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FLIPping the kill switch

The amounts of different cFLIP isoforms help cells choose between life and death.

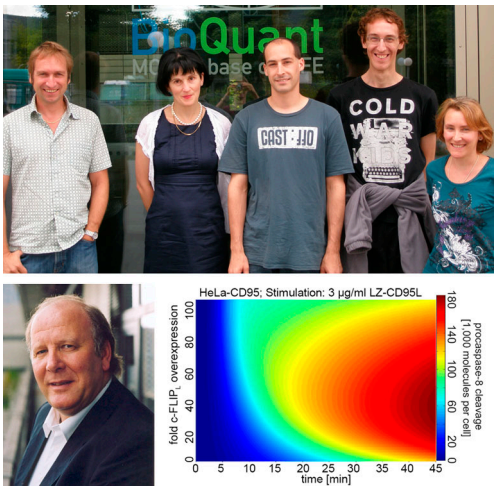
It's always wise to consult others before making a big decision, so a cell seeks input from a lot of different proteins before choosing between survival and death by apoptosis. Fricker et al. describe how one particular protein—cFLIP—can nudge a cell in either direction depending on the expression levels of its three different isoforms (1).

Cells contemplate suicide in response to a number of stimuli, including activation of the CD95 death receptor by its extracellular ligand CD95L (2). Receptor stimulation triggers the formation of a death-inducing signaling complex (DISC) at the plasma membrane containing inactive procaspase-8 molecules, which are converted by a series of self-cleavage events into active proteases that induce apoptosis (3). cFLIP, which exists as one long ($cFLIP_L$) and two short ($cFLIP_{S/R}$) splice variants, also localizes to the DISC and regulates the activation of procaspase-8. The short variants inhibit procaspase-8 cleavage and cell death, but the function of $cFLIP_L$ is more controversial: while some reports suggest $cFLIP_L$ is exclusively anti-apoptotic (4), others indicate that the longer isoform can promote apoptosis under certain conditions (5).

Inna Lavrik and colleagues at the German Cancer Research Center in Heidelberg decided to take a more quantitative approach to the problem. "Previous studies simply overexpressed or down-regulated cFLIP," explains Lavrik. "Ours is the first quantitative study to really show the conditions in which $cFLIP_L$ promotes apoptosis."

Fricker et al. found that $cFLIP_L$ could either block or accelerate procaspase-8 processing and cell death depending on its expression level and the strength of CD95 stimulation. The researchers then measured the absolute concentrations of all the important proteins known to function at the DISC in HeLa cells and generated a mathematical model describing how cFLIP influences the cell's life-and-death decision. The approach revealed a narrow window in which intermediate amounts of $cFLIP_L$ boost procaspase-8 cleavage and apoptosis.

Focal Point



Nicolai Fricker (middle), Inna Lavrik (second from left), and colleagues quantify components of the CD95 death-inducing signaling complex and generate a mathematical model to explain the complex effects of cFLIP isoforms on procaspase-8 activation and apoptosis. A long splice variant, $cFLIP_L$, can either promote or inhibit cell death depending on its own concentration, the amounts of shorter cFLIP isoforms, and the strength of signaling by the CD95 death receptor. The graph shows the model's experimentally verified prediction that only an intermediate amount of $cFLIP_L$ will boost procaspase-8 activation (indicated by warmer colors), while higher $cFLIP_L$ concentrations inhibit the initiation of cell death.

Nevertheless, says Lavrik, the results show that "the same molecule can have different functions in a signaling pathway, depending on its own concentration and the concentration of other proteins in the same pathway."

The reason for this seems to lie in two opposing properties of $cFLIP_L$. Like its shorter variants, $cFLIP_L$ has a high affinity for the DISC and competes with procaspase-8 for recruitment to the death receptor complex. Thus, at high concentrations, all cFLIP isoforms block procaspase-8's activation at the DISC. Unlike $cFLIP_{S/R}$ however, $cFLIP_L$ can form a catalytically active heterodimer with procaspase-8 in which $cFLIP_L$ stabilizes the protease's active loop. Therefore at intermediate concentrations $cFLIP_L$ stimulates procaspase-8 processing without blocking its recruitment to the DISC. The opportunity for $cFLIP_L$ to accelerate cell death is expanded if the CD95 death receptor is strongly stimulated by its ligand: more DISCs are induced, making it harder for $cFLIP_L$ to completely block procaspase-8 recruitment.

> "The same molecule can have different functions in a signaling pathway."

The amounts of the shorter cFLIP isoforms also influence $cFLIP_L$'s effects: when $cFLIP_{S/R}$ levels are high, the amount of procaspase-8 processing at the DISC is so low that any stabilization of the protease by $cFLIP_L$ will greatly accelerate the enzyme's activation and subsequent apoptosis. The choice between life and death is thus a complex decision involving the different cFLIP isoforms and the strength of CD95 activation. "It depends on the ratio of all these proteins at the DISC," says Lavrik.

Tweaking the levels of these proteins can therefore have a major impact—tumor cells are known to regulate CD95 signaling by changing the relative expression of long and short cFLIP isoforms. Lavrik now wants to transfer her model from HeLa to cancer cells, which may use additional proteins to regulate the outcome of life-and-death decisions. "The model nicely explains our data on all known components in HeLa cells," says Lavrik. "But there may be other modulators in different cell types, which we're trying to identify through various screening procedures."

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2. Suda, T., et al. 1993. *Cell.* 75:1169–1178.
3. Lavrik, I.N., et al. 2005. *J. Clin. Invest.* 115:2665–2672.
4. Sharp, D.A., et al. 2005. *J. Biol. Chem.* 280:19401–19409.
5. Chang, D.W., et al. 2002. *EMBO J.* 21:3704–3714.