Scientists unlock secret of death protein's activation

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Scientists at Dana-Farber Cancer Institute have identified a previously undetected trigger point on a naturally occurring "death protein" that helps the body get rid of unwanted or diseased cells. They say it may be possible to exploit the newly found trigger as a target for designer drugs that would treat cancer by forcing malignant cells to commit suicide.

Loren Walensky, MD, PhD, pediatric oncologist and chemical biologist at Dana-Farber and Children's Hospital Boston, and colleagues report in the Oct. 23 issue of the journal *Nature* that they directly activated this trigger on the "executioner" protein BAX, killing laboratory cells by setting in motion their self-destruct mechanism.

The researchers fashioned a peptide (a protein subunit) that precisely matched the shape of the newly found trigger site on the killer protein, which lies dormant in the cell's interior until activated by cellular stress. When the peptide docked into the binding site, BAX was spurred into assassin mode. The activated BAX proteins flocked to the cell's power plants, the mitochondria, where they poked holes in the mitochondria's membranes, killing the cells. This process is called apoptosis, or programmed cell death.

"We identified a switch that turns BAX on, and we believe this discovery can be used to develop drugs that turn on or turn off cell death in human disease by targeting BAX," said Walensky, who is also an assistant professor of pediatrics at Harvard Medical School.

BAX is one of about two dozen proteins known collectively as the BCL-2 family. The proteins interact in various combinations leading to either the survival of a cell or its programmed self-destruction. Cancer cells have an imbalance of BCL-2 family signals that drives them to survive instead of dying on command.

The late Stanley Korsmeyer, MD, an apoptosis research pioneer and Walensky's Dana-Farber mentor, had suggested that killer proteins like BAX could be activated directly by "death domains," termed BH3, contained within a subset of BCL-2 family proteins. He hypothesized that this activating interaction was a fleeting "hit-and-run" event, making it especially challenging for scientists to study the phenomenon.

As suspected, the proposed BAX-activating interactions could not be captured by traditional methods. "When you tried to measure binding of the BH3 subunits to BAX, you couldn't detect the interaction," explained Walensky. He recognized, however, that the BH3 peptides being used in the laboratory didn't retain the coiled shape of the natural BH3 domains that participate in BCL-2 family protein interactions. Walensky and his colleagues pioneered the design of "stapled" BH3 peptides, which contain a chemical crosslink that locks the peptides into their natural coiled shape. With biologically active shape restored, the stapled BH3 peptides bound directly to BAX and triggered its killer activity.

Defining how the activating peptides docked on BAX remained a formidable catch-22. In order to solve the structure of an interaction complex, it needed to be stable enough for analysis. In this case, the BH3 binding event itself triggers BAX to change its shape and self-associate to perform its killer function, rendering the activating interaction unstable by definition.

What if, Walensky proposed, you could set up the interaction of BH3 and BAX under laboratory conditions that caused it to be more stable or

proceed in slow motion? The plan was to adjust the potency of the stapled BH3 peptide so that, according to Walensky, "it was good enough to bind BAX, yet activate it just a bit more slowly so that we could actually study the interaction." The researchers would then look for any detectable shift in the three-dimensional structure of the BAX protein to help point them to the docking site.

The researchers used nuclear magnetic resonance (NMR) spectroscopy to monitor the arrangement of atoms in the protein. First authors of the Nature paper Evripidis Gavathiotis, PhD, of Walensky's laboratory and Motoshi Suzuki, PhD, of Nico Tjandra, PhD,'s laboratory at the National Institutes of Health, succeeded in generating pure BAX protein that could be put into solution with the stapled BH3 peptide -- the latter in increasing concentrations until it initiated a BH3-BAX interaction. Gavathiotis and Suzuki used the NMR technique to spot a group of BAX amino acids, the building blocks of proteins, which were affected by the addition of the stapled BH3 peptide.

"The discrete subset of amino acids that shifted upon exposure to the stapled BH3 peptide mapped to a completely unanticipated location on BAX," said Walensky. The long-elusive binding site on BAX that initiates its killer activity was revealed. "Because BAX lies at the crossroads of the cell's decision to live or die, drugs that directly activate BAX could kill diseased cells like in cancer and BAX-blocking drugs could potentially prevent unwanted cell death, such as in heart attack, stroke, and neurodegeneration," said Walensky.

Source: Dana-Farber Cancer Institute