Smoother tumor targets

By Lev Osherovich, Senior Writer

Texas researchers have identified two kinases—GRK2 and CKI-α—that are responsible for phosphorylating smoothened, the central player in the hedgehog signaling pathway.[1] The findings could open a new front against the tumor-promoting pathway if sufficiently selective inhibitors can be developed.

Because G protein–coupled receptor kinase 2 (GRK2; GPRK2) and casein kinase 1α (CSNK1A; CKI-α) help bring smoothened (SMO) to a cellular structure in which SMO receives a proliferative signal, blocking them might overcome activating mutations in SMO that cause resistance to SMO antagonists.

Three antagonists of SMO are in Phase II testing for various solid tumors: vismodegib (GDC-0449), jointly developed by **Roche**'s **Genentech Inc.** unit and **Curis Inc.**, IPI-926 from **Infinity Pharmaceuticals Inc.** and **Mundipharma International Ltd.** and LDE225 from **Novartis AG**.

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> ***—Jin Jiang, The University of Texas Southwestern Medical Center at Dallas***

Last week, Roche presented additional data from a Phase II trial of vismodegib in basal cell carcinoma (BCC). The trial met its endpoints of increased overall response rate and progression-free survival. Roche expects to submit an NDA for vismodegib to the FDA this year.

Chugai Pharmaceutical Co. Ltd., which is majority owned by Roche, has Japanese rights to the compound.

Despite promising data for the class of SMO inhibitors, drug resistance could become an issue. Over the last two years, Genentech and Novartis researchers have independently uncovered evidence that mutations in *SMO* or other components of the hedgehog pathway can lead to resistance to SMO inhibitors.[2,3] The threat of resistance has companies and academics looking for backup targets in the pathway.

The discovery by the Texas team, led by Jin Jiang, professor of developmental biology and pharmacology at **The University of Texas Southwestern Medical Center at Dallas**, might provide those backup targets: GRK2 and CKI-α, kinases upstream of SMO.

Jiang's work fleshes out previous reports that identified both kinases as modifiers of hedgehog signaling. "While these kinases were known to be involved in hedgehog signaling, the mechanism was not clear," said Frederic de Sauvage, VP of molecular biology at Genentech.

Turn up the smooth

Jiang's previous studies showed that SMO was phosphorylated during fruit fly development. Thus, the team suspected that kinases could also play a part in the mammalian version of the hedgehog pathway.

"Although it's been anticipated that SMO activation may involve phosphorylation, this has not yet been fully demonstrated" in mammalian cells, said Jiang. "There was some evidence in flies that SMO is activated by phosphorylation, but the sites aren't conserved in mammals."

To test whether phosphorylation was indeed driving SMO activity, Jiang's team developed an *in vitro* test and found the protein could indeed be phosphorylated by GRK2 and CKI-α.

The team then systematically mutated the C-terminal portion of SMO in cell culture to identify how the kinases regulate SMO, which turned out to be through multiple partially overlapping phosphorylation sites.

This overlap may explain why previous studies by other teams overlooked the critical role of GRK2 and CKI-α in hedgehog pathway signaling.

"There is redundancy between the two kinases," said Jiang. "That's why genetic loss of function of GRK2 had such a weak effect" on hedgehog signaling in previous studies.

Jiang noted that CKI-α previously came up in a 2008 study[4] by de Sauvage's team that profiled kinases that affect hedgehog signaling. However, the target's precise role was unknown.

Engineered mutations in *SMO* that abolished multiple phosphorylation sites prevented activation of SMO, whereas mutations that mimicked phosphorylation led to constitutive activation.

The team went on to show that phosphorylation by GRK2 and CKI-α was essential for SMO's ability to activate glioma-associated oncogene homolog 1 zinc finger protein (GLI1), a transcription factor that enacts the proliferative program triggered by hedgehog signaling.

Finally, the researchers found that SMO phosphorylation coincides with the protein's migration to the primary cilium, a projection at the cell surface. That structure harbors proteins involved in cellular differentiation and serves as the docking site for sonic hedgehog homolog (SHH), the paracrine hormone that initiates the signaling pathway in mammalian cells.

Altogether, the findings suggest that phosphorylation of SMO by GRK2 and CKI-α at the primary cilium renders SMO receptive to SHH signaling (*see* **Figure 1, "New targets in the hedgehog pathway"**).

Results were published in *Public Library of Science Biology*. Jiang did not patent his findings.

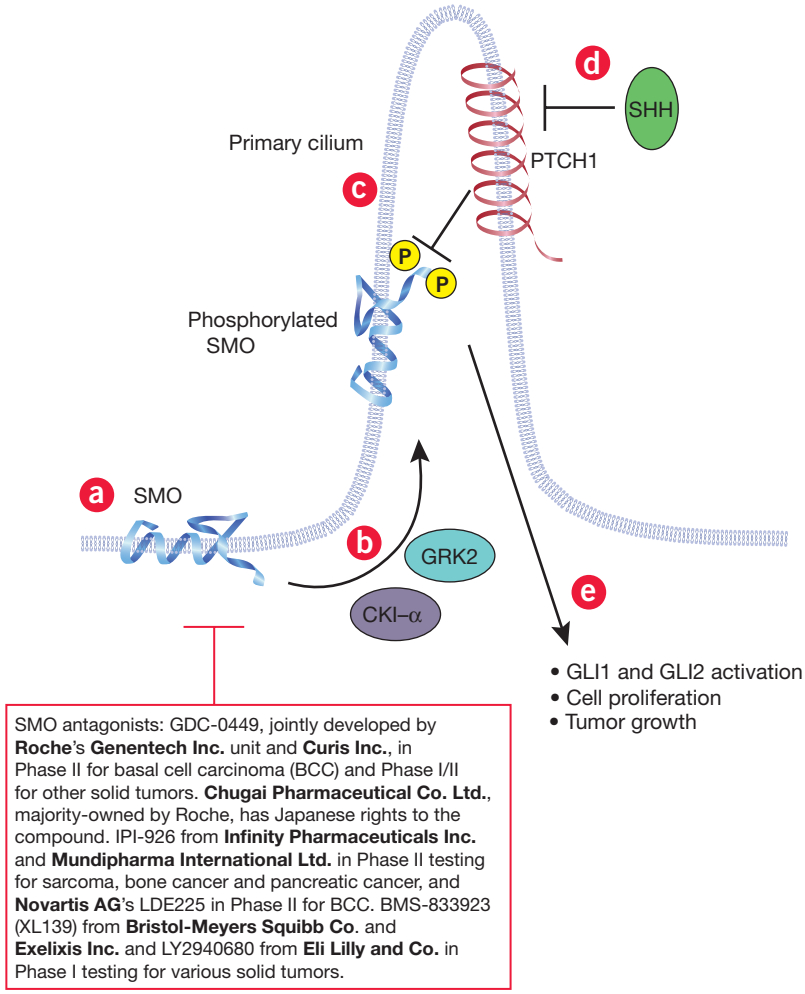
Don't be cilium

Although Jiang has not yet tested the effect of inhibiting GRK2 and CKI-α on tumor growth *in vivo*, it seems likely the kinases influence the growth of tumors driven by excessive SHH activity.

Figure 1. New targets in the hedgehog pathway. Chen *et al.* have identified a pair of kinases needed for activation of the hedgehog pathway, which drives a range of solid tumors.

The findings suggest that smoothened (SMO) at the cell surface [**a**] can be phosphorylated by two kinases, G protein–coupled receptor kinase 2 (GRK2; GPRK2) and casein kinase 1α (CSNK1A; CKI-α) [**b**]. Phosphorylation causes SMO to migrate into the primary cilium [**c**], a projection on the cell surface that coordinates proliferative signals. Phosphorylated SMO then becomes activated when sonic hedgehog homolog (SHH) binds and inhibits patched 1 (PTCH1) [**d**], leading to activation of oncogenic transcription factors glioma-associated oncogene homolog 1 zinc finger protein (GLI1) and GLI2, which promote cell proliferation and tumor development [**e**].

GRK2 and/or CKI-α inhibitors could be alternatives or adjuncts to SMO inhibitors currently in the clinic.



"Inhibiting these two kinases may have a therapeutic effect" in tumors, said Jiang. Because of the overlapping functions of the two kinases, Jiang thinks the best results will come from "combinations of GRK2 and CKI-α inhibitors, perhaps also in combination with SMO inhibitors."

Jiang noted that blocking the two kinases may even help to overcome or delay resistance to SMO inhibitors, as tumor cells with activating mutations in SMO will not have a chance to grow if SMO is kept out of the primary cilium as a result of blocking the kinases.

But according to de Sauvage, blocking the kinases would be helpful only in the subset tumors in which SMO inhibitor resistance is caused by mechanisms upstream of SMO localization to the primary cilium.

For example, de Sauvage noted that in tumors rendered resistant to SMO inhibitors because of the amplification of GLI1, inhibiting upstream players like GRK2 and CKI-α is unlikely to be of much help.

James Chen, associate professor of chemical and systems biology and chemistry at **Stanford University School of Medicine**, noted that hitting GRK2 and CKI-α may be most effective in tumors driven by excessive extracellular SHH, including certain forms of pancreatic and prostate cancer.

According to Chen, the quickest way to test the utility of knocking down the two kinases is another cell culture experiment.

"The thing I'd do first is to take cell lines that are resistant to SMO inhibitors and do siRNA knockdown of these kinases," said Chen. If Jiang is right, doing so might restore SMO inhibitor sensitivity or even hinder cell growth outright.

"The question is whether these kinases can be targeted in a way that doesn't cause effects on other pathways" also regulated by GRK2 and CKI-α, said Chen. He noted that both kinases are involved in a multitude of other cellular processes, so it's hard to predict the effects of decreasing their activity.

"The main challenge is that these are not unique to the hedgehog pathway but participate in many other signaling cascades," agreed de Sauvage.

Meanwhile, Jiang hopes to use his *in vitro* assay of SMO phosphorylation to screen for small molecules that affect the pathway, potentially including inhibitors of GRK2 and CKI-α.

Chen said he has already made progress on this front and has identified compounds that modulate SMO by hitting an undisclosed target.

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COMPANIES AND INSTITUTIONS MENTIONED

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