NOTCH in HCC

By Lauren Martz, Staff Writer

Researchers at **Stanford University** have shown that activation and not inhibition of the *NOTCH* oncogene may actually protect against hepatocellular carcinoma.¹ The results are the second set of findings in less than a month that argue for ramping up NOTCH signaling to treat some cancers.² The Stanford team now plans to tease out the precise mechanism responsible for NOTCH's antitumor effects.

Excessive activity of the NOTCH receptor originally was implicated in aberrant cell proliferation and tumorigenesis, and this prompted companies to develop NOTCH inhibitors for multiple cancer indications.

Recently, however, researchers at **Vanderbilt University** identified NOTCH ligands that lead to decreased tumorigenesis when they bind the receptor, suggesting that NOTCH may act as a tumor suppressor in some contexts.^{2,3}

Now, Julien Sage and colleagues at Stanford have found additional evidence that NOTCH may be just as much of a tumor suppressor as an oncogene. Sage is associate professor in the Departments of Pediatrics and Genetics and a member of the Stanford Cancer Institute.

Sage's team set out to identify dysfunctional genes in hepatocellular carcinoma (HCC) and developed a genetic mouse model of the human cancer by knocking out three retinoblastoma family genes in the liver with an adenovirus vector. Retinoblastoma genes are inactivated in most human HCCs.

The HCC triple knockout mice developed liver tumors with gene expression profiles similar to those of human HCC. In the HCC mouse livers, proliferation of cancer stem and progenitor cells was greater than that in the livers of control mice.

The group found that expression of *Notch1* and other genes in the Notch pathway was higher in the knockout mice than in control mice, suggesting that the NOTCH pathway is activated during HCC.

The key is that activation of the pathway was not a bad thing for the mice. Indeed, animals receiving a γ -secretase inhibitor, which blocks the NOTCH pathway, had more and larger tumors than mice given vehicle control.

In isolated tumor cells from the livers of HCC mice and in human HCC cell lines, compared with healthy cells, overexpression of the NOTCH intracellular domain (NICD) inhibited cancer cell proliferation and induced apoptosis. NOTCH signaling occurs when the receptor binds to one of its ligands, which triggers cleavage of the receptor into its NICD form. The NICD form is responsible for the effects of NOTCH on gene expression.

These results suggest increasing NOTCH activity in the tumor cells directly induces tumor cell death.

Finally, the team analyzed data from 91 human HCC samples in predefined classes of disease severity and found that cancers with greater NOTCH pathway gene expression were associated with longer patient survival than cancers with lower expression.

Results were published in *The Journal of Experimental Medicine*. The team included researchers from the **Oregon Health & Science University**, the **University of Liege** and the **NIH**.

Sage told *SciBX* the mechanism at play in his group's HCC studies is likely to differ from the mechanisms the Vanderbilt group described relating to NOTCH's antitumor activity in fibrosarcoma and lung cancer. That work was published in *Cancer Research* in August.

The Vanderbilt researchers reported that NOTCH signaling might inhibit cancer growth by reversing the effects of tumors on the immune system and restoring antitumor immunity.

"In our studies, we noted a cell-intrinsic mechanism in which NOTCH activation in the tumor cells themselves reduced proliferation and induced apoptosis. We expect that it was not an effect mediated by immune cells because the effect was present in isolated tumor cells without the surrounding tumor microenvironment," Sage said.

"This is exciting, though, because our work, when considered with the work from

the previous *Cancer Research* paper, suggests that NOTCH could have tumor-suppressor effects both directly and indirectly through altering immune cell function, suggesting an even stronger case for its antitumor activity," he added.

Activation vs. inhibition

Despite the new findings, Sage thinks there is no reason for companies to abandon NOTCH pathway inhibitors.

"Certain cancers and tissue types will benefit from pathway activation, while others will benefit from pathway inhibition," he said. "Both activating and inhibiting the pathway are worth exploring for companies. The questions now will be how to determine which cancers to target, how to specifically target them and what the proper dosing regimen will be."

He told *SciBX* his team's next steps are to study the downstream activities of NOTCH to better understand what mechanisms are promoting some cancers and what mechanisms are inhibiting others.

"We have evidence on both sides of the coin; we simply need to test the effects of NOTCH modulation in the human cancer. The logical way to start is to compare both activation and inhibition of NOTCH signaling side by side in human xenograft models of particular cancers to determine which effect will be dominant," said Patrick Zweidler-McKay, associate professor in the Division of Pediatrics and section chief of pediatric leukemia and lymphoma at **The University of Texas M.D. Anderson Cancer Center**.

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The University of Texas
M.D. Anderson Cancer Center**

Patient benefits

Although researchers continue to flesh out the effects of NOTCH signaling in various cancers, the findings in the *JEM* paper could be used to determine the aggressiveness of HCC and thus which type of treatment to use.

“Our work is also relevant in terms of prognosis and treatment decisions. We found that NOTCH helps predict how fast or slowly a patient will die from the disease. Detecting levels of NOTCH, with other biomarkers, could help determine how aggressively to treat HCC patients,” said Sage.

Zweidler-McKay added that these findings suggest that for now, HCC patients should use caution and consider the risks of enrolling in trials with compounds that inhibit NOTCH signaling, such as γ -secretase inhibitors.

“The fact that they used γ -secretase inhibitors in this study and saw a worsening of the tumors does make you pause about whether you want to allow HCC patients to enroll in the ongoing Phase I trials, which study solid tumors in general. There is a concern that what is happening in the mice could happen in patients,” he said.

Sage added that based on his results, the study also suggests patients with a type of cancer that could be affected by NOTCH inhibitors,

such as T cell acute lymphoblastic leukemia (T-ALL), should seek alternative treatment if they are at increased risk for liver cancer due to alcoholism or hepatitis.

Sage told *SciBX* his work has not been patented.

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