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Innovations

Imaging Cancer's Blood Supply

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In the earliest stages of angiogenesis, vascular endothelial cells respond to chemical signals and start to proliferate. Before long, the cells send the integrin $\alpha v\beta 3$ and the platelet endothelial cell adhesion molecule CD31/PECAM onto their outer membranes like tiny flags. Even though the endothelial cells have not become polarized (oriented in a certain direction), the $\alpha v\beta 3$ molecules are expressed within what will eventually become the lumen of the vessel and the outside of the vessel wall. Researchers aiming to trace the origins of angiogenesis for heart disease or cancer start in the same place because each of these diseases depends on the same blood vessel formation process. Plaques and tumors need a new blood supply to grow and survive. Detecting and even quantifying the earliest expression of the cascade of molecules during angiogenesis requires probes or contrast agents specific enough to detect surface molecules that signal vessel formation.

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Such detection requires probes that can home in on these molecules and imaging methodology sensitive enough to locate the probes, allowing researchers to watch changes in vessel formation over time and illuminate the relationship between angiogenesis and cancer and the level of malignancy and metastasis. Those same probes that can glom onto a surface molecule in the earliest stages of angiogenesis can in turn deliver doses of chemotherapy that could halt angiogenesis and block a tumor's blood and thus its energy supply. Because these probes play a dual role as therapeutics and diagnostics, they have been dubbed "theranostics."

Thanks to a flurry of research on a variety of probes and imaging methods, researchers today have probes in the preclinical stage than can indeed lock onto molecules expressed during angiogenesis and detect the probes down to a spatial sensitivity of around 30–50 μm.

A variety of imaging modalities is necessary because each technique has its strengths and weaknesses, says Mario Bourdon, Ph.D. founder and president of BioLaurus Inc., a contract research laboratory in San Diego, California, that specializes in molecular imaging. An assortment of probes in the preclinical stage is being tested via many different imaging technologies.

Start with the Heart

At Kereos Inc., a St. Louis, Missouri, biotech company, researchers are developing and testing a product based on perfluorocarbon nanoparticles that recognize the activated form of $\alpha v\beta 3$ integrin on budding endothelial cells.

The Kereos researchers modified a nanoparticle that was originally tried as a blood substitute. The nanoparticle could circulate in the bloodstream and is biologically inert but didn't deliver oxygen well to tissues. Although the nanoparticle didn't work as a blood substitute, Kereos scientists modified the blood substitute for magnetic resonance imaging, or MRI, by replacing the hydrogen atoms with fluorine.

"We're able to image both the proton images that normal MRIs would see and the fluorine images simultaneously because there's no background fluorine in the body," says Gregory Lanza, M.D., Ph.D., Kereos cofounder and professor of medicine at Washington University School of Medicine in St. Louis. The resulting image shows "hot spots" in the vessels where the fluorine nanoparticles are deposited.

The nanoparticles are currently in a Phase 1 clinical trial aimed at looking at the relationship to carotid artery disease, a condition in which fatty plaques build up inside the carotid artery and raise the risk of stroke. Researchers are looking both for safety and for the ability to see signals from carotids due to homing of the ligand. Lanza says he and colleagues hope that with angiogenesis imaging they can identify the people who have had a plaque hemorrhage and who would most likely go on to have stroke as opposed to those who wouldn't; right now, about 50% of those patients can't be identified well enough. The current study, done in collaboration with Phillips Healthcare, encompasses patients who could have significant carotid disease.

"The beauty of angiogenesis is that is shows exactly what's going on in the wall directly. If you give a drug that is supposed to improve the complication of atherosclerosis from diabetes rather than looking at glucose and trying to guess what happened in nonspecific inflammatory markers, you can use imaging before and after to follow whether atherosclerosis or cancer is progressing, for example, by determining whether angiogenesis is getting greater or is subsiding," says Lanza.

Once the safety of the nanoparticles is well established, Lanza says the team will expand to a cancer angiogenesis and other applications.

Chicks and Eggs

Researchers at Innovascreen, Inc., in Halifax, Nova Scotia, have "gone to the birds" by fine-tuning an avian model system that has been around for a long time. For thousands of years, people cracked open eggs to learn how living systems are put together; Innovascreen researchers are doing much the same by studying the chicken chorioallantoic membrane (CAM). This membrane supplies the developing chick with oxygen via gas exchange across the eggshell. Because the CAM has many blood vessels and sits against the eggshell, it is a great

model for angiogenesis because it is a self-sustained living organism that allows researchers to study blood vessel formation, says Andries Zijlstra, Ph.D., Chief Scientific Officer, Innovascreen.

By growing the CAM as a free-floating organ in a culture dish, researchers have a surface area of about 10 cm^2 available for bioassays. This amount of surface area comes in handy during the screening steps of Innovascreen's assay. First, the team transplants human tumors into the CAM. By placing the culture dish on the stage of a microscope hooked up for intravital microscopy, researchers can watch cellular movement and blood vessel formation in real time. The CAM model also allows the team to deliver antitumor drugs via nanoparticles. John Lewis, Ph.D., and the company's chief executive officer, developed an assay to assess how deep the nanoparticles penetrate into the tumor. "This is a robust in vivo system that we've transformed into a higher throughput model. Angiogenesis isn't static, it's a dynamic process, and we can capture those dynamics and simultaneously watch what a drug is doing," says Lewis.

Such an assay allows Innovascreen's team access to high-resolution imaging at the microscopic level. Not only does the CAM model support the growth of human tumor cells, it's less expensive than using mice and surgery and anesthesia are unnecessary, says Lewis, and the team can monitor the assays for up to three days and then quantify the results.

Tiny Bubbles

An emerging imaging technology being used for angiogenesis is gas-encapsulated microspheres that work as contrast agents with ultrasound technology. Sonograms (or ultrasounds) work by shooting sound waves into the body toward a tumor, or a baby, or a beating heart. The resulting image is captured as the ultrasound machines detect the sound waves as they bounce back. Traditional ultrasound is not sensitive enough to detect molecular events such as angiogenesis, which happen on a very small scale. Gas microbubbles boost this echo by providing a contrast that is many times brighter than anything else in the body, explains Joshua (Jack) Rychak, Ph.D., and vice president for research of Targeson, a San Diego-based biotechnology company.

Ligands specially attached to the microbubbles work as contrast agents by targeting angiogenic endothelium in tumors and can thus be used as both molecular imaging probes and a way to deliver drugs. The data that emerge from these studies is in real time and on a molecular scale. Another advantage of this technique is that ultrasound machines are portable and low cost compared to MRIs or CT scans, says Rychak.

Targeson has developed two angiogenesis-targeted agents. One agent seeks out integrins and the other, the company's lead technology, targets vascular endothelial growth factor receptor (VEGFR2). Thus far, both targets have been validated in mice and are commercially available for research use.

Here's how the microbubbles work: Rychak injects a dispersion containing the bubbles into the tail vein of a mouse. The target is not actually the tumor. Microbubbles are coated with a ligand that binds to receptors on the newly forming blood vessels, which is a hallmark of a growing tumor, and provides a way to differentiate the tumor from healthy surrounding tissue. The team coated microbubbles with an integrin-binding ligand (an RGD pentapeptide) and found that mouse tumors took up the bubbles at a high, specific rate (Anderson et al., 2011). "This was definitely a happy surprise for us," Rychak says, still amazed with the results because RGD is a common motif in the body, and he cautions that the high binding could just be happening in mice.

Rychak's team has recently completed a study in companion dogs designed to detect spontaneous prostate cancer. "We've already confirmed that when we implant a tumor in a mouse or rat, we can image it with our agents," says Rychak. "It's easy when you already know where the tumor is. The real challenge for us now is proving that we can find a spontaneously occurring cancer." The canine study looked at naturally developing prostate cancer and evaluated whether the agents can differentiate prostate cancer from benign disease. Although the data are still being prepared for publication, Rychak says his team was able to successfully detect naturally occurring prostate cancer at relatively early stages of development in dogs.

For now, researchers are using the microbubbles for both basic science studies and in the drug discovery process. Many questions still need to be answered on the kinetics and biomechanics of adhesion of the microbubbles to the receptors. Because Targeson is putting their agents out into the market to make the technology available to other researchers, "a fairly unique commercial strategy," says Rychak, that may help answer some of these questions and explain the progression of the technology.

"One company and one lab won't be able to answer all of these questions. So if we can get the microbubble out into the field and get others using it in a meaningful way, then we're contributing to the overall field of knowledge," says Rychak.

REFERENCE

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