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Delineating molecular mechanisms of c-FLIP isoforms as control checkpoints of DED filament assembly and caspase-8 activation

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Induction of apoptosis via CD95 and TRAILR1/R2 is triggered by the formation of Death-Inducing Signaling Complex (DISC) and death effector domain (DED) filaments. Caspase-8 activation at the DISC is largely controlled by c-FLIP proteins. Despite apparent progress in understanding the assembly of CD95-activated platforms and DED filaments, the detailed molecular mechanism of c-FLIP action remains elusive. To get more insight into this question we have created several mathematical models of CD95 network. The modeling has pointed out that c-FLIP is a key target in the network. Subsequently, we have rationally designed a chemical probe targeting c-FLIPL in the heterodimer caspase-8/c-FLIPL. This rationally designed small molecule was aimed to imitate the closed conformation of the caspase-8 L2' loop and thereby increase caspase-8 activity after initial processing of the heterodimer. In accordance with *in silico* predictions, the small molecule enhanced caspase-8 activity in the DISC, CD95L/TRAIL-induced caspase activation and subsequent apoptosis. A generated computational model provided further evidence for the proposed effects of the small molecule on the heterodimer caspase-8/c-FLIPL at the DED filaments. In particular, the model demonstrated that boosting caspase-8 activity at early time points after DISC assembly by caspase-8/c-FLIPL heterodimer is crucial to promote apoptosis induction. To further address the mechanism of action of c-FLIP isoforms, we have investigated their role at the DED filaments using quantitative mass spectrometry and structural modeling. These analyses uncovered that the overexpression of both c-FLIP isoforms leads to the formation of shorter DED filaments and an increase in the DISC quantities. Our data strongly indicate that c-FLIP can bind to both FADD and procaspase-8 at the DED filament. Moreover, the constructed *in silico* model shows that c-FLIP proteins can serve as bridging motifs for building a cooperative DISC network, in which adjacent CD95 DISCs are connected by DED filaments. These findings provide new insights into DISC and DED filament regulation and open innovative possibilities for targeting the extrinsic apoptosis pathway.