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NSD2 momentum

By Chris Cain, Senior Writer

Although NSD2 has been genetically linked to multiple myeloma for more than 15 years, drug discovery efforts against the target have lagged behind other histone methyltransferases including DOT1L and EZH2. Now, a **Novartis Institutes for BioMedical Research** and **Broad Institute of MIT and Harvard** collaboration and an independent **Northwestern University** team have identified activating mutations in *NSD2* that drive a subset of leukemias,[1,2] and a second Novartis team has made the best case to date that inhibiting the protein could help treat MM.[3]

DOT1L (histone methyltransferase DOT1L) and EZH2 (enhancer of zeste homolog 2) drive genetically defined subsets of leukemia. In the last year, **Epizyme Inc.** and its partners **Celgene Corp.** and **Eisai Co. Ltd.** have advanced inhibitors of the proteins into the clinic, and multiple companies have inhibitors of EZH2 in preclinical development.

Based largely on preclinical results and high expectations for these programs, Epizyme went public in June and now boasts a market cap of more than $1 billion.

NSD2 (nuclear SET domain–containing protein 2; MMSET; WHSC1) also drives a genetically defined subset of blood cancers, but until recently comparatively little was known about its functional role in disease. About 20% of multiple myeloma (MM) cases are caused by the t(4;14) chromosomal translocation, one result of which is increased expression of *NSD2*.

However, the translocation also drives expression of *fibroblast growth factor receptor 3* (*FGFR3*; *CD333*), and for many years the contribution of NSD2 to tumor development in these patients remained unclear.

Recent studies have strengthened the case that NSD2 drives tumorigenesis by increasing histone H3K36 dimethylation,[4,5] but the therapeutic potential of the target is relatively unexplored.

To build the case for targeting NSD2 in MM, a team of Shanghai-based Novartis researchers set out to detail the effect of inhibiting the target *in vivo* and confirm the mechanism by which it acts.

In bone marrow stromal adhesion assays, small hairpin RNA knockdown of *Nsd2* in multiple t(4;14)$^+$ MM cell lines decreased adhesion and proliferation compared with no knockdown, and knockdown had no effect in t(4;14)$^-$ lines. In mice injected with t(4;14)$^+$ MM cells, shRNA knockdown of *Nsd2* delayed tumor formation and disease progression.

t(4;14)$^+$ MM cells with *Nsd2* knockout did not form tumors in mice. Adding back wild-type *Nsd2* restored tumor formation, but adding back a catalytically dead mutant version of *Nsd2* did not. This strongly suggests that NSD2's methyltransferase activity is required to drive MM.

Finally, the team showed that the PHD2 domain of NSD2, which is one of many regions that mediate its binding to histones, also is required for oncogenic activity. The group thus suggested that disrupting the catalytic nuclear SET domain or the PHD2-substrate binding interface could effectively inhibit the target.

Results were published in *Cancer Research*. The team was led by Min Hu, an oncology investigator at the Shanghai campus of the Novartis Institutes for BioMedical Research (NIBR).

Activating NSD2

A separate collaboration between the Broad Institute and NIBR, as well as an independent team from the **Northwestern University Feinberg School of Medicine**, have bolstered the case for targeting NSD2 by uncovering a second genetically defined cancer driven by its activity.[1]

The NIBR-Broad team made the discovery as part of a large-scale effort to catalog histone modifications in the Cancer Cell Line Encyclopedia (CCLE).

To accomplish this, the researchers turned to targeted mass spectrometry, which enabled them to measure 42 distinct combinations of histone H3 modifications in 115 cell lines.

"The key advantage of the global mass spectrometry profiling is that it overcomes the requirement for highly selective antibodies and allows for the monitoring of several combinatorial histone marks," Frank Stegmeier, director of oncology at NIBR and co-corresponding author of the work, told *SciBX*.

The team identified multiple clusters of cell lines with known mutations in histone-modifying enzymes. For example, one cluster had increased H3K27 trimethylation and decreased H3K27 mono- and dimethylation, a hallmark of *EZH2* mutation. As expected, this cluster was comprised entirely of B cell lymphomas with a mutation in *EZH2* that alters its histone substrate specificity.

A second cluster showed increased H3K36 dimethylation, but unexpectedly only 6 of the 13 lines in that cluster bore the t(4;14)$^+$ translocation that drives *NSD2* overexpression. The team sought to explain the pattern in the other seven cell lines and thus analyzed their gene expression, copy number and mutational status.

Surprisingly, these seven lines all contained an E1099K mutation in *NSD2*, and six of the seven were acute lymphoblastic leukemia (ALL) lines that were primarily of pediatric origin. Additional sequence analysis of 1,021 pediatric cancer samples identified the mutation in 18 out of 239 B cell ALL cases and in no other pediatric cancer types.

The Northwestern team independently came to the same conclusion by analyzing the publically available database of genetic alterations in the CCLE and confirming increased H3K36 dimethylation and decreased H3K27 methylation in cells bearing the mutation.[2] Jonathan Licht, chief of the division of hematology/oncology at Feinberg and the **Robert H. Lurie Comprehensive Cancer Center of Northwestern University**, told *SciBX* that in their study his team also sequenced 200 adult patients with ALL and found no instances of the mutation.

Thus, he argued that it is largely confined to pediatric cases of ALL, though he noted that the mutation had been previously found in single

cases of chronic lymphocytic leukemia (CLL) and lung and stomach cancer. Licht was a co-corresponding author on the study, which was published in *Leukemia*.

The NIBR-Broad team went a step further and characterized the effect of the mutation in detail. *In vitro* assays showed that the E1099K mutation increased the activity of NSD2 on nucleosome substrates compared with wild-type NSD2.

Finally, the group sought to determine the therapeutic potential of inhibiting E1099K NSD2 in ALL. In six cell lines carrying the mutation, shRNA knockdown decreased growth compared with no knockdown. In mice with a subcutaneously injected E1099K ALL cell line, shRNA knockdown reduced tumor growth.

Results from the Novartis-Broad team were published in *Nature Genetics*. The Broad group was led by Levi Garraway, who is an associate professor at **Harvard Medical School**, an assistant professor at the **Dana-Farber Cancer Institute** and a principal investigator at the Broad Institute.

"The data presented in this paper provide compelling evidence of a driver role for the E1099K mutant of NSD2 in a subset of pediatric ALL," said Epizyme EVP and CSO Robert Copeland.

He added that the approach used in the paper may have broad utility for target discovery. "I think the mass spectral method used here is quite elegant and may prove useful in broad efforts at target identification and target credentialing."

"Druggability of the histone methyltransferase protein family seems variable, and NSD2 lies on the more challenging end of the spectrum."

—Harren Jhoti, Astex Pharmaceuticals Inc.

NSD2 challenges

Although the evidence that inhibiting NSD2 could have therapeutic benefit continues to build, the protein is still a challenging target.

Novartis is tight-lipped about any plans relating to NSD2 inhibitors, and although at least two academic teams and two companies are pursuing the target, their results have not yet borne fruit.

Licht said that his team is attempting to discover inhibitors of NSD2 using peptide-based screening and computational modeling. He suggested that progress in developing compounds that hit the target may be slow because it is hard to work with *in vitro*. "It is a difficult protein to work with, it seems to fold poorly *in vitro* and it is more active on assembled nucleosome substrates than on peptides," he said.

Astex Pharmaceuticals Inc. president Harren Jhoti agreed. "Druggability of the histone methyltransferase protein family seems variable, and NSD2 lies on the more challenging end of the spectrum. High throughput screening approaches are problematic because NSD2 is a weakly active enzyme in the recombinant setting and requires whole nucleosomes as substrates," he said.

He added, "Corporate compound collections may not contain the diversity required to find tractable hits. On the other hand, structure-based approaches are hampered by the lack of a crystal structure for NSD2, and although a structure is available for the close homolog NSD1, there are likely to be significant differences."

Licht said that a crystal structure would help, although attempts to crystalize NSD2 thus far have been unsuccessful.

In 2012, Astex began collaborating with **The Institute of Cancer Research** and **Cancer Research Technology Ltd.** to develop NSD2 inhibitors.[6] Astex is being acquired by **Otsuka Pharmaceutical Co. Ltd.**

Despite the challenges, Jhoti said that the clear next step "is to validate NSD2 as a therapeutic target by testing the effects of a small molecule inhibitor in E1099K-mutant ALL and t(4:14) multiple myeloma tumor models."

Irfan Asangani, a research investigator at the **University of Michigan Medical School**, told *SciBX* that it is only a matter of time before potent NSD2 inhibitors are developed. "I predict we will see a great NSD2 inhibitor within the next couple of years," he said.

Asangani is named on a patent filed by the University of Michigan covering inhibitors of NSD2, but thus far no data for the compounds have been published. Earlier this year, Asangani published work showing that NSD2 acts downstream of EZH2 in some cancers.

OncoFusion Therapeutics Inc., a spinout of the University of Michigan Medical School founded by Asangani's mentor, professor of pathology Arul Chinnaiyan, is developing NSD2 inhibitors. The company did not return calls seeking comment.

Asangani said that his lab is continuing to develop NSD2 inhibitors independently from the company. He added that *NSD2* is overexpressed in many cancer types, as is *EZH2*, so inhibitors of NSD2 could have utility outside of these genetically defined populations.

Nevertheless, Copeland said that interest in NSD2 as a drug target would largely be driven by the MM population. "While this is an interesting example of a genetic lesion conferring dependence of a specific cancer on a particular histone methyltransferase, I think that the unmet medical need and patient population size of t(4;14)$^+$ multiple myeloma will remain the key driver for drug discovery and development of inhibitors of NSD2. If such compounds also inhibit the E1099K mutant of NSD2, there would be opportunity to test the compounds as therapeutics for this pediatric indication."

Copeland did not disclose whether Epizyme is developing inhibitors of NSD2.

Novartis also did not disclose whether it is developing NSD2 inhibitors, but Stegmeier told *SciBX* that a patent has been filed by the NIBR-Broad team covering the discovery of oncogenic NSD2-E1099K mutations and molecular chromatin signatures that predict response to NSD2 inhibition.

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COMPANIES AND INSTITUTIONS MENTIONED

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