

ARRESTED DEVELOPMENT?

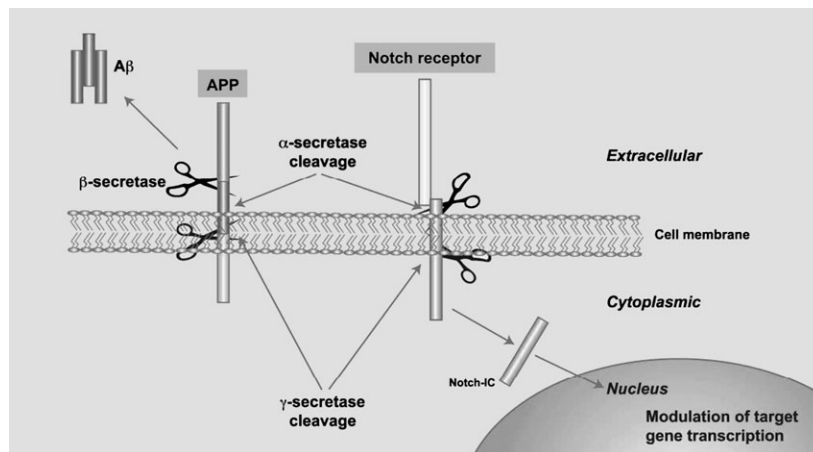
Notch Emerges as New Cancer Drug Target

By Ken Garber

A metaphor for cancer is normal development gone awry. All tissues in the body arise from embryonic stem cells that gradually give rise to specialized cells. Cancer resembles a case of arrested development because less differentiated tumors are almost always the most aggressive. So it's no surprise that genes regulating normal fetal and childhood development have been implicated in adult cancer. The question now: If a gene regulating development can cause cancer, can blocking that gene eradicate the tumor?

One such gene is Notch. First described almost a century ago, Notch has become the object of intense study in cancer. Evidence is growing that Notch signaling can drive the growth of a wide range of tumors, from leukemia to breast cancer. At least two cancer clinical trials are under way using Notch inhibitors, and several others are pending. "This is an exploding field," says Lucio Miele, M.D., Ph.D., a cancer researcher at Loyola University Medical Center in Maywood, Ill.

Notch was first identified in 1919 by geneticist Thomas Hunt Morgan, Ph.D., of Columbia University in New York. (Morgan named the gene after the notches that appeared in the wings of fruit flies lacking a gene copy.) In 1937, Donald Poulson, Ph.D., of the Carnegie Institution in Baltimore showed that defects in Notch caused cells originally destined to be skin to instead become neural tissue. This innovation established Notch's role in determining a cell's fate. Notch signaling usually blocks differentiation, thus allowing orderly deployment of diverse cell types in tissues and organs. For



Snip, snip. To enable Notch signaling—implicated in many cancers—gamma secretase must first cut the receptor, sending intracellular Notch to the nucleus. Gamma secretase inhibitors are already being tried as Notch inhibitors in cancer patients.

example, Notch signaling from developing neurons in chicks keeps adjacent cells from also turning into neurons and allows them to become glia, the brain's support cells.

Notch is a relative newcomer to the cancer field. In 1991, Notch1 (one of four Notch receptors in humans) was identified at a translocation breakpoint in rare cases of T-acute lymphoblastic leukemia/lymphoma (T-ALL). The following year, Notch4 was found at the site of a retroviral gene insertion that leads to breast cancer in mice. The 1999 discovery that gamma secretase cleaves Notch receptors, triggering Notch signaling, has led to an explosion of cancer research using gamma secretase inhibitors originally developed in the late 1990s to treat Alzheimer disease. (Gamma secretase enables amyloid plaques to build up in the brain in Alzheimer disease.) Many groups have now shown that these drugs can block tumor growth in cell culture.

Keeping Stem Cells Alive

A direct causal role for Notch in cancer remained elusive until 2004, when a group led by Jon Aster, M.D., Ph.D., at Harvard showed that more than 50% of human

T-ALLs have activating mutations in Notch. "Gains in Notch1 signaling clearly have a very central role" in T-ALL, Aster said. Still uncertain is whether activated Notch induces T-ALL or just boosts growth of existing tumors. "We really don't know, in the human disease, what's the chicken and what's the egg," Aster said. "I would like to think that Notch is an initiating event that's also going to turn out to be required for tumor maintenance, but that's not yet been

proven." Even if Notch ends up as just a collaborator, not an initiator, it's still an attractive new drug target. Merck has already launched clinical trials of a gamma secretase inhibitor in T-ALL and in solid tumors.

Studies of leukemia are also revealing how Notch activation may lead to cancer. During normal development, Notch directs immune progenitor cells in the thymus to become T cells instead of B cells; in T-ALL, Notch signaling appears to be stuck at one stage of this process, leading to uncontrolled proliferation of these immature T cells. Aster expected that blocking Notch in cells would restart the differentiation process—but it doesn't. "The most important effects of Notch are actually on cellular metabolism, which is a big surprise," Aster said. "When you withdraw Notch ... the cells basically go into something that looks like a state of hibernation."

Blocked differentiation and altered metabolism are just two ways that Notch may be driving tumor growth. Another scenario is that Notch signaling promotes the survival of cancer stem cells. In 2003, Max Wicha, M.D., and Michael Clarke, M.D., of the University of Michigan in Ann Arbor

identified likely breast cancer stem cells. The following year Wicha showed that Notch signaling promotes survival and proliferation of normal breast stem cells. “We now have good tumor data, too, that when tumors expand they have activation of the Notch pathway,” Wicha said. Signaling through either Notch or Hedgehog, another important developmental gene, leads to activation of Bmi-1, a crucial stem cell regulator.

“We think that these [breast] malignancies are really initiated by expansion of these stem cells,” Wicha said, “and then they produce cancers that are driven by [a] small component of stem cells, which are the ones that have active Notch signaling—Notch or Hedgehog.” Wicha’s group is preparing a multicenter clinical trial using a gamma secretase inhibitor with chemotherapy to treat metastatic breast cancer. Experiments show that Notch inhibition causes breast cancer stem cells to differentiate into progenitor cells that are then sensitive to chemotherapy. Wicha helped devise the stem cell theory of cancer, which holds that chemotherapy ultimately fails because it hits only differentiated cells and leaves cancer stem cells unharmed to repopulate the tumor. By first driving stem cells to differentiate and then hitting them with chemotherapy, his breast cancer trial could help validate the theory—if patients benefit.

This crucial role for Notch in breast cancer stem cell self-renewal, though, has yet to be proven. “It’s a very intriguing idea,” Aster said. “But it just hasn’t been clearly documented that Notch is really having such a role.” Wicha agrees. “It’s far from proven that ... Notch is absolutely required or that the effects we’re seeing with chemotherapy in the mouse will translate into humans,” he said.

Whether it works through stem cells or not, Notch signaling appears to be important in breast cancer. About half of breast cancers have low levels of Numb, a Notch antagonist. Notch expression is associated with poor prognosis, and a group at the University of Manchester in the U.K. recently showed that Notch is required for the growth of stemlike cells in ductal carcinoma in situ, the precursor breast cancer lesion. (*see* J Natl Cancer Inst 2007;99:616–

27) “This [Notch] pathway doesn’t just act on the bulk population of cancer cells but is one of the half-dozen pathways that appears to be truly important for tumor-initiating cells or cancer stem cells,” Miele said. Miele and Kathy Albain, M.D., are planning a breast cancer clinical trial at Loyola using gamma secretase inhibitors.

Targeting Notch: Risks and Rewards

Brain tumors are another focus of research. In 2004, two groups showed that treating medulloblastoma, a rare childhood tumor, with gamma secretase inhibitors blocked tumor growth in cells and in mice. Last year one of these groups, led by Charles Eberhart, M.D., Ph.D., of Johns Hopkins University in Baltimore, showed that Notch inhibition depleted cancer stem cells in medulloblastoma cell lines.

Although Notch inhibitors have potential in medulloblastoma, there are only about 350 new cases a year in the U.S.—most of them already curable—which limits the prospects for clinical trials. Glioblastoma, on the other hand, is common (10,000 cases a year) and deadly. Eberhart has unpublished data showing that Notch signaling is also important in glioblastoma and that a Notch inhibitor can block tumor growth. “We think it’s doing the same thing in glioblastoma as in medulloblastoma—that is, killing the stem cells,” he said. “My guess is the first clinical use of Notch inhibitors as an anti-stem cell agent in the brain will be in glioblastoma.” Targeting Notch in other brain tumors might also work. “Basically, in any brain tumor under the sun, you can find dysregulation of Notch expression,” Eberhart said.

There is also evidence that activated Notch is important in lung cancer, melanoma, pancreatic cancer, ovarian cancer, and multiple myeloma. But no activating Notch mutations have been found yet except in T-ALL. In their absence, there will always be a question whether Notch activation is really driving these tumors, although high levels of Notch ligand (expressed in surrounding tissue) could be what’s activating Notch.

Drugs blocking Notch have great potential but some pitfalls. Notch signaling is complex and multifaceted. “The ultimate

result of activation of Notch is a cascade of hundreds of genes, many of which are transcription factors themselves,” Miele said. “So it has the potential to affect the expression profile of a significant portion of the genome.” Blocking Notch should inhibit multiple pathways affecting cell growth and differentiation, thus helping circumvent tumor drug resistance. On the other hand, side effects are certain and could involve killing normal stem cells or blocking their ability to differentiate.

For example, Notch signaling is required for proper cell differentiation in the rapidly self-renewing cells of the small intestine. Blocking Notch shifts the balance of cell types from nutrient-absorbing cells to mucus-secreting cells. The result: massive diarrhea, often seen in patients taking gamma secretase inhibitors. While dosing changes can reduce diarrhea, gamma secretase inhibitors are not smart bombs, and giving them chronically is likely to be toxic. Short-term treatment is preferable. “It is possible—but by no means assured—that one might succeed in targeting a relatively small population of cancer stem cells in the body, without impairing the differentiating ability of normal stem cells,” Miele said.

A potential solution is direct tumor targeting (Miele is developing a nanoparticle delivery system) or more specific drugs. At least one lab—Aster’s—and several pharmaceutical companies are trying to develop drugs against specific Notch receptor subtypes, which could prove safer than the current generation of gamma secretase inhibitors, which target all Notches and have many non-Notch targets. Meanwhile, companies are developing antibodies against a Notch ligand, DLL4, which plays a key role in tumor angiogenesis (*see* J Natl Cancer Inst 2007;99:991–5). Biotech companies Oncomed (cofounded by Wicha) and Stemline Therapeutics are also working on new kinds of Notch inhibitors.

So these drugs should get a thorough tryout in cancer patients. The Merck trials are just the start. “It’s really the opening of what we hope will be a productive new area of cancer therapeutics,” Aster said.