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Activating activators is strategy against apoptosis defectors

By Anette Breindl, Senior Science Editor

By combining an activator of the pro-apoptotic protein Bax with an inhibitor of the anti-apoptotic protein BCL-XL, researchers at Albert Einstein College of Medicine have been able to overcome resistance to apoptosis in both a wide range of cell lines and animal studies.

The team reported its findings in the March 7, 2022, issue of *Nature Communications*.

Programmed cell death, or apoptosis, is one of the key sticking points in cancer, and one of the hallmarks of cancer first proposed by Douglas Hanahan and Robert Weinberg in 2000.

Regardless of the molecular underpinnings, for tumors to grow, there has to be a mismatch between proliferation and cell death. Which means that in theory, reinstating apoptosis is a broadly useful strategy against tumors.

In practice, that promise has been tough to realize. There is one approved agent, Venclexta (venetoclax, Abbvie Inc.), that targets the anti-apoptotic protein BCL-2.

But there has also been a string of failures, owing to both toxicity and resistance issues.

Venclexta itself is approved in acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL), but not in any solid tumors where it has hit trouble in breast and small-cell lung cancers.

Because of its importance, apoptosis is tightly regulated by a multilayer system of pro-apoptotic and anti-apoptotic proteins. The so-called BH3-only proteins inhibit a group of anti-apoptotic proteins that normally bind to the pro-apoptotic Bax. When Bax is activated, it pierces the mitochondrial membrane in cooperation with another pro-apoptotic protein, Bak.

That piercing leads to leakage of mitochondrial proteins into the cytosol, which is a strong signal for cells to self-destruct.

Venclexta binds to BCL-2, pushing cells toward apoptosis by preventing BCL-2 from inhibiting Bax.

That strategy of "inhibiting the inhibitor" works to a degree. But because there are multiple anti-apoptotic proteins that can substitute for BCL-2, "now you can get apoptotic suppression by one of the brothers or sisters," Loren Walensky told *BioWorld*. "It's a whack-a-mole game, literally, and it's going on in real time in patients as we speak."

Walensky is a co-founder and chair of the scientific advisory board of Bakx Therapeutics Inc., which has licensed the technology underlying the findings. He is also a professor of pediatrics at Harvard Medical School, and a pediatric oncologist.

In contrast to those multiple brakes on apoptosis, Evripidis Gavathiotis told *BioWorld*, "there is one throttle, which is Bax."

Gavathiotis is the paper's senior author, professor of biochemistry and of medicine at Einstein, and is co-founder and scientific adviser of Bakx.

In the therapeutic world, activators have historically been much more challenging to develop than inhibitors, because binding sites for inhibitors tend to be more tractable by the classical tools of medicinal chemistry.

As a result, Gavathiotis said, drug discovery efforts have been concentrated "around one half of the mitochondrial apoptosis pathway… Everyone had worked around an anti-apoptotic protein" which would require the more tractable development of inhibitors to target.

In 2008, Gavathiotis, Walensky and their colleagues were able to identify a binding site for the peptide BH3 on Bax by using so called "stapled peptides," peptides that have been shape-stabilized by adding chemical bridges between specific amino acids.

BH3 is a peptide and one part, or domain, of the anti-apoptotic BCL-2 protein family. BH3-only proteins are a group of proteins that consist largely of the BH3 domain and bind to BCL-2 – and to Bax directly, but with key differences.

The site where the BH3 peptide binds to anti-apoptotic proteins "is a deep groove," Walensky explained. "And these peptides, when they went into the groove, they underwent folding. This Bax site was more shallow than that, so when people were doing peptides, they never picked up on this binding site…. The prefolding of the helix enabled it to bind to that groove."

Using a peptide that was partially prefolded by stapling (or, technically speaking, by Ruthenium-catalyzed olefin metathesis)

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was "the key that opened the door for this discovery," Gavathiotis said. "That was the 'aha' moment for this entire project."

Binding site identified, the team proceeded to develop compounds that could directly activate Bax. In the work now published in *Nature Communications*, they showed that by combining a third-generation Bax activator, BTSA1.2, with the BCL-XL inhibitor and Venclexta precursor Navitoclax, they were able to kill multiple solid tumor cell lines that were resistant to either approach alone, as well as cell line and patient-derived xenografts of colorectal tumors in animal models.

Bakx is in preclinical studies with its Bax activator, BKX-001, in both solid tumors and hematological cancers. "Our immediate target is certain heme indications where we believe we have certain advantages, and a clinical path," co-founder and CEO Sree Kant told *BioWorld*.

In July 2021, Bakx inked a deal with Ipsen Pharma SA around BKX-001 that could eventually be worth more than $800 million in royalties and milestone payments. Ipsen was also an investor in the company's $25 million series A round, along with AB Magnitude Ventures Group and Sherpa Healthcare Partners. The series A round was announced in November 2021.

Targeting Bax is a possible strategy to get around the redundancy of anti-apoptotic proteins, which include BCL-2, BCL-XL, MCL-1 and others.

The company is also working on other apoptosis proteins.

"If you are a Lord of the Rings fan – we've got the one ring to rule them all, but we've got the other rings to get as well," Kant said. "It's the intent of the company is to bring all of these rings together."