nerve was anatomically intact in 85% of patients. He does not state what the functional condition of the nerve is at that point. The rate of hearing preservation was 36% and the mortality rate 2.4%. He concluded that the surgical treatment of NF2-associated vestibular schwannoma was more difficult than unilateral vestibular schwannoma. Slattery (Slattery et al., 1998) reported that in 23 tumors removed from 18 patients via the middle cranial fossa (MCF) approach, that the facial nerve as preserved at a House grade I in 100% of cases and that hearing preservation was excellent in 48% and serviceable in 65%. They concluded that their outcomes were not substantially worse than their unilateral schwannoma population. My personal experience more closely follows that of Dr. Samii (Neff and Welling, 2005). Our NF2 patients were more likely to have facial nerve weakness and hearing loss following surgery than were their unilateral vestibular schwannoma counterparts.

Stereotactic radiation, delivered either in a single dose or fractionated, offers precise delivery of radiation to the tumor with the goals of long-term tumor control and preservation of cranial nerve function. I have listed what I feel are the advantages and disadvantages as follows:

**Advantages**
- 95% tumor control in short-term follow-up (33% unchanged, 62% smaller
- 1 day hospital stay
- Rapid return to work
- No surgery required
- May be used for recurrent tumor following surgery

**Disadvantages**
- Unknown long-term tumor control
- Malignant change estimated at 0.1%
- Tumors should be < 3 cm in size
- Ataxia
- Hydrocephalus
- 79% House grade I if the tumor is limited to the IAC
- Hearing may be lost over time
- Difficult dissection if surgical intervention is necessary
- NF2 and cystic tumors have poorer outcomes with radiation

(See Pendl et al., 1995; Shirato et al., 2000)

Some radiation induced tumors do not become evident for up to 30 years, therefore current follow-up in the literature is not sufficient to know the true outcome. We are therefore reluctant to recommend stereotactic radiation for our younger patients, and are more likely to recommend it for the patient over 65 years of age or the medically infirm.

A case of malignant change in a vestibular schwannoma reported by Shin et al. in 2002 was reviewed wherein a 26 year-old woman had a vestibular schwannoma subtotally resected and then radiated. Within several years, the tumor recurred. When
removed again, a malignant change was noted and the tumor now showed a p53 mutation which was not present in the specimen removed initially.

In conclusion, we find it valuable to continue to study vestibular schwannoma because:

1) There is serious morbidity and mortality associated with these tumors in spite of current treatment techniques. The outcomes of our current treatment regimens are inadequate and poorly studied.

2) Prospective case control studies should be performed on current treatment options both to better understand how to counsel patients and to prepare to be compared to new treatment options as they become available.

3) Improved treatment outcomes depends upon improved understanding of the pathogenic process of vestibular schwannoma formation at the molecular level.

4) Lastly, a conclusion from my earlier presentation on cochlear implantation in the profoundly deafened NF2 patient was included. It stated that early surgical intervention is an important consideration in the NF2 patient for the potential preservation of the cochlear division of the 8th cranial nerve and cochlear implantation.

Future studies will include evaluation of identified regulators of Schwann cell and schwannoma cell cycles. We hope to be able to offer new treatment modalities for our patients who suffer from vestibular schwannoma.

I wish to acknowledge the contributions of many members of our laboratory and clinical staff who have made these studies possible. In particular I want to acknowledge the work of Dr. Long-Sheng Chang as my collaborator, mentor and colleague. I also acknowledge the support of the National Institute of Health Deafness and Communicative Disorders and the Congressionally Directed Medical Research Program administrated by the Department of Defense.

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