

Nanotechnology Tackles Brain Cancer

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Brain cancer can be counted among the most deadly and intractable diseases. Often diagnosed after a patient exhibits symptoms such as nausea, dizziness, uncharacteristic behavior changes, or paralysis, the growing mass of a brain tumor will continue to squeeze out normal tissue and degrade the brain's function if left untreated. But treatment is elusive. Tumors may be embedded in regions of the brain that are critical to orchestrating the body's vital functions, while they shed cells to invade other parts of the brain, forming more tumors too small to detect using conventional imaging techniques. Brain cancer's location and ability to spread quickly makes treatment with surgery or radiation like fighting an enemy hiding out among minefields and caves, and explains why the term "brain cancer" is all too often associated with the word "inoperable." Making treatment even more challenging, there is a system of blood vessels and protective cells in the brain — the blood-brain barrier — that admits only essential nutrients and oxygen, and keeps out everything else, including about 95 percent of all drugs. This natural barrier puts serious limits on how much a patient can benefit from traditional chemotherapy and new cancer drugs.

In an ideal situation, we would have a "smart" drug that could cross the blood-brain barrier, zero in on the cancer cells, mark their location clearly for surgery, or destroy them with such precision that it would leave surrounding, normal brain cells intact. Until now, such a scenario seemed so far-fetched. But using nanotechnology, NCI-supported researchers at the University of Michigan, the University of Washington, the University of Texas M. D. Anderson Cancer Center, Virginia Polytechnic Institute, and Virginia Commonwealth University are creating ultrasmall particles that can target and destroy cancer cells in the brain, even those in tumors too small to be removed surgically.

Getting Into the Brain — "Ticket" Required

Among the properties of nanoparticles that make them ideal candidates for recognizing and treating brain cancer, their ability to deliver a wide variety of payloads across the blood-brain barrier is perhaps the most important. Understanding how some nanoparticles achieve this special "permission" to enter the brain requires a closer look at how the blood-brain barrier works.

The blood-brain barrier permits the exchange of essential nutrients and gases between the bloodstream and the brain, while blocking larger entities such as microbes, immune cells and most drugs from entering. This barrier system is a perfectly logical arrangement, since the brain is the most sensitive and complex organ in the human body and it would not make sense for it to become the battleground of infection and immune response. This biological "demilitarization zone" is enforced by an elaborate and dense network of capillary vessels that feeds the brain and removes waste products. Each capillary vessel is bound by a single layer of endothelial cells, connected by "tight junctions," thereby making it very difficult for most molecules to exit the

capillaries and permeate into the brain. Outside of the central nervous system, capillaries have fenestra (the latin for “window”), which are the cracks between the cells in the vessel wall. Both small and large molecules and even cells can leave the capillary and enter into the surrounding tissue. Instead of “leaking” material, brain capillary walls closely regulate the flow of material using molecular pumps and receptors that recognize and transport nutrients such as glucose, nucleosides, and specific proteins into the brain. In other words, substances need to be pre-recognized to enter.

So what allows some nanoparticles to get into the brain? Nanoparticles that successfully cross the barrier are often coated with polyethylene glycol (PEG), polysorbate, or other polymer or surfactant (a detergent-like substance). The exact mechanism of nanoparticle transport into the brain is not fully understood, but it is thought to depend on the particle’s size, material composition, and structure. In some cases, it appears that a specialized coating of polymer or surfactant allows nanoparticles to mimic molecules that would normally be transported into the brain. For example, polysorbate-coated nanoparticles are thought to mimic low-density lipoproteins (LDL), allowing them to be transported across the capillary wall and into the brain by hitching a ride on the LDL receptor.

In another example, nanoparticles were “decorated” with opioid peptides, short pieces of protein that act as natural painkillers. The opioid peptides bind to specific receptors on the capillary walls, which help carry the nanoparticles into the brain. In other cases, no special tricks are needed: larger tumors can disrupt the local vasculature, creating leaky vessels through which nanoparticles and other molecules can easily penetrate. Once inside the brain, a nanoparticle can deliver a wide variety of payloads to detect and treat cancer.

Throwing PEBBLES at Brain Cancer

When the team of Raoul Kopelman, Ph.D., at the University of Michigan, thought of a tool to diagnose and treat the most virulent forms of brain cancer, they thought of pebbles. That is, 20 to 200 nanometer diameter nanoparticles they dubbed Probes Encapsulated by Biologically Localized Embedding (PEBBLEs).³

Kopelman designed the PEBBLEs to carry a variety of agents on their surface, each with a unique function. Therein lies another major potential advantage of using nanoparticles to treat cancer: multifunctionality. One target molecule immobilized on the surface could guide the PEBBLE to a tumor. Another agent could be used to help visualize the target using magnetic resonance imaging (MRI), while a third agent attached to the PEBBLE could deliver a destructive dose of drug or toxin to nearby cancer cells. All three functions can be combined in a single tiny polymer sphere to make a potent weapon against cancer. Kopelman introduced the common MRI contrast element — gadolinium — to the PEBBLEs. When injected into the bloodstream, the nanoparticles wend their way through the bloodstream. But because they can transverse the blood-brain barrier, and because they have a targeting agent attached, the PEBBLEs accumulate in the brain tumor — enabling a clear MRI image within just a few hours.

The next functional step is a remarkable feat of nano-engineering. Each PEBBLE carries a photocatalyst . When stimulated by a light source through a micrometer-sized fiber-optic probe inserted into the skull, the photocatalyst converts oxygen into a so-called singlet state, which effectively “bleaches” and destroys nearby cells. The PEBBLEs are inert and harmless until the light is turned on. Used in combination with MRI imaging, one could now kill cancer cells at will, while tracking the effectiveness of the treatment with imaging. The targeted treatments

using nanoparticles may offer a number of advantages over traditional chemotherapy. In chemotherapy, the drugs permeate cells throughout the body to damage their DNA and prevent rapid growth, and are only moderately more toxic to cancer cells over normal cells. That is why patients suffer so many side effects of chemotherapy including nausea, hair loss, and anemia. In contrast, PEBBLEs are highly localized to the cancer target, and do very little damage to surrounding healthy tissue.

PEBBLEs and other nanoparticle drugs could also avoid another serious problem occurring in traditional chemotherapy — for some cancers, over 50 percent of patients become non-responsive to treatment due to the development of multi-drug resistance (MDR). MDR occurs when cancer cells mutate and begin to pump the chemotherapy drugs back out before they can destroy the cell. The cancer becomes “immune” to the drug. But PEBBLEs act on the outside of the cell, and the toxic payload of oxygen that they deliver acts quickly, without giving the cancer much chance to survive and develop resistance. In rat models of a type of brain cancer called 9L-gliosarcoma, PEBBLE-based treatment can significantly increase survival time. Patients with this particularly aggressive form of cancer rarely live more than four months after diagnosis without treatment. For rats, the clock runs out in about five days. When rats were treated with PEBBLEs targeted to 9L-gliosarcoma tumors, some were still thriving after two months, and an MRI image of their brain revealed that the tumors had been eliminated.

Kopelman and collaborators (including Martin Philbert, Ph.D., at the University of Michigan School of Public Health), hope ultimately to prove the utility and safety of this approach to treating brain cancer in humans.

The Illuminated Brain

Unfortunately, the most common form of primary brain cancer, glioblastoma, is also the most aggressive and lethal. Glioblastoma tumors emerge rapidly and spread throughout the brain. About 20,000 Americans are diagnosed each year, and more than half of those patients will die within 18 months. Surgery is limited in its effectiveness because it is difficult to differentiate visually between cancerous and normal brain tissue, and any cancer cells left behind are likely to proliferate and form new tumors. In order to improve the odds of eliminating all the cancer during surgery and avoid removing healthy brain tissue, researchers have devised a number of fluorophores, or “glowing” molecules that mark the tumor boundaries for removal.

But the fluorescent probes are difficult to locate and use within the brain during surgery. A multidisciplinary team at the **University of Washington** and the Fred Hutchinson Cancer Research Center in Seattle, wanted to see if surgical outcomes could be improved by a single probe that accurately marks the location of a tumor in pre-operative MRI scans, while guiding the surgeon to those same locations in the exposed brain. To materials scientist **Miqin Zhang**, Ph.D., radiologist Raymond Sze, M.D., and oncologist Jim Olson, M.D., nanotechnology was the perfect solution for creating such a multifunctional probe.⁴ Starting with a 10 nanometer diameter iron oxide core that serves as an MRI contrast material, **Zhang** and colleagues coated the nanoparticles with polyethylene glycol and modified them with a fluorescent molecule called Cy5.5. Cy5.5 gives off light at near-infrared wavelengths, which — unlike visible light — can penetrate several centimeters through brain tissue.

In order to selectively light up glioma tumors through imaging, a targeting agent had to be attached. **Zhang** and colleagues selected chlorotoxin, a peptide derived from the venom of the giant Israeli scorpion, which binds specifically to a tumor surface marker found in the vast

majority of gliomas. At 15 nanometers, the final particle size and composition gave it the best chance for crossing the blood-brain barrier, and homing in on its target. Tested on cultured cells, the nanoparticles performed remarkably well. Studies clearly demonstrated uptake by glioma cells, but not healthy brain cells, and the nanoprobe was readily detectable by both MRI and near-infrared fluorescence. More work remains to be done in animal studies, but **Zhang** and coworkers are driven by a vision that some day a surgeon will be able to use MRI to map out a surgical plan, and then have a visual guide for that plan in real time, while operating on the brain.

According to Chun Li, Ph.D., at the University of Texas M. D. Anderson Cancer Center, and Eastman Kodak, near-infrared emitting nanoparticles could be a valuable tool for outlining the margins of a tumor, and helping the surgeon to avoid cutting into healthy tissue. Better cameras and detection methods will be needed before these nanoparticles are used in a real operation, but a photographic technology expert such as Eastman Kodak seems ideal to address these challenges.

Kodak's engineering and chemistry teams are backed by a long history of expertise in dye chemistry. The company is supplying high-quality near-infrared emitting nanoparticles with well-controlled properties. Li's multidisciplinary approach, pooling the expertise of engineers, chemists, and biologists, is a good example of the multidisciplinary research collaborations needed to move nanotechnology research forward in the area of cancer.

Buckyballs Pack Heat

In the 1970s, a new variety of carbon was discovered that formed hollow nanometer-scale cage-like spheres. They were called buckminsterfullerenes or buckyballs, after the architect who built domed structures of a similar shape, and were hypothesized to have great potential for their ability to carry atoms and molecules with useful properties. Then in 1999, Virginia Tech chemists Harry Dorn, Ph.D. and Harry Gibson, Ph.D. created the first buckyballs encapsulating rare earth metals, the kind that can easily be recognized as a contrast agent for MRI. The attachment of targeting agents to the outside of the carbon cage allowed the buckyballs to accumulate in tumors.

Now, Panos Fatouros, Ph.D., and neurosurgeon William Broaddus, M.D., Ph.D., both at Virginia Commonwealth University, are collaborating with Dorn and Gibson on a project using buckyballs to improve the ability of MRIs to locate brain tumors, and deliver a payload of radiation to destroy them. Experiments on rats have shown that buckyballs packed with the MRI contrast metal gadolinium can increase the sensitivity of MRI detection by at least 40-fold. This level of precision is reaching a point at which cancer cells that have spread beyond the margins of the tumor may become visible. Stray cells, left behind after surgery, are thought to be responsible for tumor relapse. Finding and removing these cells could improve a patient's chance of survival.

Fatouros and colleagues have created a modified version of the buckyballs with a fluorescent metal atom called terbium. A glowing buckyball could guide surgeons to remove tumors with greater precision. Addition of yet another metal, lutetium, would deliver a lethal dose of radiation to the cancer cells, including those missed by the surgeon. The research is about three to five years away from testing in humans, but the possibilities seem remarkable.

Detect, Treat, Track

There is a growing consensus that brain cancer is a problem in need of a radically different solution, and that nanotechnology fits the bill. Functionalized nanoparticles could provide precision detection, targeted treatment, and real-time tracking that conventional technology lacks. For a disease in which only 5 percent to 32 percent of patients are likely to survive after five years, large hope is riding on the potential success of “small” technology.

Brain Cancer Facts 5-6-7

Brain cancer is a complex disease, classified into 120 different types. So-called non-malignant (benign) brain tumors can be just as life-threatening as malignant tumors, as they squeeze out normal brain tissue and disrupt function. The glioma family of tumors comprise 44.4% of all brain tumors. Glioblastoma is the most common glioma at 51.9%, followed by astrocytoma at 21.6%.

Brain tumors are the leading cause cancer death in children under the age of 20. They are the second leading cause of cancer death among 20-29 year old males. Metastatic brain tumors result from cancer that spreads from other parts of the body into the brain. About 10-15% of people with cancer will eventually develop metastatic brain tumors. The five-year survival rate following diagnosis of a primary malignant brain tumor is about 32.7%. The five-year survival rate for some forms of cancer, such as glioblastoma, can be as low as 5%.

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