Putting SMYD3 on the MAP

By Lev Osherovich, Senior Writer

Stanford University and GlaxoSmithKline plc researchers have uncovered the mechanism of action of SMYD3, a histone lysine methyltransferase overexpressed in many lung and pancreatic tumors.¹ The surprise was that SMYD3 acts in the cytoplasm to regulate the MAPK pathway and not in the nucleus as previously thought. The discovery implies that inhibiting the enzyme can counter activating mutations in MAPK pathway components such as K-Ras.

SMYD3 (SET and MYND domain containing 3) is a member of a family of histone lysine methyltransferases that typically are epigenetic regulators of chromatin structure in the nucleus.²

Now, a team co-led by Julien Sage and Or Gozani has found a previously unknown function for SMYD3 in cancer. Rather than working in the nucleus as expected, SMYD3 promotes cancer growth by regulating a branch of the cytoplasmic MAPK signaling pathway.

Sage is an associate professor of pediatrics

“The beauty and the weakness of hitting SMYD3 is that this is not an essential protein for MAPK signaling. Thus, drugs against SMYD3 are not going to have as many side effects as direct inhibition of the pathway. On the other hand, they may not be as effective as drugs that hit the main components of the pathway.”

—Julien Sage,

Stanford University School of Medicine

Figure 1. SMYD3 in cancer. Mazur *et al.* have identified the role of SET and MYND domain containing 3 (SMYD3) in K-Ras (KRAS)-driven lung and pancreatic cancers.

In certain lung and pancreatic tumors, activating mutations in *KRAS* or *BRAF* (a) lead to excessive signaling through downstream MAPK signaling pathways. One branch of the pathway utilizes mitogen-activated protein kinase kinase 2 (MAP3K2) (b) to activate downstream effectors mitogen-activated protein kinase kinase 5 (MAP2K5; MEK5) and MAP kinase 7 (MAPK7; BMK1; ERK-5) (c), which drive tumor growth (d).

The team uncovered that the histone lysine methyltransferase SMYD3 can methylate (CH₃) MAP3K2 (e), leaving the kinase stuck in an active state to promote hyperactive proliferative signaling.

and genetics at the **Stanford University School of Medicine**. Gozani is an associate professor of biology at Stanford University.

The findings suggest that SMYD3 inhibitors could complement MAPK pathway inhibitors such as GSK's Mekinist trametinib. Mekinist is marketed to treat melanoma with activating mutations in the upstream MAPK pathway regulator BRAF. The drug is in Phase II testing for solid tumors including lung cancers with K-Ras (KRAS)-activating mutations. At least 18 cancer therapeutics targeting the MAPK pathway are in development or on the market (see Table 1, “Selected MAPK pathway compounds in cancer”).

“SMYD3 is acting in the cytoplasm to methylate a specific target in the MAPK cascade,” said Robert Copeland, EVP and CSO of **Epizyme Inc.** “The demonstration of a broader role for this enzyme beyond just methylating histones speaks to the interplay between chromatin-remodeling factors and other important signaling pathways.”

Under a 2011 deal, Epizyme and GSK are discovering compounds against three undisclosed epigenetic targets.

SMYD range

As part of an ongoing collaboration to characterize the function of

Table 1. Selected MAPK pathway compounds in cancer.

Source: BCIQ: BioCentury Online Intelligence

Company	Compound	Indication	Target	Status
Amgen Inc. (NASDAQ:AMGN)	Nexavar sorafenib	Advanced cancers including colorectal, liver, breast, renal and ovarian cancers	CRAF (RAF1); VEGF receptor	Marketed
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)	Tafinlar dabrafenib	Melanoma with a BRAF V600E or V600K mutation	BRAF	Marketed
	Mekinist trametinib	Melanoma with a BRAF V600E or V600K mutation	MAP kinase kinase 1 (MAP2K1; MEK1); MEK2 (MAP2K2)	Marketed
Roche (SIX:ROG; OTCQX:RHHBY); Daiichi Sankyo Co. Ltd. (Tokyo:4568); Chugai Pharmaceutical Co. Ltd. (Tokyo:4519)	Zelboraf vemurafenib	Melanoma with a BRAF V600E mutation	BRAF	Marketed
Amgen	Nexavar sorafenib	Thyroid cancers	CRAF; VEGF receptor	Approved
GlaxoSmithKline	Tafinlar dabrafenib plus Mekinist trametinib	Melanoma with a BRAF V600E or V600K mutation	MEK1; MEK2; BRAF	Registration
Amgen	Nexavar sorafenib	Breast, ovarian and liver cancers; colorectal cancer (CRC)	CRAF; VEGF receptor	Phase III
AstraZeneca plc (LSE:AZN; NYSE:AZN); Array BioPharma Inc. (NASDAQ:ARRY)	Selumetinib (AZD6244)	Non-small cell lung cancer (NSCLC)	MEK1; MEK2	Phase III
Exelixis Inc. (NASDAQ:EXEL); Genentech Inc. unit of Roche	Cobimetinib (RG7421)	Melanoma with a BRAF V600E mutation	MEK1; MEK2	Phase III
GlaxoSmithKline	Tafinlar dabrafenib	Solid tumors with BRAF mutation	BRAF	Phase III
Novartis AG (NYSE:NVS; SIX:NOVN)	Encorafenib (LGX818)	Melanoma with BRAF mutations	BRAF	Phase III
Novartis; Array BioPharma	Binimetinib (MEK162)	Melanoma with BRAF mutations; ovarian cancer	MEK1; MEK2	Phase III
AstraZeneca; Bayer AG (Xetra:DAYN)	Refametinib (BAY 86-9766)	Liver cancer	MEK1; MEK2	Phase II
AstraZeneca; Array BioPharma	Selumetinib (AZD6244)	Leukemia; melanoma; thyroid cancer; breast cancer; CRC	MEK1; MEK2	Phase II
GlaxoSmithKline	Tafinlar dabrafenib	NSCLC with BRAF mutations	BRAF	Phase II
	Tafinlar dabrafenib plus Mekinist trametinib	CRC	MEK1; MEK2; BRAF	Phase II
	Mekinist trametinib	Relapsed or refractory solid tumors	MEK1; MEK2	Phase II
		Second-line treatment of NSCLC with <i>K-Ras</i> (KRAS) mutations		
Merck KGaA (Xetra:MRK)	Pimasertib (AS703026)	Pancreatic cancer	MEK1; MEK2	Phase II
Novartis	Encorafenib (LGX818)	Melanoma; pancreatic and ovarian cancer	MEK1; MEK2	Phase II
Roche; Daiichi Sankyo; Chugai Pharmaceutical	Zelboraf vemurafenib	Solid tumors	BRAF	Phase II
AstraZeneca; Bayer	Refametinib (BAY 86-9766)	Thyroid cancer with a BRAF V600E mutation	BRAF	Phase II
AstraZeneca; Bayer	Refametinib (BAY 86-9766)	Pancreatic and biliary duct cancers	MEK1; MEK2	Phase I/II
BioMed Valley Discoveries Inc.	BVD-523	Solid tumors	MAP kinase 1 (MAPK1; ERK-2); MAPK3 (ERK-1)	Phase I/II
GlaxoSmithKline	Tafinlar dabrafenib	Metastatic CRC (mCRC)	BRAF	Phase I/II
	Mekinist trametinib	mCRC	MEK1; MEK2	Phase I/II
Novartis	Encorafenib (LGX818)	Solid tumors with BRAF V600 mutations	MEK1; MEK2	Phase I/II
Novartis	Encorafenib (LGX818)	CRC	BRAF	Phase I/II

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Table 1. Selected MAPK pathway compounds in cancer. (continued)

Company	Compound	Indication	Target	Status
Teva Pharmaceutical Industries Ltd. (NYSE:TEVA); Ambit Biosciences Corp. (NASDAQ:AMBI)	CEP-32496	Melanoma	BRAF	Phase I/II
Eli Lilly and Co. (NYSE:LLY); Deciphera Pharmaceuticals LLC	LY3009120 (DP-4978)	Melanoma; CRC	BRAF; CRAF; ARAF	Phase I
Exelixis; Genentech	Cobimetinib (RG7421)	Solid tumors	MEK1; MEK2	Phase I
Merck; BeiGene Co. Ltd.	BeiGene-283	<i>BRAF</i> - or <i>KRAS</i> -mutation-positive cancers	BRAF	Phase I
Pfizer Inc. (NYSE:PFE)	PD-0325901 plus PF-05212384	Solid tumors	MEK1; MEK2; mammalian target of rapamycin (mTOR; FRAP; RAFT1); phosphoinositide 3-kinase (PI3K)	Phase I
Roche; Chugai Pharmaceutical	RG7304	Solid tumors	MEK1; CRAF	Phase I
	RG7167	Solid tumors	MEK1; MEK2	Phase I
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502)	TAK-733	Solid tumors	MEK1; MEK2	Phase I

epigenetic targets, the Stanford-GSK team started by analyzing gene expression data for 54 histone lysine methyltransferases in a panel of pancreatic cancers with high *KRAS* activity.

SMYD3 levels were consistently higher in the tumors than in healthy tissue, suggesting that the enzyme might contribute to cancer growth. To test this possibility, the team introduced conditionally activated *Kras* mutations into a *Smyd3* knockout mouse. The animals were less susceptible to pancreatic and lung tumors caused by hyperactive *Kras* than controls with normal *Smyd3*. The team observed similar effects with shRNA knockdown of *SMYD3* in cultured, *KRAS*-driven human pancreatic and lung cancer cells and in mouse xenografts.

The next step was to figure out how SMYD3 promotes tumor growth. The team knew that the enzyme methylated something, but identifying the substrates required informed guesswork. One clue came from immunohistochemical experiments in murine pancreatic tumors that showed *Smyd3* was predominantly localized in the cytoplasm, not the nucleus.

An *in vitro* screen for cytoplasmic proteins that could be methylated by SMYD3 pointed to mitogen-activated protein kinase kinase 2 (MAP3K2), a key component of a MAPK pathway branch that is hyperactive in some tumors.

The team went on to show that SMYD3 methylates MAP3K2 in tumor cells, leading to higher MAPK pathway signaling than unmethylated MAP3K2. Surprisingly, the team found that methylation of MAP3K2 by SMYD3 did not directly activate the kinase. Rather, methylation prevented MAP3K2 from being inactivated by a downstream phosphatase called protein phosphatase 2 (PPP2CA; PP2A).

“We expected that the methylation mark would recruit something, but in fact it’s the opposite—exclusion of the inhibitor PP2A,” said Sage.

Altogether, the findings suggest that SMYD3 renders MAP3K2 constitutively active, leading to broad effects on downstream effector proteins that drive tumor growth (see **Figure 1, “SMYD3 in cancer”**).

Results were reported in *Nature* and are not patented.

Been there, SMYD that

SMYD3 thus appears to be a good target for a subset of *KRAS*-driven tumors. “If you have cancers driven by *KRAS* and have high levels of SMYD3 or methylation of MAP3K2, these are good candidates for SMYD3 inhibition,” said Gozani.

“This study defines a patient population that might benefit from SMYD3 inhibitors,” said coauthor Olena Barbash, an oncology R&D investigator at GSK.

For drug developers, the key question is how targeting SMYD3 compares to hitting MAP3K2 or other targets in the MAPK pathway. “The beauty and the weakness of hitting SMYD3 is that this is not an essential protein for MAPK signaling,” said Sage. “Thus, drugs against SMYD3 are not going to have as many side effects as direct inhibition of the pathway. On the other hand, they may not be as effective as drugs that hit the main components of the pathway.”

One concern is that SMYD3 could be a player in just one branch of the multipronged MAPK pathway. If so, compensatory changes to other branches of the pathway could render SMYD3 inhibitors ineffective.

However, the Stanford-GSK team showed that *SMYD3* knockdown reduced phosphorylation of multiple downstream MAPK targets, suggesting that SMYD3 plays a broad role in amplifying proliferative signaling. Thus, it is possible SMYD3 has other downstream effectors besides MAP3K2.

The principal advantage of hitting SMYD3 over MAP3K2 may be a better safety profile. Sage said that *Smyd3* knockout mice appear to be healthy, so pharmacological blockade of the target is likely to be well tolerated.

“If you have cancers driven by *KRAS* and have high levels of SMYD3 or methylation of MAP3K2, these are good candidates for SMYD3 inhibition.”

— Or Gozani, Stanford University

Sage and Gozani think that SMYD3 inhibitors should be paired with compounds such as Mekinist that target core components of MAPK signaling. Indeed, in the *Nature* study Sage cited evidence that SMYD3⁻ cells were especially sensitive to Mekinist. *In vitro* and in mouse xenografts, growth of tumor cells lacking SMYD3 was inhibited by a smaller dose of the compound than that needed for wild-type tumor cells.

“We showed deleting SMYD3 could improve the effective dose of MEK inhibitors,” said Sage. He noted that drug resistance is more likely to develop when a single drug is given at high doses, so achieving a potent antiproliferative effect with a lower dose of Mekinist “together with SMYD3 inhibitors could reduce the potential for resistance.”

The next biology question is whether SMYD3 plays a role in other tumors besides the *KRAS*-mutant pancreatic and lung cancers examined in the *Nature* paper. “It will be important to understand whether the modulation of MAPK activity by SMYD3 could be used

outside of *KRAS*-driven tumors,” said Barbash. She noted that MAPK pathway abnormalities also occur in some liver cancers.

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