How proteins talk to each other: Caspase-3 cleaves in unforeseen ways

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Investigators at Burnham Institute for Medical Research have identified novel cleavage sites for the enzyme caspase-3 (an enzyme that proteolytically cleaves target proteins). Using an advanced proteomic technique called N-terminomics, Guy Salvesen, Ph.D., professor and director of the Apoptosis and Cell Death Research program of Burnham's NCI-designated Cancer Center, and colleagues determined the cleavage sites on target proteins and found, contrary to previous understanding, that caspase-3 targets α-helices as well as unstructured loops. In addition, researchers found that caspase-3 and the substrates it binds to co-evolved.

The paper was published on September 20 in the journal *Nature Structural & Molecular Biology*.

Prior to this study, scientists believed that proteases primarily cleave in unstructured loops, unstable parts of proteins that are readily accessible. The discovery that caspase-3 also cleaves α-helices contradicts a current dogma and offers new insights into protein signaling pathways.

"This was a big surprise because there shouldn't be anything for a protease to grab onto in a helix," said Dr. Salvesen. "We found that the basic concept that they don't cleave to helices is wrong. However, though we've found that proteases can cleave helices, we don't believe that's their biological function."

In addition to determining cleavage sites, the team also determined

which interactions were "biologically significant." In other words which cleavages altered the function of the target protein and which ones had little impact.

The team tested the human caspase-3 and the Staphylococcal protease glutamyl endopeptidase (GluC) against the *Escherichia coli* (*E. coli*) proteosome. In a second set, the human caspase substrate was challenged with human caspase-3 . The researchers found cleavage sites using N-terminal proteomics (N-terminomics), in which cleaved substrates are tagged at an exposed edge (N-terminal) and analyzed though mass spectrometry. The data from these assays were then matched against lists of substrates in the Protein Data Bank. Notably, caspase-3 did not cleave *E. coli* proteins as effectively as it did human proteins. However, when hybrid human/*E. coli* proteins were constructed, cleavage was greatly improved, leading researchers to conclude that caspase-3 co-evolved with its human substrates.

Because they alter the functions of other proteins, proteases like caspase-3 are critical to cell signaling. Understanding how and where they interface with target proteins enhances our ability to understand the progress of diseases.

Source: Burnham Institute (news : web)