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New Hope for Brain Cancer Therapy

Lynn Yarris, lcyarris@lbl.gov

Glioblastoma multiforme, the most common of malignant brain tumors in adults, is one of the deadliest of all forms of cancer. Striking some 18,000 new victims in the United States every year, the disease is always fatal, usually within six months of onset. Surgery and conventional radiation therapies may prolong life for up to a year, but cannot stop the tumors from continuing to spread throughout the brain.

Some anticancer drugs show promise against brain tumors, but getting drugs past the blood-brain barrier is a major challenge. Now a collaboration of researchers from Lawrence Berkeley National Laboratory and the Children's Hospital of Oakland Research Institute (CHORI) have demonstrated the potential for nanosized synthetic particles of low density lipoprotein, or LDL, to deliver anticancer drugs to glioblastoma multiforme tumors safely and effectively.

Trudy Forte (right) and Mina Nikanjam led the study showing that nanosize particles of synthetic low density lipoprotein selectively bind to tumor cells of a deadly brain cancer.

"We have identified LDL receptors on glioblastoma multiforme tumor cells that can serve as specific molecular targets," says lipoprotein research specialist Trudy Forte.

LDL receptors are sparse in normal brain tissue but elevated in tumor cells; the hope is that the synthetic nano-LDLs (nLDLs) can deliver drugs to glioblastoma multiforme tumors while sparing healthy cells. Forte, who has joint appointments in Berkeley Lab's Life Sciences Division and CHORI, led the study with colleagues Mina Nikanjam, Eleanor Blakely, Kathleen Bjornstad, Xiao Shu, and Thomas Budinger.

Glioblastoma multiforme is a cancer of the glial cells, which support the neurons and make up about 90 percent of the cells in the brain. As the term multiforme suggests, these cells can take on a wide variety of shapes, making detection difficult until tumors become large.

Tendrils of malignant cells can extend into healthy brain tissue. If removal or destruction of the main tumor mass leaves tendrils intact, like the mythical Hydra the tendrils will sprout new tumors.

One solution would be to follow surgery with anticancer drugs. However, drugs infused in the blood encounter the blood-brain barrier, a tightly knit membrane of cells at the boundary between the central nervous system and the rest of the body that protects the brain from harmful substances in the blood. Because it also blocks anticancer drugs, researchers have long sought a means of circumventing the blood-brain barrier.

Previously Forte and coauthor Blakely were part of a team that characterized a tumor-seeking compound known as boronated (proto)-porphyrin, or BOPP, known to concentrate in glioblastoma multiforme tumors. Using the specialized equipment at Berkeley Lab's Life Sciences Microscope Resource, they identified the chemical sites where BOPP binds to glioblastoma multiforme tumors. These binding sites turned out to have the same receptors that take low density lipoproteins into the cell.

"Tumor cells generally have high cholesterol requirements as they are rapidly dividing, and LDLs are the major transporters of cholesterol in the plasma," explains Forte.

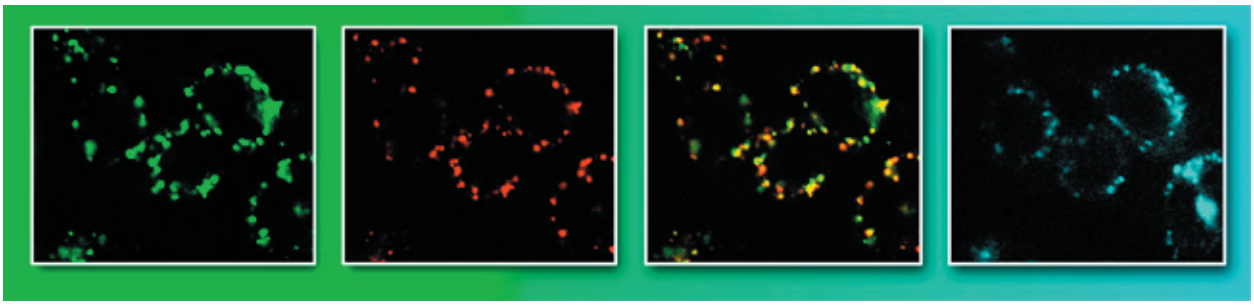
Using seven lines of human glioblastoma multiforme cells, Forte and Blakeley and their collaborators found them to harbor anywhere from 125,000 to 950,000 LDL receptors (LDLRs) per cell. Because previous studies with monkeys and rats indicated that normal brain tissue, particularly neurons, harbor few LDLRs, these receptors are an inviting target for drug delivery. Natural LDLs are variable in size and composition, however, and difficult to isolate in quantity.

Forte and Mina Nikanjam, a guest researcher at Berkeley Lab, a member of Forte's research group at CHORI, and a doctoral student in UC Berkeley's Department of Bioengineering, synthesized a peptide featuring two functional regions, one that binds to LDLRs and one that binds to the fatty, water-insoluble molecules known as lipids. This synthetic bifunctional peptide was combined with a lipid emulsion to produce particles measuring 10 nanometers in diameter, about half the size of natural LDLs.

"Using a fluorescent dye as a model drug, we were able to confirm that in cell cultures the synthetic nLDLs efficiently bind to the surface of glioblastoma multiforme cells," says Forte.

Says Nikanjam, "We settled on the method of creating our synthetic nLDLs very quickly and stuck with it throughout."

Synthetic nLDLs could serve as drug delivery vehicles because once they have bound to a malignant cell's receptors they are taken into the cell by endosomes, membrane-bound compartments inside the cells. There, the receptors would be dissociated from the nLDLs and recycled back to the cell surface, while the nLDLs will be shuttled into the lysosome for digestion—along with their anticancer drug charge.



Confocal microscopy indicates that fluorescently labeled nLDL particles are taken into the cell by LDL receptors and are found together in the cell's lysosomes, sac-like organelles which contain enzymes that can break down and destroy cellular components. Images on the left show peptides (green) and lipids (red), components of the nano-LDLs. When these images are merged (third from left), the yellow/orange color indicates that the peptides and lipids are in the same places in the cell. The final image (right) reveals that the sites where the nLDL peptides and lipids are localized are in the lysosomes, here outlined in blue.

Even at 10 nanometers the synthetic nLDLs are still too large to cross the blood-brain barrier. But they could be carried to the cancerous cells via a technique called "convection-enhanced delivery," in which one or more catheters are implanted into the brain and pressure is used to infuse particles into a target area.

"The catheters can be put into place after the surgical removal of the main glioblastoma multiforme tumor mass," says Forte. "We could then use convection-enhanced delivery to send in the nLDLs with their charge of anticancer drug and destroy any remaining cell islets."

Can synthetic nLDLs carry a big enough drug payload to kill glioblastoma multiforme cells? Preliminary studies in vitro are promising and show that a modified form of the anticancer drug taxol can be transported into cultured glioblastoma multiforme cells by nLDL and can kill them.

Although Forte and her colleagues are focusing on glioblastoma multiforme, their nLDLs should also be applicable as drug delivery vehicles for other forms of cancer with elevated numbers of LDLRs.

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