Inhibiting E3 ligases

By Tim Fulmer, Senior Writer

Separate groups of researchers have identified highly selective small molecule inhibitors of E3 ubiquitin ligase,^{1,2} a large multi-subunit complex involved in protein degradation that has been considered difficult, if not impossible, to target. The compounds could represent a new class of cancer therapeutics that are more specific for malignant cells than previous drugs directed toward the proteasome.

The E3 ubiquitin ligase complex is a key component of the ubiquitin-proteasome system, the cell's chief mechanism of breaking down proteins. Dysfunction of the pathway occurs in a variety of cancers, including lymphomas and prostate, bladder and breast cancer.^{3,4}

There is one marketed drug that targets the proteasome—Velcade bortezomib—and there are no disclosed compounds in the clinic against E3 ubiquitin ligase. Velcade is marketed in the U.S. by **Takeda Pharmaceutical Co. Ltd.'s Millennium Pharmaceuticals Inc.** subsidiary to treat multiple myeloma (MM) and mantle cell myeloma. **Johnson & Johnson** has rights outside the U.S.

Despite its commercial success, Velcade leaves room for improvement, as the drug has poor selectivity for tumor cells over normal cells, resulting in off-target toxicity. Thus, the challenge in developing next-generation inhibitors of the ubiquitin-proteasome system is to find compounds that selectively target protein degradation pathways unique to tumors.

The latest work by two teams of researchers takes a new tack. Rather than go after the proteasome, the most downstream component of the protein degradation pathway, the groups focused on the E3 ubiquitin ligase complex, which is immediately upstream of the proteasome.

Unlike the proteasome, tremendous variation exists in the E3 ubiquitin ligase family, with at least 600 different complexes identified so far.^{5,6} Thus, targeting a specific E3 ligase associated with a particular cancer could reduce the likelihood of off-target toxicity.

The open question was whether such compounds existed or could be designed.

In the first study, a group led by Peter Kaiser and Jing Huang used a yeast-based screen to identify compounds that increased the activity of the mammalian target of rapamycin (mTOR; FRAP; RAFT1) inhibitor Rapamune rapamycin (sirolimus). Kaiser is an associate professor of biological chemistry at the **University of California, Irvine School of Medicine**, and Huang is an associate professor of molecular and medical pharmacology at the **University of California, Los Angeles**.

In a screen of 30,000 molecules, the researchers identified 86 small molecule enhancers of rapamycin, dubbed SMERs, that were lethal to

yeast in combination with rapamycin. Of those 86 molecules, SMERs 1–5 were chosen for further study based on their levels of lethality and structural features.

To hone in on the possible targets of those SMERs, the researchers used DNA microarrays to study the compounds' effects on genome-wide protein transcription. The standout was SMER3, which had a transcription profile distinct from the others.

In a reaction mixture, SMER3 blocked ubiquitination of transcriptional activator Met4, a substrate of an E3 ubiquitin ligase called SCF^{Met30}, which is a key regulator of the cellular pathway that controls homeostasis of sulfur-containing compounds such as methionine in yeast.

The compound did not block ubiquitination reactions catalyzed by SCF^{Cdc4}, another E3 ligase. Thus, SMER3 was deemed a potent and selective inhibitor of SCF^{Met30}, which plays a critical role in cell division and could contribute to a proliferative phenotype in cancer.

The California researchers, reporting in *Nature Biotechnology*, concluded that “SMER3 and SMER3-like molecules represent a class of E3 ubiquitin ligase inhibitors that can potentially be used as anticancer drugs in the future.”

In the second study, researchers led by Mike Tyers, professor of cell biology at **The University of Edinburgh**, and Frank Sicheri, professor of molecular genetics at the **University of Toronto**, focused on identifying inhibitors of SCF^{Cdc4}, which is the yeast homolog of human SCF^{FBW7}, an E3 ligase implicated in cancer,⁷ metabolic disease⁸ and stem cell renewal.⁹ Sicheri and Tyers are also senior investigators in the Samuel Lunenfeld Research Institute at **Mount Sinai Hospital** in Toronto.

The researchers previously determined the X-ray crystal structure of a portion of SCF^{Cdc4} bound to a phosphorylated peptide derived from one of its substrates.¹⁰ In their new paper, published in *Nature Biotechnology*, the group screened a 50,000-compound library for inhibitors of that interaction.

There were 44 compounds that inhibited the SCF^{Cdc4}-substrate interaction by at least 50%. One of the top hits, denoted SCF-I2, blocked ubiquitination of SCF^{Cdc4} substrates Sic1 and Far1 without showing activity against SCF^{Met30}, a closely related and structurally similar E3 ligase.

Subsequent X-ray crystallography studies showed that SCF-I2 worked by an allosteric inhibitory mechanism.

In a commentary accompanying the two papers, John Lydeard and J. Wade Harper from **Harvard Medical School** wrote that the ability to target individual E3 ubiquitin ligases “would be expected to cause fewer side-effects than general blockade of the entire class of E3 ligases or the proteasome itself.”¹¹

Optimization

Going forward, both groups plan to optimize the compounds and do further testing in animal models of cancer.

The California team will continue to screen for SMERs and characterize SMER3 in rodent cancer models, corresponding author Huang told *SciBX*. She said the group also is working with Michael Jung,

professor of organic chemistry at UCLA, to design analogs of the SMERs with potentially improved potency and selectivity, she said.

Huang said the patent and licensing status of their findings is undisclosed.

The Canada-U.K. team will run additional screens to identify small molecule inhibitors of SCF^{FBW7}, said corresponding author Sicheri. In parallel with those screens, the researchers plan to use SCF-I2 as a scaffold to design inhibitors with better selectivity and potency. To help them do that, they will collaborate with Mark Bradley, professor of high throughput chemical biology at the University of Edinburgh.

Sicheri said his team's findings are not patented and are unavailable for licensing.

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REFERENCES

1. Aghajan, M. *et al. Nat. Biotechnol.*; published online June 27, 2010; doi:10.1038/nbt.1645
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2. Orlicky, S. *et al. Nat. Biotechnol.*; published online June 27, 2010; doi:10.1038/nbt.1646
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3. Eldridge, A.G. & O'Brien, T. *Cell Death Differ.* **17**, 4–13 (2010)
4. Hoeller, D. *et al. Nat. Rev. Cancer* **6**, 776–788 (2006)
5. Nalepa, G. *et al. Nat. Rev. Drug Discov.* **5**, 596–613 (2006)
6. Skaar, J.R. *et al. Cell* **137**, 1358–1358.e1 (2009)
7. Welcker, M. & Clurman, B.E. *Nat. Rev. Cancer* **8**, 83–93 (2008)
8. Sundqvist, A. *et al. Cell Metab.* **1**, 379–391 (2005)
9. Reavie, L. *et al. Nat. Immunol.* **11**, 207–215 (2010)
10. Orlicky, S. *et al. Cell* **112**, 243–256 (2003)
11. Lydeard, J.R. & Harper, J.W. *Nat. Biotechnol.* **28**, 682–684 (2010)

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