Radiobiology of vestibular schwannomas: mechanisms of radioresistance and potential targets for therapeutic sensitization

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Vestibular schwannomas (VS) are benign tumors arising from the Schwann cells of cranial nerve VIII. Historically the prevailing therapy for patients with VS has been microsurgical resection. More recently, stereotactic radiosurgery (SRS) and fractionated stereotactic radiotherapy have gained acceptance as effective alternatives. Although the side effect profile and rates of tumor control appear to be favorable for SRS, there is a subset of radioresistant tumors that continue to progress despite properly administered radiation treatment. In this review, the authors summarize what is known about the mechanism of radioresistance in VS at the clinical and molecular level. An improved understanding of the radiobiological behavior of VS may help guide appropriate patient selection for SRS and potentially aid in the design of novel therapies to treat radioresistant tumors. (DOI: 10.3171/2009.9.FOCUS09185)

KEY WORDS vestibular schwannoma • radiotherapy • resistance • merlin • neurofibromatosis Type 2

Vestibular schwannomas are the most common tumors of the cerebellopontine angle and account for 6–8% of all intracranial tumors. Tradition-}
ally, the mainstay of VS management has been microsurgical resection by an experienced surgical team. More recently, focal radiotherapy has emerged as an alternative to surgery in selected patients. Radiotherapy is delivered using one of two techniques. Stereotactic radiosurgery targets tumor tissue by delivering a large single dose of ionizing radiation. In contrast, fractionated SRT utilizes multiple treatment sessions to deliver therapeutic doses of radiation to the tumor. Regardless of technique, treatment-related morbidity is generally more favorable than that for microsurgical intervention.

Treatments for VS such as SRS and SRT are becoming increasingly accepted, but tumor control rates are not 100%, suggesting that radiosensitivity can vary among patients with tumors of similar size and histological type. In the present paper we review what is known about VS biology and potential mechanisms of radiation resistance. We begin by discussing the problem of radioresistance in VS and what is known about the histopathological fea-
sensitivity in VS may be related to an inherently low proliferation index. If only a small percentage of cells within a given VS are dividing, the bulk of the tumor may be radioresistant, especially when radiation is delivered at low doses.20

The reasons for these treatment failures are likely multiple. For example, factors such as tumor size35 and hypoxia caused by inadequate vascular supply2 can both play a role in a lesion’s unresponsiveness to radiation. In 2003, Lee and colleagues18 performed a review of histopathological features in 4 patients who underwent salvage microsurgical resection of VSs after primary SRT. Light microscopy confirmed the presence of viable VS tumor cells in all cases. All tumors were moderately cellular, exhibiting varying degrees of nuclear pleomorphism with hyperchromasia and vascular hyalinization with surrounding hemosiderin deposition. No specimens exhibited necrosis, zones of scar proliferation, or any evidence of malignant transformation. The authors attributed the lack of significant degenerative tumor changes to global tumor radiation resistance, radiation resistance in a subpopulation of tumor cells followed by expansion of resistant clones, or insufficient radiation dose delivered to all or part of the tumor. Other studies describing histopathological features of VS after SRS have demonstrated varying degrees of treatment-related changes.20,15,30

We analyzed histological specimens obtained in 4 of our patients who underwent microsurgery for VS after failed SRS. The histological H & E features were similar for all 4 specimens. All tumors had a central area of fibrosis, suggesting radiation effect. The periphery of these tumors had hypercellular areas of neoplastic cells with an appearance typical for schwannoma (Fig. 1). Interestingly, we frequently noted nests of tumor cells within the fibrotic regions (Fig. 1). We also noted extensive vascular hyalinization, which in our experience is not specific for radiation-treated tumors and can be seen in untreated VSs. We did not note significant regions of necrosis in these specimens, but given that radiation may work in these tumors by inducing cell-cycle arrest, as opposed to necrotic cell death, it is not certain if this absence of necrosis is a function of radioresistance or if the absence of necrosis would be expected in tumors responsive to SRS.

**Molecular Biology of Radioresistance**

Perhaps the most important factors that determine the sensitivity of a tumor to radiation relate to specific genetic features that have cellular consequences. Differential tissue-specific gene expression, including oncogenes and tumor suppressor genes, may result in variations of radiation-resistant cellular phenotypes seen clinically.4,27 Support for the role of differential gene expression in determining radiation sensitivity comes in part from observations that tumors from different patients with the same histological diagnosis can show varied responses to ionizing radiation.33 Such differential radiosensitivity can also be present within a single tumor. For example, Weichselbaum and colleagues33 reported that 4 cell lines clonally derived from the same squamous cell carcinoma showed differential radiation sensitivities. In summary, these studies suggest that the expression of apoptotic markers, growth factor receptors, and angiogenic and

**Fig. 1.** Hematoxylin and eosin–stained sections from 2 different tumor specimens demonstrating the typical histological features we have observed following failed SRS. The predominant histological feature we noted was a broad central area of eosinophilic fibrosis (single wide arrow) with nests of neoplastic schwannoma cells (single narrow arrow) inside the fibrotic region. The tumor periphery consistently contained large regions of tumor cells, which appeared typical for VS (triple arrows). Original magnification x 100.
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The molecular parameters that determine how a cell becomes more or less sensitive to DNA damage induced by radiation or chemotherapeutic agents are poorly understood. The status of cell-cycle checkpoint signaling pathways is one possible crucial determinant of the response to DNA damage. Supporting evidence for this mechanism includes the findings that mutations in checkpoint components are prevalent in human cancers. Tumor cells might exhibit growth arrest or apoptosis in response to cytotoxic therapies, depending on the functional state of checkpoint pathways. Similarly, in other systems using nontransformed cells, incomplete mechanisms of DNA repair occurring during checkpoint phase delay increase the tendency toward apoptosis.

The phosphatidylinositol kinase–related protein ATM is a signal transducer initiating cell-cycle changes after ionizing radiation–induced DNA damage. Ionizing radiation rapidly induces protein kinase activity of the ATM gene, which in turn interacts with a broad network of proteins to block progression through the cell cycle. This hiatus allows for DNA repair. The ATM activates both p53 and CHK2, leading to either a G1/S or G2/M cell-cycle block, depending on interactions with downstream target genes.

Multiple pathways are involved in the maintenance of genetic integrity after exposure to ionizing radiation, most of which are related to the cell cycle. Cells commonly respond to DNA-damaging agents by activating cell-cycle checkpoints. These checkpoints provide for a controlled temporary arrest at a specific stage of the cell cycle to allow the cell to correct possible defects. Ionizing radiation induces arrests in G1, S, and G2 phases of the cell cycle. The G1 checkpoint prevents the replication of damaged DNA before the cell’s entry into S phase, and the G2 checkpoint prevents the segregation of aberrant chromosomes during M phase.

It has been shown that the pRb-CDK pathway tightly regulates G1 to S phase progression. The relevance of this pathway to radioresistance in VS is outlined in Fig. 2. In this model, loss of retinoblastoma function combined with decreased CDK2 levels in VS, can prevent the normal cell-cycle arrest that occurs after radiation-induced cellular injury, allowing these cells to avoid radiation-induced quiescence. Several studies have suggested an interaction between merlin and cell-cycle regulation via the pRb-CDK pathway. Lasak and colleagues have used microarray technology to study the G1 to S phase cell-cycle pathway in VS tissue. Microarray chips with a large number of genes known to be important to the pRb-CDK pathway were generated and hybridized to cDNA from VS. The authors demonstrated downregulation of this pathway in all 8 VS samples. Downregulation of the pRb-CDK pathway may relate to the characteristic slow growth of these tumors.

Merlin functions as a negative regulator of Rac-dependent signaling. In addition to regulating cytoskeletal organization, Rac activates an array of intracellular signaling pathways involved in cellular proliferation, transformation, and transcriptional activation. Downstream signaling regulated by Rac includes the JNK, p38, and NF-κB pathways. Activation of JNK and p38 cascades stimulates the activity of several transcriptional factors such as Jun and ATF2. Active, dephosphorylated merlin inhibits Rac-induced signaling, and inactive phosphorylated merlin potentiates Rac function. The JNK pathway in particular has been implicated in radiation-induced apoptosis and cell-cycle arrest, probably through the Rac pathway.

This overactivity of Rac would be predicted to lead to increased radiosensitivity, but merlin is also known to inhibit the function of the ERK pathway, which has been shown to interact with the JNK pathway to promote survival in response to radiation-induced cell injury.
In 2002, Lee and colleagues evaluated the proliferation potential of recurrent VS following Gamma Knife surgery compared with microsurgery. They concluded that recurrent VSs treated with Gamma Knife surgery have a lower proliferation rate, as assessed by proliferating cell nuclear antigen, than those treated with microsurgery. This supports the idea that radioresistance is mediated in part by a relative lack of cell division in some tumors and that recurrent tumors represent expansion of slowly dividing, radioresistant cell populations.

Subsequent work by Hansen and colleagues demonstrated this concept in vitro. The authors exposed VS cells in culture to escalating radiation doses and found the expected reduction in proliferative rate and induction of apoptosis with increasing doses. They subsequently demonstrated that inhibition of the growth-stimulating protein ErbB2 led to increased radioresistance and lower rates of radiation-induced apoptosis. They hypothesized that by preventing cell proliferation via ErbB2, they had induced radioresistance by interfering with cell-cycle arrest.

Angiogenesis Mediators

With the advances in the understanding of the molecular biology of cancer, it has become well recognized that both tumorigenesis and the development of radioresistance are related to the dysregulation of specific genes and a change in the tumor environment from hypoxia and acidosis. Tumor cell hypoxia may result in part from a tumor growth rate that exceeds the regional distribution of blood supply. Thus, faster growing tumors may also develop radioresistance due to inadequate angiogenesis and local tumor hypoxia.

Several pro-angiogenic factors have been identified, including the well-described and potent VEGF-A. Vascular endothelial growth factor causes vasodilation, increases vascular permeability, induces angiogenesis through endothelial cell proliferation and migration, and thus plays an important role in regulating angiogenesis. It promotes extravasation of plasma proteins from tumor vessels to the extravascular matrix, favoring inward migration and proliferation of endothelial cells.

In VS, a relationship exists between the number of vessels, the growth rate, and the size of the tumor. The expanding surface zone of the tumor is the region of neovascularization. A recent study demonstrated that VEGF was expressed in VS, and the intensity of immunohistochemical expression correlated positively with the growth rate of the tumor. There was no relationship between expression of VEGF and tumor size or duration of symptoms. These observations were further confirmed in patients with VS in a recent trial of an antiangiogenesis agent, which demonstrated an antitumor effect in the vast majority of patients.

Apoptotic Markers and Radiation-Induced Cell Death

Irradiation induces both single- and double-strand DNA breaks. The double-strand breaks are generally considered the lethal event. Studies have shown that severe combined immunodeficient mice are exquisitely sensitive to radiation. These mice are deficient in DNA-dependent...
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protein kinase, which functions in a complex at the site of DNA double-strand breaks to promote repair.9 This suggests that the type of nuclear damage and the nature of DNA repair processes together determine the response of cells to ionizing radiation.

Multiple genes that regulate apoptosis have been discovered. The p53 gene is one such regulator that has been particularly well characterized. Ionizing radiation–induced DNA damage activates p53, which then activates the proapoptotic Bax protein. This leads to the release of several proteins, including Cytochrome c, from the mitochondria into the cytoplasm. Cytochrome c activates the caspase cascade leading to cell death. Additionally, Fas, a cell-surface protein that triggers apoptosis when it binds to its ligand, is encoded by a target gene transcriptionally activated by p53. Despite p53’s known interaction with all of these antiapoptotic genes, none of them appears to be the principal mediator of the p53 apoptotic signal.26 This leaves open the possibility that p53’s targets vary between different tissues or cell types and vary in their regulatory response to ionizing radiation.6

Multiple studies have substantiated the absence of significant alterations of the p53 gene in VS.11 Monoh and colleagues23 investigated alterations of the p53 tumor suppressor gene in 21 cases of VS by using polymerase chain reaction-restriction fragment polymorphism and single-strand conformation polymorphism. No mutations or deletions were found. In 13 informative cases, no loss of heterozygosity was confirmed. These results further substantiate that p53 mutations are unlikely to contribute to the pathogenesis of vestibular schwannomas.

Although p53 itself has not proven itself to be vital in VS tumorigenesis, other apoptotic markers may play a more significant role. The induction of apoptosis by Bax has been shown to be independent of other important upstream and downstream components of the apoptosis pathway, such as p53 and caspases. Marwin and colleagues22 investigated the expression of the antiapoptotic factor Bcl-2 and the proapoptotic factor Bax in 14 sporadic VSs. They found Bcl-2 expression in the cytoplasm of 9 tumors (64%), and Bax was found in 10 (71%) of 14 schwannomas. Research has demonstrated that the inability of Bax to induce the activity of Bax in specific neoplastic cells is associated with the development of radioresistance in malignant gliomas. However, there are no studies to date regarding the role of Bax in conferring radiosensitivity or radioresistance to VS.

Conclusions

Despite the widespread use of radiation therapy as both a primary and secondary treatment modality for patients with VS, the radiobiology of VS is poorly understood, and translational research on VS is limited. Although apoptotic markers, cell-cycle regulators, growth factor receptors and Schwann cell proliferation mediators, and angiogenesis mediators have been identified in VSs, the role that they play in conferring radiosensitivity and radioresistance has not been well studied. Clearly, identification of molecular markers that can be used to predict tumor radiosensitivity and radioresistance would be important for optimizing treatment protocols. Intraoperatively, these markers may be used to guide the extent of excision. For radioresistant tumors, the choice of limited surgery may be appropriate. For exquisitely radiosensitive tumors, the choice of limited surgery to reduce the risk of cranial nerve dysfunction may be the better choice. At this time, there is no known molecular or genetic radiosensitive marker to guide intraoperative decision-making, and the development of such a test is limited by the need for rapid results and improved sampling techniques to correct for the inhomogeneous expression of the candidate radiosensitive marker. Regardless, currently no clear candidate target exists, and thus further work to elucidate mechanisms of VS radiosensitization is warranted.

Disclosure

Michael Sughrue was supported in part by the AANS NREF. Ari Kane was supported in part by the Howard Hughes Medical Institute, and the Ivy Foundation. Andrew Parsa was funded by the Georgiana and Reza Khatib endowed Chair of Skull Base Surgery.

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