Drugging the Wnt Pathway: Problems And Progress

By Ken Garber

Developmental pathways have become important targets for new cancer drugs. Three such pathways—Notch, Hedgehog, and Wnt—have been strongly implicated in cancer, and drug companies have targeted them all. Inhibitors of Notch and Hedgehog are already in clinical trials, including a Hedgehog inhibitor from Genentech that has shown early promise in advanced basal cell carcinoma. (J. Natl. Cancer. Inst. 2008;100:692–3, 697.)

But Wnt has lagged behind. No specific Wnt pathway inhibitor has yet entered clinical testing, even though more than 80% of colorectal cancers are driven by Wnt pathway mutations.

“For many, many years the rate of progress was extremely slow in developing anything resembling a therapeutic for this pathway,” said Randall Moon, Ph.D., of the University of Washington in Seattle. The lack of progress, in large part, was due to the lack of obvious enzyme targets in the pathway, which was not considered “druggable.”

Several new discoveries have changed that. “The idea that this is a nondruggable pathway is, at this point, thrown out the window,” Moon said. Several academic groups have published proof-of-concept reports on small-molecule Wnt pathway inhibitors, and a few biotech companies (along with an unknown number of pharmaceutical companies) are actively developing these and other compounds. At the same time, the number of tumor types known to be dependent on Wnt signaling, in addition to colorectal cancer, has been growing. The list includes pancreatic cancer, lung cancer, prostate cancer, and leukemia.

At least two companies have publicly disclosed plans to move Wnt pathway inhibitors into the clinic by early 2010. Genentech also is involved, although the company declined an interview request because “there is very little we can discuss publicly,” according to spokesperson Robin Snyder, Ph.D. But at the 2007 annual meeting of the American Association for Cancer Research, Genentech researcher Paul Polakis, Ph.D., described an extensive program. “We hope to find drugs that will inhibit [Wnt signaling], because the big score is there,” he said.

Key to Colorectal Cancer

Wnt was first isolated in 1982, when Roel Nusse, Ph.D., a postdoc in the lab of Harold Varmus, M.D., at the University of California, San Francisco, found the gene at the integration site of a retrovirus that causes mammary tumors in mice after infection. Nusse and Varmus named the gene int-1. In 1987 int-1 was found to be the mammalian homolog of the Drosophila gene Wingless, first described in 1973 in fly mutants lacking wings. Combining the names Wingless and int-1 yielded Wnt.

Wnt signaling is crucial throughout embryonic development, beginning with axis specification (determining where the head and tail and left and right sides will be), through formation of the mesoderm, the germ layer that gives rise to connective tissue, muscles, the circulatory system, and the urinary and reproductive systems. It also helps direct patterning of the nervous system. Wnt signaling is context dependent and tightly regulated.

Although Wnt is much quieter in adults, it can lose its tight regulation via mutation or other means and trigger several diseases, including cancer. In 1993 Bert Vogelstein, M.D., and Ken Kinzler, Ph.D., at Johns Hopkins, and Polakis, then at Onyx Pharmaceuticals, separately reported that the adenomatous polyposis coli (APC) tumor suppressor gene, which is mutated in about 80% of sporadic colorectal cancers, interacted with the key Wnt pathway component β-catenin: the first Wnt link to human cancer.

Over the last 15 years, the main components of canonical Wnt signaling (signaling that depends on β-catenin), have been worked out. Without secreted Wnt proteins, β-catenin in the cytoplasm joins APC and Axin in a “destruction complex” that enables β-catenin’s subsequent degradation. β-Catenin target genes stay turned off. But when Wnt proteins bind to their receptors on the cell surface, the destruction complex is inactivated and β-catenin goes to the nucleus, where it interacts with T-cell factor (TCF) transcription factors,
triggering transcription of target genes, including several oncogenes. (Noncanonical Wnt signaling in cancer is less well understood.)

But understanding the pathway does not mean controlling it. Because APC-activating mutations are so common in colorectal cancer, “what everybody’s looking for ... is to somehow find a way to block, downstream of APC, this activation,” said Lawrence Lum, Ph.D., a cancer researcher at the University of Texas Southwestern Medical Center in Dallas. “This is difficult to do because APC is fairly downstream within the pathway. So you’re left with very few chemically tractable targets.” These involve almost exclusively protein–protein interactions, which are extremely hard to target pharmacologically with small molecules, because large protein surface interactions must be blocked.

**Upstream or Downstream?**

One of the first signs that Wnt could be successfully targeted came in 2004 when Michael Kahn, Ph.D., then at the Institute for Chemical Genomics in Seattle, reported a compound that specifically blocked β-catenin/TCF-dependent gene transcription. Kahn’s group then identified the drug’s target, a coactivator of β-catenin. Kahn’s compound bound to the coactivator and caused the cell to switch from a proliferation program to differentiation. (Lack of differentiation is a hallmark of cancer-initiating cells.)

Kahn, who is now at the University of Southern California in Los Angeles, has since developed similar anticancer compounds 15–20 times more potent than the original. Even though Wnt signaling is crucial for stem cell viability, “we find very, very good safety margins with these compounds, at least in dog studies that have been done to this point,” Kahn said. The compounds have been licensed to an undisclosed company and should begin clinical trials at the end of 2009 or the beginning of 2010, according to Kahn, first in leukemias and then in colorectal cancer.

Theriac Pharmaceutical Corporation in Seattle, a subsidiary of Korean drugmaker Choongwae Pharma, has a similar compound in development that could enter the clinic in 2010. “Whether or not that or any other compound is going to find itself as really useful is still out there,” said Moon. “The jury’s not really clear.” Moon is co-founder of a San Diego company, Fate Therapeutics, that is also developing cancer therapies targeting Wnt/β-catenin signaling.

Genentech is taking a different approach by targeting Wnt signaling upstream, at the level of the Wnt ligand. The company created a decoy receptor that sequesters Wnt, preventing it from binding its receptor and triggering the signal cascade. Certain tumors seem to make their own Wnt, becoming dependent on Wnt production, and Genentech’s Polakis, at the 2007 meeting, reported that a Wnt inhibitor is effective in such ovarian and testicular cancer cell lines. “Obviously where we want to go is to branch out into breast cancers and other cancers,” Polakis said. Genentech also has small-molecule Wnt pathway inhibitors against an undisclosed target.

Targeting the Wnt pathway upstream of APC is controversial because downstream activating mutations in APC would, in theory, drive cancer despite upstream inhibition. “Antagonists should probably be designed for being downstream,” Moon said. “To cover the broadest number of mutations that are activating, it seems that the ideal antagonist of the pathway would be one that works in the nucleus.” But the Genentech experiments suggest that upstream targeting can work, at least in some cases. Overexpression of an endogenous Wnt antagonist can also shut down colorectal cancer in animal models. So researchers continue to explore both approaches.

For example, Naoaki Fujii, Ph.D., of St. Jude Children’s Research Hospital in Memphis, Tenn., is developing upstream compounds that block the pathway at the level of the Wnt receptor complex. Downstream of APC, Eric Fearon, M.D., Ph.D., at the University of Michigan in Ann Arbor is targeting the β-catenin/TCF interaction with small-molecule inhibitors. And Avalon Pharmaceuticals in Germantown, Md., has compounds that act on the proteasome, the enzyme complex that breaks down cellular proteins, causing “a very dramatic and very rapid reduction in β-catenin protein,” said Avalon CEO Kenneth Carter, Ph.D. The company hopes to begin clinical trials by 2010.

**New Tools, New Targets**

In other work, researchers have found two new ways to block Wnt signaling, both upstream and downstream of APC. In the February 2009 issue of *Nature Chemical Biology*, Lum’s group in Dallas reported a class of small-molecule compounds that specifically target the protein porcupine, which is essential for Wnt secretion. These compounds “argue that you can successfully target pathways like the Wnt pathway at the level of ligand production,” Lum said.

Lum is using the compounds to probe the function of various Wnt ligands. There are 19 Wnt genes in the human genome, and some may contribute to cancer via unknown pathways. Until now “we’ve been looking ... from a very small window, which is Wnt/β-catenin signaling,” Lum said. “Wnt proteins that control other signaling pathways may be playing just as potentially important roles in promoting cancer cell growth.”

Lum’s other new compound class (also reported in *Nature Chemical Biology*) blocks downstream Wnt/β-catenin signaling by binding to Axin proteins, which are part of the β-catenin destruction complex. The compounds stabilize Axin and essentially build a new destruction complex to eliminate β-catenin, even in the presence of APC mutations. Lum said that in cancer cell lines and other models with decreased APC function, “in every case these compounds seem to bring down β-catenin levels and this activation of target genes associated with colorectal cancer.” His group has provisional patents for these compounds but has not formed a relationship with a drug or biotechnology company to develop them.

**Toxicity Pitfalls**

Lum’s group also found that the negative effects of these compounds on normal cells
were reversible. That’s important because the Wnt pathway is critical for tissue regeneration and for the ability of stem cells in the bone marrow and gut to self-renew. Wnt pathway inhibitors could therefore have serious and lasting side effects, including anemia and immune suppression, as well as damage to the intestinal tract. “It is a complete black box as to what will happen to an adult mammal when this pathway is shut down,” said Lum.

His experiments offer some clues, though. When he added the drugs to the aquarium water of zebrafish, after cutting off part of the fishes’ tailfins, the compounds blocked tailfin regeneration as Wnt pathway inhibitors should. But after the chemicals were removed from the water, the fish regrew their fins, showing that the defect was temporary. And though drug treatment led to gastrointestinal damage and caused the fish to lose energy and appetite, these effects at least partly disappeared when treatment ended. “The [gastrointestinal] tract does not look great, but, remarkably, after we removed these chemicals, the fish not only regenerate tails but they feed and thrive,” said Lum.

Side effects in humans are inevitable, though. “I’m not sure at what [dosage] level you’ll see these effects, but they will occur,” said Polakis at the 2007 meeting. Moon speculated that toxicity in humans might be manageable as long as the pathway isn’t blocked. “Even if you knock down signaling 75%, the remaining 25% might be fine for [stem cells] to get by on,” he said. But Wnt pathway inhibitors may not cause frank tumor regression, even at high doses. “With targeted agents like this … I think there’s a question about whether any of these agents are going to lead to sort of cytotoxic levels of tumor regression,” said Carter, who added that these drugs will probably be used in combination with others.

Another challenge for Wnt researchers is pathway variability across tumor types. Because Wnt signaling is so context dependent, tumor models will have to closely mimic the human situation to accurately predict response. “It’s important for people to realize that the pathway is not identical between all cell types,” Moon said.

Despite pitfalls, researchers and companies are moving steadily forward with Wnt pathway inhibitors. And at the very least, Wnt drugs will get a thorough testing in clinical trials. “We will see actual therapies,” predicted Moon.