Down Syndrome Offers Fresh Clues to Angiogenesis

By Karen Rowan

People with Down syndrome, who have an extra copy of chromosome 21, rarely develop solid tumors, and researchers are starting to put together the pieces of this genetic puzzle to learn why.

In a recent study led by Sandra Ryeom, Ph.D., a researcher at Children’s Hospital Boston, scientists identified two genes on chromosome 21, known as DSCR1 and Dyrk1a, that may play crucial roles in inhibiting the growth of new blood vessels. Ryeom’s team found evidence that the single extra copy of DSCR1 present in the cells of people with Down syndrome suppresses angiogenesis—a crucial ingredient in tumor growth.

“This was an absolutely fascinating study,” said Nancy Demore, M.D., a surgical oncologist at the University of North Carolina, who worked in the late Judah Folkman’s lab in the 1990s. Folkman, who founded angiogenesis research, long suspected that one could find clues to angiogenesis by studying Down syndrome, or trisomy 21, said Demore, who was not involved with this study. “He would talk about the fact that kids with Down syndrome had lower rates of solid tumors and hypothesized that this difference could be due to genes on chromosome 21. It’s fascinating to see a paper published in 2009 completely validating his hypothesis from then.”

Antiangiogenesis research is currently booming, but according to Demore, Ryeom’s work differs from the scores of trials under way that are focused mainly on inhibiting vascular endothelial growth factor (VEGF) with monoclonal antibodies. “This is another way to go: to look at the factors that stimulate angiogenesis and try to block multiple angiogenic factors,” she said.

The study revealed that the two genes both encode proteins that disrupt the calcineurin pathway, which is involved in angiogenesis. It also showed that, compared with control subjects, DSCR1 protein levels are increased in the tissues of people with Down syndrome and in Ts65Dn mice—the mouse model of Down syndrome that has three copies of 104 of the 231 genes on human chromosome 21.

Further, the researchers compared the growth of two common tumors, Lewis lung carcinoma and B16F10 melanoma, in the Down syndrome mice and in control subjects. Growth of tumors was suppressed, and the density of microvessels was statistically significantly lower in the Ts65Dn mice than in the diploid control mice. Also, when induced pluripotent stem cells derived from an individual with Down syndrome were injected into immunodeficient mice, the tumors that grew had reduced microvessel densities—less angiogenesis—compared with those grown from induced pluripotent stem cells derived from a person without Down syndrome.

The next step was to find a link between the suppressed angiogenesis and the extra copy of DSCR1. The DSCR1 gene was first identified in 1997 and was implicated in the signaling pathway of VEGF, a key pathway involved in angiogenesis, in 2004. But the gene’s role in angiogenesis was not well understood.

“We took a different approach because not a lot was known about the intracellular pathways that inhibit blood vessel growth,” said Ryeom. VEGF is well studied, but the VEGF signal acts at the cell membrane, and the pathway she was interested in is downstream of the VEGF signal.

To show that extra DSCR1 could inhibit angiogenesis, the researchers created a transgenic mouse with an extra copy of that gene only. In a normal cell, VEGF signaling activates calcineurin, which dephosphorylates a transcription factor known as nuclear factor of activated T cells (NFAT). NFAT then moves into the nucleus and the transcription of genes needed for angiogenesis begins. Ryeom’s team found that NFAT remained in the cytoplasm of cells with the extra DSCR1 gene, indicating that the calcineurin pathway was disrupted. When melanoma and carcinoma tumors were planted in the mice with the extra gene, the number of endothelial cells that line the blood vessels within the tumors was statistically significantly lower than that in control mice—an indication that less angiogenesis had occurred in the transgenic mice. “Most of us have two copies of DSCR1, and a tumor signal can override this. But three copies is enough to block the VEGF signal,” Ryeom said.

On the basis of the data from mice showing that DSCR1 affects the location of NFAT within cells, Ryeom decided to examine the expression of another gene on chromosome 21 and also known to regulate NFAT. She and her team found that increased expression of this gene, Dyrk1a, further reduced angiogenesis in the transgenic mice.

And she believes there are more to be found. “We are really now trying to identify the other genes; we think there are four or five,” she said. Although DSCR1 is critical, the most effective treatments would involve finding all.

Research is increasingly demonstrating that calcineurin is a critical component in mediating angiogenesis. In a report published in June in Cancer Research, Demore

Nancy Demore, M.D.
and her colleagues identified a protein called SFRP2 that also stimulates angiogenesis via the calcineurin pathway. SFRP2 activates calcineurin, just as DSCR1 does, she said. Therefore, blocking VEGF-mediated calcineurin or inhibiting VEGF may not work to suppress angiogenesis. But, “if you instead target calcineurin, you potentially inhibit both VEGF and SFRP2 signaling,” she said, which could have more widespread effects. “Maybe calcineurin is a final common pathway mediating angiogenesis.”

But other scientists say that the DSCR1 protein may be acting via a different pathway. Bharat Aggarwal, Ph.D., professor of experimental therapeutics at the University of Texas M. D. Anderson Cancer Center in Houston, pointed to findings that patients given cyclosporin A, a widely used drug that inhibits calcineurin signaling in T cells and suppresses the immune systems of patients receiving organ transplants, have greater chances of developing cancer than the general population. “The paradox is that the inhibition of calcineurin also increases the cancer risk,” he said.

However, Aggarwal said that Ryeom’s evidence that DSCR1 triploidy is involved in angiogenesis inhibition is convincing. “Both the mouse model [of Down syndrome] and the transgenic mouse show that the gene they’ve identified is the gene that suppresses angiogenesis,” he said. “This is a very, very important study.”

Aggarwal thinks that the gene must be working through a pathway other than the calcineurin pathway or that DSCR1 must have multiple targets within the cell. “The most important question,” he said, is, “Can you still use this to target tumor growth, if cyclosporin is the best agent known?”

But cyclosporin may not be the only agent. Demore said that tacrolimus, a newer immunosuppressant drug, also targets calcineurin and, according to her research, inhibits the growth of angiosarcomas in mice. “[Ryeom and her colleagues] are right in thinking that this pathway is extremely important in angiogenesis,” she said.

In cancer cells, multiple signals and pathways act in concert to cause angiogenesis, Aggarwal said, and therefore, targeting any one pathway will not be effective. No single inhibitor of angiogenesis has done anything because of the bypass pathways, he said, and hitting one target pathway will not be the solution.

Targeting calcineurin only in cancer cells could resolve the problem of targeting calcineurin and causing increased malignancies, suggested Svetomir N. Markovic, M.D., Ph.D., a hematologist and oncologist at the Mayo Clinic in Rochester, Minn. “There is a wealth of literature showing that chronic use of cyclosporin or [tacrolimus] is associated with increased risk of malignancy,” he said. “It’s the old question of specificity. We need ways to target this pathway in only the right cells.”

The idea of searching chromosome 21 for clues to angiogenesis will lead to clinical applications, said Markovic, who added that Ryeom’s work was “a really great find—it brings chromosome 21 back.” One approach would be to develop treatments that target the calcineurin pathway and mirror the natural expression of the genes as it occurs in people with Down syndrome. But the delivery of an agent that disrupts calcineurin signaling also must be considered, he said, pointing out that cyclosporin interrupts the signaling at several levels, depending on the dose.

Ryeom said that individuals with Down syndrome have increased expression of DSCR1 from birth, so any clinical application may have to reflect this lifelong exposure to increased levels of the protein. For example, this work could lead to an agent that women with BRCA1 mutations might take as a prophylactic to prevent tumors from growing into large masses.

Ryeom added that there are two forms of the DSCR1 protein, one of which is found only in endothelial cells, a fact that could help with targeting. She added that further developments would also rely on advanced imaging techniques that allow one to see and analyze new blood vessel growths.

The study will have basic scientists rethinking chromosome 21 and trisomy 21, said Markovic. He expects that it will drive attention to Down syndrome genes and give more insight into the intricacies of vascular biology and the regulation of vascular growth. “It gives us a reasonable door to go through,” he said.