Hedgehog joins the resistance

By Lev Osherovich, Senior Writer

Increased glucuronidation by activated hedgehog signaling turns out to be the root of drug resistance for at least two compounds in acute myeloid leukemia.[1] Inhibiting the pathway could avoid the toxicity of general glucuronidase suppression and boost the efficacy of standard acute myeloid leukemia therapies.

The Canadian team behind the study now plans to test a combination of ribavirin, a chemotherapeutic nucleoside analog, with an inhibitor of smoothened (SMO), a druggable player in the hedgehog pathway.

Glucuronidation by UDP glucuronosyltransferase (UGT) enzymes represents one of the key metabolic clearance mechanisms used by the liver to eliminate drugs.

Although cancer cells are well known to develop drug resistance through overexpression of P glycoprotein (MDR1; ABCB1; P-gp; CD243)—an efflux pump involved in drug disposition—this is the first report of cancer cells hijacking UGT enzymes to nullify drugs.

"The link between the glucuronosyltransferase and AML was a complete surprise to us," said Katherine Borden, lead author on the study. "These enzymes have been known since the 1950s, and people think of them as being important in steady-state drug metabolism in the liver. The idea that glucuronidation could be evolved by a cancer cell to neutralize a drug is totally new." Borden is a professor of pathology and cell biology at the **University of Montreal**.

Her team found the connection when they noted that all initial responders in a Phase II acute myeloid leukemia (AML) trial of ribavirin monotherapy subsequently relapsed. Ribavirin was provided by Canadian generics maker **Pharmascience Inc.**, which also participated in the new study.

To investigate what might explain the relapse, Borden's group created resistant cells from cancer cell lines that normally respond to ribavirin. In the resistant cells, ribavirin no longer interacted with its target, eukaryotic translation initiation factor 4E (eIF4E).

Next, the team sequenced mRNA from drug-resistant cells and saw higher levels of *glioma-associated oncogene homolog 1 zinc finger protein* (*GLI1*) than those in ribavirin-sensitive controls. *GLI1* mRNA levels also were greater in leukemic cells from relapsed patients with AML than in responding patients or healthy controls.

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In vitro, overexpression of *GLI1* induced resistance to both ribavirin and cytarabine—another drug associated with resistance in AML—whereas siRNA knockdown of *GLI1* restored sensitivity to both drugs.

GLI1 is a transcription factor involved in hedgehog signaling. To find what was driving the GLI1-induced resistance, the team looked upstream in the signaling pathway and found that the SMO inhibitor Erivedge vismodegib restored sensitivity to both ribavirin and cytarabine.

Erivedge is marketed by **Roche** for basal cell carcinoma (BCC). Erivedge and at least five other inhibitors of hedgehog signaling, including **Pfizer Inc.**'s PF-04449913, are in various stages of clinical testing for a range of cancers.

Finally, Borden's team looked for the target of GLI1 responsible for drug resistance. They noted that levels of eIF4E remained elevated in ribavirin-resistant cells despite the protein's inability to associate with the drug. That suggested GLI1 might have modified the ribavirin target.

The team looked at drug-metabolizing enzymes and found higher levels of UDP glucuronosyltransferase 1 family polypeptide A1 (UGT1A1) in resistant cells than in controls. Those levels were decreased by *GLI1* knockdown. In addition, both ribavirin and cytarabine were glucuronidated in resistant cells, and inhibition of hedgehog signaling with Erivedge eliminated ribavirin glucuronidation.

Borden and colleagues concluded that GLI1 promotes resistance to ribavirin and cytarabine by stimulating UGT1A1 activity to glucuronidate the drugs and prevent them from interacting with their target.

Results were reported in *Nature*.

Metabolic resistance

Borden's study is the latest example of how the hedgehog pathway helps cancers evade therapeutics, but this is the first time the pathway has turned up as a driver of drug metabolism.

"Here, hedgehog signaling clearly contributes to removal of the drug," said Tannishtha Reya, a professor of pharmacology at the **University of California, San Diego**. Reya led a team that discovered the involvement of the hedgehog pathway in drug-resistant chronic myeloid leukemia (CML).[2] The hedgehog-dependent mechanism of CML drug resistance does not appear to involve glucuronidation.

The hedgehog pathway "is clearly integral to the evolution of drug resistance, and an inhibitor of this pathway could extend survival," she added.

Borden's findings argue for combining inhibitors of hedgehog signaling with conventional chemotherapy to combat drug resistance in AML. Along those lines, Pfizer is testing PF-04449913 in combination with cytarabine and other chemotherapeutics in an open-label Phase Ib/II trial.

Borden and Pharmascience have filed for authorization in Canada to repeat their ribavirin AML trial in combination with a hedgehog pathway inhibitor such as Erivedge. **Genentech Inc.**, the Roche unit that developed Erivedge along with partner **Curis Inc.**, declined to comment on Borden's study.

Stephen Morris, senior director of research and innovative drug development at Pharmascience, said that his company played a supporting role in Borden's study and might play a more active role in Borden's planned combination trial.

"We stumbled into this," said Morris. "Borden approached us some time ago to test her hypothesis that ribavirin could be useful for treating AML. Borden and her clinical collaborators noticed the emergence of resistance in the trial. Our contribution was to determine that the drug was being modified by glucuronidation." Morris said that it would be worthwhile to study whether hedgehog-mediated glucuronidation occurs in other tumor types or with other chemotherapeutics besides cytarabine and ribavirin.

Meanwhile, Borden wants to know whether inhibiting glucuronidation directly, rather than by hitting hedgehog signaling, could overcome resistance.

"We're also interested in targeting glucuronosyltransferases themselves," said Borden. "This is a family of ten enzymes, but we think that only a subset is responsible for drug resistance. We're trying to set up an NMR-based fragment screen for glucuronosyltransferase inhibitors."

Borden has filed a patent on the use of hedgehog pathway inhibitors to prevent drug resistance in AML. The patent, which is co-owned by Pharmascience, is available for licensing.

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   Contact: Katherine L.B. Borden, University of Montreal, Montreal, Quebec, Canada
   e-mail: katherine.borden@umontreal.ca
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COMPANIES AND INSTITUTIONS MENTIONED

Curis Inc. (NASDAQ:CRIS), Lexington, Mass.
Genentech Inc., South San Francisco, Calif.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Pharmascience Inc., Montreal, Quebec, Canada
Roche (SIX:ROG;OTCQX:RHHBY), Basel, Switzerland
University of California, San Diego, La Jolla, Calif.
University of Montreal, Montreal, Quebec, Canada