Myosin II Implicated in Malignant Gliomas

Researchers at the Brain Tumor Center, part of the Herbert Irving Comprehensive Cancer Center at NewYork-Presbyterian Hospital/Columbia University Medical Center, have discovered that the enzyme myosin II is essential for the movement of malignant gliomas through the brain. Deactivation of myosin II might limit tumor spread and make malignant gliomas more amenable to therapy, according to Steven Rosenfeld, MD, PhD.

Malignant gliomas are diagnosed in approximately 10,000 people each year. This year, one of them was Sen. Edward Kennedy. Malignant gliomas carry a devastating prognosis that has changed little over the years; they remain remarkably resistant to treatment with surgery, radiation, and chemotherapy, with a median patient survival time after diagnosis of 1 to 1.5 years. Dr. Rosenfeld faced the consequences of these grim statistics firsthand; his mother died of a malignant glioma at age 51.

“We need to significantly improve the survival of people with malignant gliomas,” he said.

In a recently published study (Mol Biol Cell 2008 [Epub ahead of print]), Dr. Rosenfeld and colleagues examined the migration of glioma cells in living brain tissue. Dr. Rosenfeld explained that when observed on a coverglass, glioma cells migrate like fibroblasts and do not require myosin II. Only when they were studied in 3-dimensional rat and mouse models—developed by Peter Canoll, MD, PhD, also a member of the Columbia neuro-oncology group—did the role of myosin II become apparent.

“These models are the most reliable ones for the study of the human disease,” said Dr. Rosenfeld. “We wouldn’t have been able to figure out how myosin works any other way.”

Dr. Rosenfeld outlined the tactical advantage of working at a comprehensive neuro-oncology center. “We can go from the clinic back to the laboratory, try and figure out the pathophysiology of our patients’ diseases, and ultimately develop new treatments. Our group is currently involved in 10 clinical trials and is enrolling new patients for brain tumor therapy. That’s the unique strength of the Columbia brain tumor program. It’s one of the reasons I came to NewYork-Presbyterian Hospital 3 years ago,” he said. “If I had a brain tumor, I would want to go to a place that not only treats a lot of brain tumors, but does research as well,” he added.

Novel Technique Removes Giant Brain Tumor

A failed operation in Costa Rica to remove a giant tumor in his clivus, sella, and suprasellar cistern led a 50-year-old man on a journey to seek out surgeons who could help him (Figure 1, page 7). Eventually, he found the neurosurgical–otolaryngologic team of Theodore Schwartz, MD, and Vijay K. Anand, MD, who successfully removed the massive tumor through his nose.

“Minimally invasive surgery has been a real revolution in the way we perform pituitary and skull base surgery. Patients tolerate it better and go home sooner. Visualization is actually better with the endoscope than the operating microscope because the endoscope can be angled and look around corners. Although the endoscope’s opening is smaller, one can actually see more,” said Dr. Schwartz. For the last 5 years, Dr. Schwartz has been working in tandem...
Gene Therapy Clinical Trial Yields Promising Results

Recent research led by Ronald Crystal, MD, suggests that a genetically engineered virus is safe and may prevent progression of neurologic impairment in children with late infantile neuronal ceroid lipofuscinosis, also known as Batten disease (Hum Gene Ther 2008;19[5]:463-474).

The study included 10 children with Batten disease who received an average dose of $2.5 \times 10^{12}$ particle units of AAV serotype 2 vector expressing human CLN2 cDNA (AAV2.hCLN2). The virus was administered through 6 burr holes, 3 on each side of the brain, through fine flexible glass catheters that infused the virus at a rate of 1 to 2 mcL per minute over 70 minutes. There were 2 infusion areas in each burr hole for a total of 12 infusion sites. Four of the children were from the United States, 2 from Australia, 2 from England, and 2 from Germany. Mark Kaplitt, MD, PhD, performed the surgery with Mark Souweidane, MD. Dr. Kaplitt explained that advanced stereotactic neurosurgical methods were used to plan the virus delivery.

“In the operating room, we use the computer to navigate into the brain to find any spot in 3-dimensional space,” said Dr. Kaplitt. He emphasized that precise targeting of the virus is important because the virus needs to enter the brain substance and not just spill into the cerebrospinal fluid (CSF) space in order to have maximal efficacy. Additionally, if the virus enters the CSF, it is more likely to cause an undesired immune reaction. None of the patients developed infections from the procedure or clinically recognized hemorrhage.

After the procedure, the subjects spent a couple of days in the intensive care unit and typically left the hospital after 1 week. The children were followed clinically and with serial magnetic resonance imaging scans for 18 months. One child died 49 days following treatment after developing status epilepticus that was thought to be due to the underlying Batten disease.

Barry Kosofsky, MD, PhD, who also participated in the study, outlined some of the major milestones: “First, we revised the scale for assessing disease progression, which we call the modified Hamburg or the Weill Cornell Batten Disease Scale. Second, we developed brain imaging as a surrogate marker for disease progression. Third, we developed some very sophisticated computer-based methods to quantitate how fast the brain is deteriorating.”

The study showed that the procedure was safe, and in the moderate cases, Dr. Kosofsky suggested, “It probably delayed progression of disease for 3 to 9 months.”

Dr. Souweidane noted, “This study served as a fantastic springboard for the institution,

“...The excitement of Dr. Crystal’s study is the development of a technology to introduce a gene into the brain of a child who has a genetically determined disease.”

—Darryl C. De Vivo, MD

Dr. Crystal and colleagues in the Department of Genetic Medicine at Weill Cornell Medical College conducted the innovative gene therapy study. “Because of a genetic defect, the neurons of children with Batten disease are deficient in the enzyme tripeptidyl peptidase. We were able to put the normal gene that will manufacture this enzyme into an adeno-associated virus [AAV] carrier and administer the virus directly to the brain. This project required a large collaborative effort with a team of neurosurgeons, neuroradiologists, pediatric neurologists, gene therapists, and others over several years to get to our first Phase I trial,” said Dr. Crystal.

Darryl C. De Vivo, MD, who treats children with Batten disease at NewYork-Presbyterian Hospital/Columbia University Medical Center said, “The excitement of Dr. Crystal’s study is the development of a technology to introduce a gene into the brain of a child who has a genetically determined disease.”

Batten disease is a rare, autosomal recessive disease that presents in 4 different clinical forms depending on the age of onset. “The disease is characterized by the inappropriate premature accumulation of ceroid and lipofuscin in the lysosome within the cell,” added Dr. De Vivo. “The subjects of Dr. Crystal’s study were children with late infantile neuronal ceroid lipofuscinosis who usually present at age 18 months to 4 years with seizures, delayed development, and decreased life expectancy. About 40% of the children with Batten disease have this late infantile form. There is no cure, and only supportive treatment is available.”
Dr. Kosofsky emphasized the advantages of performing this type of research at NewYork-Presbyterian/Weill Cornell Medical Center: “We have the laboratory at the Belfer Gene Therapy Core Facility where Dr. Crystal can develop the virus; Dolan Sondhi, PhD, and Neil Hackett, PhD, run the animal models; Doug Ballon, PhD, John Dycke, PhD, Henning Voss, PhD, Dikoma Schungu, PhD, and Linda Heier, MD, run the brain-imaging analysis; and we have high-quality neurosurgeons, pediatric neurologists, and the rest of the team.”

Drs. Souweidane and DeVivo noted the promise of this innovative gene therapy and its potential use in other diseases besides Batten disease. “My research for the past 8 years has focused on the delivery of therapeutic molecules to the brain via cannulas for inoperable brain tumors in children,” said Dr. Souweidane. “The technical advances that we developed during this study are directly applicable to treating children with brain cancer, as well as other processes that may benefit from local delivery techniques.”

Dr. DeVivo observed that it might be possible to introduce a functioning gene for Tay Sachs disease, Canavan’s disease, spinal muscular atrophy, or glucose transporter type I deficiency. “Most of [the children with these diseases] are born clinically normal and only become symptomatic postnatally. Consequently, if the genetic abnormality was determined at birth, an effective treatment could be instituted, preventing the development of symptoms and allowing these children to lead normal lives,” he concluded.
For Philip Meyers, MD, arteriovenous malformations (AVMs) are an uncommon vascular phenomenon that provide a living laboratory in which to study the intricacies of functional brain organization.

Dr. Meyers explained that the presence of AVMs, which occur in approximately 4.3% of the population, may result in an abnormal wiring of the brain that impairs cognition, reasoning, and memory. These deficits may be subtle and elicited only with fairly complex instruments for neuropsychological testing. However, if and when an AVM ruptures, causing bleeding in the brain, the deficits can be magnified and extended (Figure 1).

Currently, Dr. Meyers participates in the ARUBA (A Randomized Study of Unruptured Brain AVMs) trial (http://www.arubastudy.org), formulated by the staff of the Doris and Stanley Tananbaum Stroke Center of the Neurological Institute and the InCHOIR Clinical Trial Center, both at NewYork-Presbyterian Hospital/Columbia University Medical Center. The ARUBA study, funded by the National Institutes of Health, has been undertaken to determine whether early treatment of unruptured brain AVMs is better than a “wait-and-see” approach. Patients with AVMs that have never ruptured are eligible to participate in the study, which randomizes them to observation or interventional therapy and follow-up for as long as 7.5 years. The plan is to enroll 800 patients.

Dr. Meyers emphasized that the risk that an AVM will hemorrhage appears to depend on its anatomic characteristics. For example, very small AVMs carry a high risk, probably because the pressures inside the abnormal vessels are greater than they are in larger AVMs. Many AVMs are associated with aneurysms or deep venous drainage, which also appears to increase the risk for hemorrhage. The results of the ARUBA study may provide more detailed information regarding the risk for hemorrhage of individual AVMs.

“The risk of hemorrhage may be as high as 35% per year in AVMs that have ruptured previously. By contrast, for AVMs that have never ruptured, the hemorrhage risk may be much lower. It is important to optimize treatment outcomes in both circumstances,” said Dr. Meyers.

As an interventional neuroradiologist, Dr. Meyers uses endovascular methods to treat an AVM, blocking the blood flow within its nidus (Figure 2). By threading a catheter from the femoral artery into the nidus of the AVM in the brain, he can inject an embolization agent like N-butyl-cyanoacrylate or ethylene vinyl copolymer through a microcatheter into the AVM, where it forms a precipitate and plugs the AVM. For a large AVM, the procedure may be performed in stages over several weeks to allow the brain to adapt to the abrupt changes in blood flow resulting from selective embolization of parts of the AVM. The procedure is not without risk and must be carefully controlled; a stroke can result if the glue is misplaced.

Dr. Meyers confided that one of the reasons he likes his job as an interventional neuroradiologist at NewYork-Presbyterian/Columbia is the collaboration between specialists—endovascular and cranial neurosurgeons, clinical stroke neurologists, neuroanesthesiologists, and neuropsychologists all work together to treat AVMs. The surgical team includes Robert Solomon, MD, E. Sander Connolly, MD, Sean Lavine, MD, Dr. Meyers, and Eric Heyer, MD, PhD, a neuroanesthesiologist, who all have a special interest in the care of people with AVMs.
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“The collaboration works to the patients’ advantage. The treatment outcomes are excellent,” said Dr. Meyers.

He also works closely with Ronald Lazar, PhD, M. Joanne Festa, PhD, and Emily Lantz, PsyD, all neuropsychologists. Dr. Lazar often accompany him into the operating room to administer neuropsychological tests during the procedure. Anesthetic agents are administered super-selectively into small areas of the brain surrounding the AVM to test for the potential effects of treatment before permanent occlusion. In this way, injury to the patient is avoided. Drs. Lantz and Meyers recently published a review of the neuropsychological effects of brain AVMs (Neuropsychol Rev. 2008;18(2):167-177).

Dr. Meyers’ research interest is brain neuroplasticity—specifically, the ability of one area of the brain to take over the functions of another area injured by rupture of an AVM, stroke, or other insult. In particular, he examines the phenomenon of hyperacute neuroplasticity, in which changes in functional localization occur very rapidly.

Dr. Meyers explained, “I’m a brain plumber. I go in through the vessels and perform selective testing with a microcatheter less than 0.5 millimeters in diameter. I can direct the catheter carefully to the nidus of the AVM and administer anesthetic agents to very specific locations. We usually administer amobarbital and lidocaine, which temporarily block both the gray-matter and white-matter tracts. In this way, we map the function of highly specific locations when we anesthetize a particular gyrus near an AVM.”

For example, he has seen patients with intact language function in brain tissue closely associated with an AVM. After the AVM was embolized, the language function remained intact but moved from its usual location in the temporal lobe to a different location in the frontal lobe. These changes can be visualized with functional magnetic resonance imaging. “If a primary speech area is damaged, for example, there are accessory areas that hopefully will pick up this essential function,” he said.

Dr. Meyers concluded, “Stroke is one of the developed world’s major public health problems. Some patients recover from their strokes, but many do not. My goal would be the development of stroke rehabilitation therapy to encourage this neuroplastic process. Young people have tremendous neuroplastic potential and adapt beautifully, but older people aren’t as adaptable as children. The phenomenon of neuroplasticity appears to be due to the presence of redundant functional neuronal systems throughout the brain. Right now, I am focusing on AVM patients as a model for functional neurological reorganization. We want to treat patients well, so we must maximize our understanding of brain function.”

Dr. Meyers’ research is supported by the Charles M. Dana Foundation.

Contributing faculty for this article:
Philip Meyers, MD
Researchers Create Tissue-Engineered Intervertebral Discs

A functional bioengineered intervertebral disc (IVD) is the goal of a collaborative research venture between a group of neurosurgeons and biomedical engineers. According to Roger Härtl, MD, a bioengineered disc could replace the current surgical treatment of discectomy, spinal instrumentation, and fusion. And for the 20 million people with discogenic back pain, a composite tissue-engineered IVD may provide superior relief from degenerative disc disease.

For the last year-and-a-half, Dr. Härtl has been working with Lawrence Bonassar, PhD, to develop a bioengineered disc suitable for humans. Their collaboration developed from biannual brainstorming sessions between Dr. Härtl’s team of neurosurgeons at NewYork-Presbyterian Hospital/Weill Cornell Medical Center and Dr. Bonassar’s team of biomedical engineers in the Departments of Biomedical Engineering and Mechanical and Aerospace Engineering at Cornell University.

Dr. Härtl performs about 350 operations a year and has privileged access to human disc tissue. In the collaborative research study, Dr. Härtl harvests the appropriate cells and sends them to Dr. Bonassar’s laboratory to be grown into discs. After the discs are grown, Dr. Härtl develops techniques for implantation of the discs into rats. The rats are imaged and tested to determine whether the discs are structurally and biochemically suitable to serve as replacement human discs. Ultimately, Dr. Härtl will determine how such discs can be safely implanted in humans.

Dr. Bonassar’s research group specializes in the regeneration and analysis of bone and cartilage. Using cells harvested from sheep or cows, his laboratory has already developed composite tissue-engineered discs composed of regenerated tissue that have formed the key disc structures of nucleus pulposus and surrounding annulus fibrosus. Dr. Bonassar has developed techniques known as tissue injection molding and cell-mediated sintering, which induce cultured cells to produce complex tissue structures such as IVDs. He and his team have succeeded in growing discs from bovine cells and implanting them in nude mice.

Dr. Härtl outlined the many technical challenges of developing a functional human disc from extracted human disc cells. First, there is the issue of growing a suitable disc with an anatomically correct nucleus pulposus and annulus fibrosis, which is Dr. Bonassar’s task. Second, there are surgical concerns, such as choosing the best approach to insert the disc between the vertebrae and minimizing trauma to the patient. Additionally, after the disc is placed, does it have to be stabilized with another device? Because the disc is avascular, how will it obtain the necessary nutrients from the bloodstream? Will simple diffusion be sufficient? In order to answer these and other questions, the first discs will be placed in rats. Dr. Härtl noted that experiments in larger animals, with anatomy more closely resembling that of humans, may be necessary.

Although multiple disc prostheses have been developed and are currently available, they are made of metal alloys and polymers and are subject to wear and fatigue. In some cases, the metal may leave tissue debris that can cause adverse reactions in patients. Additionally, mechanical discs do not fully integrate with patient anatomy. Dr. Härtl explained that one of the problems with the current strategy of disc removal and spinal fusion in patients with severe degenerative disc disease is that the procedure can accelerate the degenerative process above and below the surgical site. Other researchers have attempted to examine alternative ways to produce functional discs.

Chinese researchers have transplanted intact IVDs from human cadavers into living patients with cervical degenerative disc disease and herniated discs with promising outcomes (Lancet 2007;369[9566]:993-999). However, a paucity of donors is a significant practical limitation of this approach. Dr. Härtl noted that this research is headed in the right direction, however, because it focuses on biological rather than on mechanical replacement.

Another approach being attempted in a clinical trial in Germany is the injection of chondrocytes directly into patients with damaged discs, with the hypothesis that the new chondrocytes will produce functional disc material (Biomol Eng 2007;24[1]:5-21). One limitation of this approach, according to Dr. Härtl, is that many patients have severely damaged discs with bony osteophytes and significant collapse that are unlikely to respond to this type of repair.

Dr. Härtl works with the assistance of Andre Tomasino, MD, and Harry Gebhard, MD, 2 neurosurgical fellows who helped develop techniques for harvesting cells from the nucleus pulposus and annulus fibrosus. Another collaborator is Matthew Cunningham, MD, PhD, an orthopedic surgeon at the Hospital for Special Surgery, an affiliate of the NewYork-Presbyterian Healthcare System and Weill Cornell Medical College.

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which is important in 2 surgical services where the philosophy and principles are a little different.”

When operating with Dr. Schwartz, Dr. Anand’s primary role is to direct the surgical approach through the sinuses and nasal cavity to expose the tumor, which Dr. Schwartz then removes with Dr. Anand’s assistance. The last phase of the operation is reconstruction of the skull base, which is also critical to prevent a CSF leak. The team has developed novel closure techniques, including the “gasket seal,” which includes fat and fascia lata from the thigh, a piece of the vomer or high-density porous polyethylene (Porex), and a synthetic sealant such as DuraSeal (Neurosurgery 2008;62[suppl 2]:ONSE342-ONSE345). The team has also developed techniques for testing the integrity of the seal with fluorescein dye. Using these innovative CSF sealing techniques, the team has reduced its postoperative CSF leak rate to approximately 6%, far below the national average.

Figure 1. Magnetic resonance imaging depicts 50-year-old patient with giant brain tumor.

Figure 2. Endoscopic views of the surgical site demonstrating the (a) endoscopic tumor resection, (b) presence of cerebrospinal fluid leak in normal light, (c) blue light, and (d) with amber blocking filter.

Drs. Schwartz and Anand, who perform about 50 to 60 cases of endoscopic neurosurgery each year, have recently published a textbook on surgery of the skull base (Anand VJ, Schwartz TH. Practical Endoscopic Skull Base Surgery. San Diego, CA: Plural Publishing; 2007), and teach courses on dissection and protection at Weill Cornell Medical College and nationally.

They recently returned from the Third World Congress for Endoscopic Surgery of the Brain, Skull Base, and Spine, where they presented results from the NewYork-Presbyterian Hospital experience with minimally invasive endoscopic neurosurgery.

Based on their database of 225 consecutive endonasal endoscopic operations, Drs. Schwartz and Anand recently devised a classification of approaches to endoscopic cranial base surgery; this highlights the importance of understanding the 4 nasal corridors for entry and 9 approaches to the cranial base, and identifies 12 amenable intracranial targets (Neurosurgery 2008;62[5]:991-1002). In their experience, the most commonly encountered types of pathology are pituitary tumors (50%), meningioma/encephalocele (14%), craniopharyngioma and Rathke cleft cyst (10%), meningocele (8%), chordoma (5%), esthesioneuroblastoma (2%), and other (11%). The approach can even be used to access the top of the cranial spine and decompress the spinal cord in patients with odontoid fractures and congenital abnormalities of C2 (J Neurosurg Spine 2008;8[4]:376-380).

Dr. Schwartz described a recent breakthrough in endoscopic surgery. “One of the limitations of endoscopic surgery has been that endoscopes are 2-dimensional, so the image is flat because it’s seen by only 1 eye. We now have the ability to do endoscopic surgery using a special computer chip that’s been designed like an insect eye to produce 3-dimensional images, which have to be viewed on a special monitor.”

Dr. Schwartz concluded, “Minimally invasive endoscopic surgery is the state of the art. We plan to push the frontiers even farther and maximize what we can do.”

Contributing faculty for this article: Vijay K. Anand, MD, and Ted Schwartz, MD.
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Dr. Cunningham helps with the rat model. In addition, Robert Bowles, a second-year doctoral student in Biomedical Engineering, assists Dr. Bonassar in assessing the viability of cells grown in culture. The composite cells may take as long as 8 weeks to grow.

Funding for this research project has come in the form of a seed grant from Cornell University as well as from the Leonard and Fleur Harlan Clinical Scholarship in Neurological Surgery that Dr. Härnl received.

Dr. Härnl concluded, “It’s very exciting for me to work with the biomedical engineers. They are very smart people, and they have a lot of ideas that we can use to treat our patients.”

Contributing faculty for this article: Roger Härnl, MD