Targeting Vessel Abnormalization in Cancer

By Ken Garber

The role of angiogenesis in cancer is now so well accepted that it’s easy to forget how young the field is. New discoveries still have the power to change how people think about blood vessels and cancer. The latest example: delta-like ligand 4 (DLL4).

DLL4 is generating intense interest because it offers a new way to use angiogenesis, or new blood vessel formation, against tumors. At least three biotech companies are now racing each other to be first in the clinic with a DLL4-blocking drug, a milestone that will probably come sometime next year. Activity “has really heated up,” said Jan Kitajewski, Ph.D., a cancer researcher at Columbia University in New York.

One reason for the buzz is DLL4’s link to vascular endothelial growth factor (VEGF). VEGF is thought to be the dominant growth factor driving angiogenesis in cancer; bevacizumab (Avastin), Genentech’s FDA-approved colorectal and lung cancer drug, targets VEGF. VEGF induces expression of DLL4, which appears equally crucial for blood vessel creation and thus tumor survival. Yet DLL4 acts on tumor blood vessels in a completely different way from that of VEGF, thus promising a powerful alternative—or complement—to drugs like bevacizumab. Put simply, blocking DLL4 seems to entangle tumors in a chaotic snarl of newly formed but useless blood vessels, starving the tumors.

First identified and cloned in 2000, DLL4 binds to Notch, a receptor family that plays a key role in cell fate decisions in the developing embryo. In 2001 DLL4 was shown to be preferentially expressed in tumor blood vessels—the first DLL4 cancer connection. Knockout mouse experiments in 2004 showed that mice missing only one copy of the DLL4 gene died before birth—an amazing result, because for other genes a second copy almost always compensates and rescues the embryo. The only other gene known with such single-copy lethality is VEGF. The discovery of such a sensitive target, and one with VEGF-like potential, “ignited a frenzied interest,” University of Toronto angiogenesis researcher Robert Kerbel, Ph.D., wrote in an e-mail.

But these researchers got a surprise when they tried blocking DLL4 in animal cancer models. Instead of fewer blood vessels, they got more. A web of new blood vessels sprouted and spread in the absence of DLL4. Then came a second surprise: Despite the high level of new angiogenesis, these tumors stopped growing, while untreated control tumors continued expanding. “Smaller tumors but more vessels—we were perplexed,” Regeneron researcher Irene Noguera-Troise, Ph.D., said in April at the annual meeting of the American Association for Cancer Research (AACR).

Hypoxia experiments revealed that the DLL4-negative tumors were getting less oxygen than control tumors, suggesting that the new blood vessels were nonfunctional. Such vessels “can’t seem to deliver nutrients and oxygen to the tumors very well, and thus you’re blunting tumor growth,” explained George Yancopoulos, M.D., Ph.D., Regeneron’s chief scientific officer. DLL4 blockers create more vessels but nonfunctional ones, which “end up choking the tumor,” Yancopoulos said. “It’s quite remarkable.”

Researchers have half-jokingly dubbed this phenomenon “vessel abnormalization,” a reference to the vessel normalization hypothesis of Harvard cancer researcher Rakesh Jain, Ph.D. His theory holds that VEGF blockers shrink tumors by normalizing some nonfunctional blood vessels near the tumor, allowing chemotherapy drugs to better reach their target. For DLL4, researchers are hoping that the opposite is true: that anti-DLL4 drugs will unleash a burst of abnormal blood vessel growth around the tumor, starving it of oxygen, and perhaps making chemotherapy drugs even more effective by presenting them a target rich in dividing cells.

Bad Vessels Grow, Tumors Slow?

DLL4 and VEGF are the yin and yang of angiogenesis. Recent studies in mouse eyes and in zebrafish show that DLL4’s normal function is to regulate angiogenesis indirectly, by keeping VEGF expression in check to ensure that vessel development is orderly. VEGF induces DLL4, which restrains VEGF—a negative feedback loop. VEGF “is inducing its own negative regulator to make sure that the processes that it induces don’t go out of control,” Yancopoulos explained.
Until DLL4, it wasn’t clear why VEGF signaling didn’t induce new vessels sprouting everywhere on the blood vessel wall, which would destroy it. “To get a nice mature vascular network, you have to make sure that you just have single sprouts and then some spacing,” explained Judah Folkman, M.D., director of the vascular biology program at Children’s Hospital, Boston. DLL4 appears to be the spacing mechanism. Block DLL4, and VEGF runs wild, promoting sprouting everywhere, but forming abnormal blood vessels that blood can’t fill—a new antitumor mechanism.

The surprises didn’t end there. Regeneron and Genentech researchers found that DLL4 blockers worked in tumor models resistant to VEGF inhibitors. Adding VEGF inhibitors to DLL4 blockers in such models worked even better. “They work in combination even when one or the other isn’t effective,” Yancopoulos said. Such an additive effect is counterintuitive—blocking both the tumor-promoting growth factor (VEGF) and its natural inhibitor (DLL4) should blunt the antitumor effect, not strengthen it. Yancopoulos speculated that while a DLL4 blockade generates a useless network of blood vessels, a few functional vessels do get formed, giving the tumor some access to nutrients. Meanwhile VEGF production revs up in the absence of DLL4. “So you come in with the VEGF blocker, and now that’s blocking the small amounts of productive vasculature that you have in there as well,” Yancopoulos said. (Bevacizumab, for example, has been shown to directly block blood flow to tumors.) The result: a doubly starved tumor.

New angiogenesis inhibitors are badly needed. While bevacizumab, in combination with chemotherapy, extends survival in colorectal cancer patients by a median of 4.7 months, fewer than half of patients respond to the treatment, and their tumors eventually become resistant to the drug. And bevacizumab has not worked well in several other cancers. “It will be very interesting to evaluate whether anti-DLL4 therapy will work in instances where anti-VEGF therapy has failed in the clinic,” such as in pancreatic cancer, Kerbel wrote.

In theory, since DLL4 expression is limited mostly to newly forming blood vessels, DLL4 blockade therapy should be relatively nontoxic. In animal models, “We’re looking very hard [for toxicity],” Yancopoulos said. “We haven’t seen anything yet.” Inhibitors of Notch signaling used in clinical trials for Alzheimer disease have caused serious side effects, like massive diarrhea, because Notch affects gut renewal. But because DLL4 inhibitors should block only the Notch signaling that takes place in newly forming blood vessels—mainly in the growing tumor—side effects should be much more limited.

**Rethinking Angiogenesis**

There are some concerns about inducing a DLL4 blockade. For one, surrounding the tumor with nonfunctional blood vessels could keep chemotherapy drugs from reaching tumor cells. “That’s a real question,” said Yancopoulos, who nevertheless expects that some functional vessels around the tumor will form to deliver chemotherapy to the target. Genentech researcher Minhong Yan, Ph.D., reported at AACR that DLL4 blockers did not appear to interfere with chemotherapy. “We actually saw an additive effect, at least in the model we’ve used so far,” Yan said, although he did not present data. Kerbel speculated that DLL4 blockers could make tumor cells more vulnerable to chemotherapy and radiation by increasing the number of rapidly dividing endothelial cells in the expanding vascular network—provided that the drugs can reach the tumor.

Another concern is that some of the dysfunctional vessels created by a DLL4 blockade could remodel themselves and become functional after treatment ends, giving the tumor access to a blood supply and promoting new tumor growth. A similar “rebound” phenomenon is seen in colorectal cancer patients when doctors end bevacizumab treatment, Folkman said. “When they stop Avastin, the tumor explodes and kills the patient,” he pointed out. For a DLL4 blockade, “tumors may not be well-nourished, but they’re rich in endothelial cells,” Kitajewski said. “If that endothelium can reform a good vasculature after release of blockade, then that might be a concern.” Kerbel pointed out that VEGF expression also rises with a DLL4 blockade and the subsequent hypoxia. If accompanied by maturing blood vessels, he said, “this could be a recipe for a ‘perfect storm.””

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Greg Plowman, M.D., Ph.D., senior director of tumor biology and angiogenesis at Genentech, has seen no evidence in animals of the nonfunctional blood vessels normalizing and tumors returning after treatment stops. “We really don’t see this massive [tumor] rebound,” Plowman said. But only human trials will ultimately put this concern to rest.

Biological complexity is another confounding factor. DLL4, expressed on the surface of endothelial cells, presumably engages Notch receptors on nearby vascular endothelial cells and triggers Notch signaling. Notch signaling in blood vessels typically has many effects, including promoting endothelial cell survival, yet a DLL4 blockade seems to have a specific outcome: the chaotic vessel overgrowth seen in mouse models. The identity of DLL4’s receptors remains controversial, and DLL4 may or may not trigger a specific kind of Notch signaling. Also at issue is whether the tumors themselves—not just the nearby endothelial cells—express DLL4 and whether this makes them...
more or less vulnerable to anti-DLL4 therapy.

Finally, some direct effect of DLL4 on tumor growth could be taking place, in addition to its indirect vessel-spacing effect. For example, DLL4’s binding to Notch receptors on tumor cells could help maintain a tumor stem cell population, because Notch is crucial for maintaining the stem cell “niche,” or microenvironment. And DLL4–Notch binding could directly stimulate tumor growth, as Notch does in certain leukemias. All these questions await answers.

A DLL4 blockade could treat a wide range of tumor types. Noguera-Troise reported at AACR that Regeneron has seen effects on all 10 tumor lines tested in mice so far. Genentech tried a DLL4 blockade in 13 tumor models and saw tumor inhibition in all 13, according to Plowman. But most tumor lines have yet to be tested, and a DLL4 blockade is not likely to work equally well across all tumor types. “We need more information,” Kerbel said. Of course, only clinical trials can determine whether drugs that block DLL4 will benefit patients.

Human trials are on the way. Yanopoulos expects that Regeneron will have an anti-DLL4 antibody in phase I trials in 2008. Genentech and the Los Angeles biotechnology company Vasgene have active preclinical programs, and other companies are almost certainly involved. “I would presume that there are many,” Plowman said. Amgen scientists, for example, helped clone DLL4 and later collaborated in the first mouse knockout models. (The company would not say whether it has an active DLL4 program.)

At the scientific level, DLL4 has turned upside down the dogma that more angiogenesis always translates to more aggressive tumors. “We’re having to reexamine our whole definition of the process,” Plowman said. Meanwhile, the prospect of a drug that causes tumors to starve themselves in a tangle of useless blood vessels has, at least for now, captivated the angiogenesis field.

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