SKPping out on cancer

By Tim Fulmer, Senior Writer

Researchers from Harvard Medical School and The University of Texas M.D. Anderson Cancer Center have shown that knocking out a

protein called S phase kinase-associated protein 2 (SKP2) triggers a robust response against tumors in mice, apparently through increased cellular senescence.¹ Although it may be difficult to design protein-protein inhibitors to specifically target SKP2 interactions, researchers have shown proof of principle with a compound from **Millennium Pharmaceuticals Inc.** that targets the protein complex containing SKP2.

SKP2, an F-box protein that is part of the E3 ubiquitin ligase SCF^{SKP2}, has been associated with increased rates of tumorigenesis² (see **Figure 1, “Blocking SKP2 induces senescence in cancer cells”**).

A Harvard and M.D. Anderson group, led by Pier Paolo Pandolfi, had previously shown that abnormally high cytosolic levels of SKP2 correlated with the loss of function of phosphatase and tensin homolog deleted on chromosome ten (PTEN; MMAC1; TEP1), a

Figure 1. Blocking SKP2 induces senescence in cancer cells. S phase kinase-associated protein 2 (SKP2), an F-box protein that is part of the E3 ubiquitin ligase SCF^{SKP2}, has been associated with increased rates of tumorigenesis before.² New research published in *Nature* now shows that blocking SKP2 triggers cellular senescence mechanisms that can lead to reduced levels of tumorigenesis.¹

[a] SCF^{SKP2} is composed of SKP1, cullin 1 (CUL1) and the F-box protein SKP2. In addition, CUL1 interacts with ring-box 1 (RBX1; ROC1), which in turn binds to an E2 ubiquitin-conjugating enzyme. Binding of CUL1 to neural precursor cell expressed developmentally downregulated 8 (NEDD8) is further required to activate SCF^{SKP2}. SKP2 triggers the transfer of ubiquitin from the E2 ubiquitin-conjugating enzyme to one of SKP2's many substrates, including cyclin-dependent kinase inhibitor 1A (CDKN1A; p21; CIP1) and phosphorylated CDKN1B (p27; KIP1), an inhibitor of cell growth.

[b] Once ubiquitinated, these substrates are targeted to the 20S proteasome, in which they undergo proteolysis.^{9,10}

[c] The new research shows that blocking SKP2 activates cellular senescence pathways leading to reduced tumorigenesis.

tumor suppressor, in human colon and prostate cancer samples.³ Thus, they hypothesized that reducing SKP2 could generate a strong effect in cancers characterized by altered expression of tumor suppressors like PTEN.

Pandolfi is professor of medicine at Harvard Medical School and director of the Cancer Genetics Program at the **Beth Israel Deaconess Medical Center**. His group now has tested their hypothesis using *Skp2* knockout mice.

In cultured mouse embryonic fibroblasts, double knockout of *Pten* and *Skp2* led to slower growth rates and significantly increased cellular senescence compared with what was seen in single-knockout fibroblasts or wild-type fibroblasts ($p < 0.001$). The increased cellular senescence also correlated with higher levels of cyclin-dependent kinase inhibitor 1B (CDKN1B; p27; KIP1), a cell-cycle inhibitor.

Cellular senescence is a mechanism of tumor suppression triggered by a variety of oncogenic stress signals, including DNA damage, reactive oxygen species (ROS) and loss of or mutation in tumor suppressor genes such as *PTEN* and *K-Ras*.^{4,5} When loss of a tumor suppressor occurs, senescence pathways can kick in, blocking cell proliferation and preventing progression of tumor growth.

The researchers next tested whether the increased cellular senescence translated into decreased tumorigenesis in two murine models of cancer.

In *Pten*^{+/-} knockout mice, which are highly susceptible to developing adrenal tumors, additional *Skp2* knockout significantly reduced the incidence of adrenal tumors after one year compared with that in *Pten*^{+/-} knockout mice expressing *Skp2* ($p < 0.0001$). *Pten*^{+/-} and *Skp2* double knockout mice survived significantly longer than *Pten*^{+/-} single knockout controls ($p = 0.0013$).

In prostate-specific *Pten* knockout mice, which model an aggressive form of prostate cancer, additional *Skp2* knockout led to significantly lower anterior prostate tumor mass than did *Skp2* expression ($p < 0.05$).

Taken together, the experiments suggest that inhibition of SKP2 in combination with prior loss of *PTEN* leads to activation of cellular senescence and inhibition of tumor formation (see **Figure 1, “Blocking SKP2 induces senescence in cancer cells”**).

The final question was whether small molecule SKP2 inhibitors would block tumorigenesis by triggering cellular senescence pathways. However, because there are no disclosed SKP2 inhibitors in development, the team settled on a different experiment: inhibit the entire SCF^{skp2} complex and look for senescence.

To do that, the researchers turned to MLN4924, an inhibitor of NEDD8 activating enzyme (NAE), which is a component of the E3 ligase that is required for the ligase's activity. The compound, from the Millennium unit of **Takeda Pharmaceutical Co. Ltd.**, is in Phase I testing to treat cancer.

Indeed, MLN4924 triggered cellular senescence in cultured *PTEN*^{-/-} human prostate cancer cells. In xenograft mice with the same prostate cancer cells, MLN4924 significantly reduced tumor weight compared with vehicle ($p = 0.002$).

Previous work had shown that MLN4924 kills cancer cells via apoptosis.⁶ The *Nature* findings now show that the compound “can also block cancer cell growth through the induction of senescence,” Millennium CSO Joseph Bolen told *SciBX*.

Like senescence, apoptosis is an antiproliferative defense mechanism triggered by cellular stress signals. However, rather than blocking further cell growth, as occurs in senescence, apoptosis triggers outright cell death.

Because MLN4924 targets a different portion of the SCF^{SKP2} complex than a SKP2 inhibitor would, the implication was that inhibiting the SCF^{skp2} complex might generally suppress tumorigenesis via cellular senescence pathways.

The study, which was published in *Nature*, was led by Pandolfi and Hui-Kuan Lin, assistant professor of molecular and cellular oncology at M.D. Anderson.

Attacking SKP2

Lin said he is now interested in looking for SKP2 activity in a variety of other cancer tissues “to see how universal SKP2-mediated pathways are in cancer.”

Mouse models are an important part of that work. “We are conducting studies of SCF^{SKP2} complex inhibition in several mouse models of human cancer to better understand the genetic backgrounds in which the strategy works best,” said Pandolfi.

In parallel with those studies, Lin said he is also designing and screening SKP2 inhibitors, with hopes of identifying a small molecule that disrupts the function of the SCF^{SKP2} ligase and triggers tumor suppressive senescence pathways, he said.

“Identifying drug-like compounds that inhibit SKP2 function could be a daunting task” because small molecules have a hard time disrupting interactions between two proteins, noted Bolen.

According to Bolen, there are at least two reasonable approaches to inhibiting SKP2, and both would likely involve targeting protein-protein interactions. The first is blocking the protein's substrate-binding domain. The second involves altering the way SKP2 presents its substrate to the E3 ubiquitin ligase in order to prevent ubiquitination.

Lin said he is open to investigating all possible approaches and that the existence of multiple X-ray crystal structures of SKP2 bound to other proteins, including SKP1 (ref. 7), could help guide the team's discovery efforts.

Another researcher, Liang Zhu, is already using X-ray crystal structures to guide the development of small molecule SKP2 inhibitors. Zhu is professor of medicine and developmental and molecular biology at **Albert Einstein College of Medicine of Yeshiva University**.

“Using the published X-ray crystal structure of the SKP1-SKP2-p27-CKS1 complex as our guide, we have identified a protein-protein interface that we believe is amenable to inhibition by small molecules, and we are now in the process of designing and synthesizing those inhibitors,” said Zhu. p27 is a SKP2 substrate; CDC28 protein kinase

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Medicine of Yeshiva University**

regulatory subunit 1B (CKS1B; CKS1) is a cyclin-dependent kinase that interacts with SKP2 and p27.

Zhu told *SciBX* that he is not disclosing the identity of the interface at this time. However, using a combination of *Skp2* knockout mice and mutant *p27* knock-in mice, he and colleagues have shown that a phosphorylated form of p27 plays a critical part in mediating SKP2's role in tumorigenesis.⁸

The *Nature* findings are neither patented nor available for licensing, said Pandolfi.

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REFERENCES

1. Lin, H.-K. *et al. Nature*; published online March 18, 2010; doi:10.1038/nature08815
Contact: Pier Paolo Pandolfi, Harvard Medical School, Boston, Mass.
e-mail: ppandolf@bidmc.harvard.edu

2. Herskho, D. *Cancer* **112**, 1415–1424 (2008)
3. Lin, H.-K. *et al. Nat. Cell Biol.* **4**, 420–432 (2009)
4. Courtois-Cox, S. *et al. Oncogene* **27**, 2801–2809 (2008)
5. Collado, M. & Serrano, M. *Nat. Rev. Cancer* **10**, 51–57 (2010)
6. Soucy, T. *et al. Nature* **459**, 732–736 (2009)
7. Hao, B. *et al. Mol. Cell* **20**, 9–19 (2005)
8. Wang, H. *et al. Nat. Genet.* **42**, 83–89 (2010)
9. Cardozo, T. & Pagano, M. *BMC Biochem.* **8**, S9 (2007)
10. Cardozo, T. & Pagano, M. *Nat. Rev. Mol. Cell Biol.* **5**, 735–751 (2004)

COMPANIES AND INSTITUTIONS MENTIONED

Albert Einstein College of Medicine of Yeshiva University,
New York, N.Y.

Beth Israel Deaconess Medical Center, Boston, Mass.

Harvard Medical School, Boston, Mass.

Millennium Pharmaceuticals Inc. (NASDAQ:MLNM),
Cambridge, Mass.

Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan

The University of Texas M.D. Anderson Cancer Center,
Houston, Texas