Glucocorticoids get Smoothened

By Kai-Jye Lou, Staff Writer

Researchers at the **Duke University School of Medicine** have identified several FDA-approved glucocorticoids that also agonize a member of the hedgehog pathway called Smoothened and have shown that the drugs can stimulate the proliferation of primary neuronal precursor cells.¹ The findings suggest that glucocorticoids—and their potential derivatives—could be repurposed for various tissue repair and regenerative medicine applications.

Activation of the hedgehog pathway stimulates stem cell proliferation and has shown promise in diseases such as spinal cord injury (SCI), Parkinson's disease (PD), myocardial infarction (MI) and wound healing.

However, hedgehog agonists have not entered the clinic due in part to perceived safety risks, like carcinogenicity. For example, activating mutations in Smoothened (SMO) and inactivating mutations in Patched 1 (PTCH1) are associated with multiple types of cancer.² SMO is a G protein-coupled receptor that activates transcription factors in the hedgehog pathway, whereas PTCH1 is a negative regulator of the pathway.

"The hedgehog pathway was described over two decades ago, but to date there are no FDA-approved agonists that specifically target this pathway," said Wei Chen, an assistant professor in the Department of Medicine at Duke. "The development of Smoothened agonists has lagged due to concerns about their safety."

To address the issue, Chen's group ran a high throughput, image-based screen for compounds with SMO agonist activity. Four of the hits turned out to be fluorinated glucocorticoids from a library of FDA-approved drugs. The four drugs—halcinonide, fluticasone, clobetasol and fluocinonide—are used for asthma, inflammation, skin disease and other indications.

"One of the primary issues in the development of SMO agonists is safety, and the other is immediate clinical applications," said Chen. "With the current findings, we can at least jump-start the development of treatments that activate the hedgehog pathway because the safety profiles of these drugs, along with their pharmacokinetic properties, are known."

Indeed, the glucocorticoids identified in the article have had a long history of use in humans. Halcinonide, clobetasol and fluocinonide all received FDA approval before 1990, and fluticasone was approved in

1994. The side effects and pharmacokinetics of these drugs are well known and none has shown an association with cancer.

In cultured mouse cerebellar granular cell precursors, halcinonide, fluticasone and clobetasol each increased proliferation by 5- to 50-fold compared with vehicle. On its own, fluocinonide had no significant effect on proliferation. But treatment with a 2% sonic hedgehog homolog (SHH) solution and any of the four drugs increased proliferation compared with the glucocorticoid alone.

SHH activates the hedgehog pathway by inhibiting PTCH1.

Results were published in the *Proceedings of the National Academy of Sciences*.

"We found a subset of fluorinated glucocorticoids that can act as Smoothened agonists and stimulate proliferation in a cell population important for tissue repair and regeneration," said Chen, who is corresponding author on the paper. "We were very surprised by these results" because these drugs were not known to have SMO agonist activity, he said.

"These findings are interesting in the context of regenerative medicine because the hedgehog pathway is thought to be involved in tissue maintenance and repair," said Chin Chiang, a professor in the Department

of Cell and Developmental Biology at the **Vanderbilt University Medical Center (VUMC)**. "One of the more interesting findings to me is that these agonists, while moderately effective in potency by themselves, can strongly synergize with the sonic hedgehog ligand."

However, Chiang noted that the mechanism for SMO agonism is still unclear.

"At this time, we know that these glucocorticoids are activating hedgehog signaling and possibly activating Smoothened, but we don't actually know how they are doing this—we don't know for sure if these drugs are working exclusively on Smoothened," he said.

Michael Cooper, an assistant professor of neurology at VUMC, wanted to see the mechanism teased out with genetic and chemical epistasis studies, in which expression inhibitors and antagonists of other molecules in the hedgehog pathway are evaluated in conjunction with the glucocorticoids.

He added that it will be important to determine which effects of the glucocorticoids are due to signaling in glucocorticoid pathways and which are due to signaling in the hedgehog pathway.

Unlike the proliferation-modulating effects of the hedgehog pathway, activation of the glucocorticoid pathway triggers an anti-inflammatory response. Glucocorticoids are traditionally believed to work by binding to the glucocorticoid receptor (GCCR), which stimulates or suppresses the expression of anti-inflammatory or proinflammatory factors, respectively.

Getting wound up

Because hedgehog signaling can drive the differentiation of one cell type and simultaneously inhibit the differentiation of others, it will be

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important to pick an appropriate indication in which to evaluate the glucocorticoids.

Chiang thinks the glucocorticoids may be best suited for cases in which activation of the hedgehog pathway is necessary to promote proliferation of a stem cell population into the multiple cell types needed, such as in tissue repair. Models of wound healing “will probably be the most straightforward system to test the effect of these agonists, as they are simple and minimally invasive,” he said.

Indeed, a 2006 study in which topical *SHH* gene therapy accelerated wound healing suggested that the protein may be acting on an epidermal stem cell population.³

Cooper agreed that a wound-healing model might be a good starting point, given that three of the glucocorticoids are topical.

Chen, however, is tackling different indications. He said his group is currently studying the effects of glucocorticoids on neurogenesis and hair growth in wild-type mice.

“This would be important in diseases like Parkinson’s and hair loss, where patients are losing regenerative capacity,” he told *SciBX*. “In addition, we are currently collaborating with another group at Duke to evaluate these glucocorticoids in a rodent spinal cord injury model.”

Meanwhile, a handful of companies still are pursuing hedgehog pathway antagonists in oncology. Roche’s **Genentech Inc.** unit and partner **Curis Inc.** have the most advanced SMO inhibitor, GDC-0449, in Phase II trials for solid tumors.

Other SMO inhibitors being developed for solid tumors include IPI-926 from **Infinity Pharmaceuticals Inc.** and partners **Mundipharma Interna-**

tional Ltd. and **Purdue Pharma L.P.**, which is in Phase Ib/II testing, and BMS-833923 (XL139) from **Bristol-Myers Squibb Co.** and **Exelixis Inc.**, which is in Phase I trials.

LEQ506 from **Novartis AG** is expected to enter Phase I testing this year.

Duke has filed a provisional patent covering the application of halcinonide, fluticasone, clobetasol and fluocinonide in tissue repair and regeneration. The work is available for licensing. The original patents covering composition of matter for the four drugs have expired.

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COMPANIES AND INSTITUTIONS MENTIONED

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Genentech Inc., South San Francisco, Calif.
Infinity Pharmaceuticals Inc. (NASDAQ:INFI), Cambridge, Mass.
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