Measuring and Modeling Cell Death Pathways in Single Cells

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Receptor-mediated apoptosis is mediated by soluble ligands of the tumor necrosis factor (TNF) family and by a set of conserved receptors, caspases and regulatory proteins. The biochemical functions of these many of proteins have been determined but it remains poorly understood how they interact to create regulatory networks controlling responsiveness to natural ligands and therapeutic molecules. In particular, when a homogenous population of cells is exposed the TNF-related protein TRAIL, only a subset of cells die and those that do, die at very different times. If surviving cells are recovered and replated, they exhibit fractional killing indistinguishable from that of the parental cell population. Thus, cell-to-cell variability in the time and probability of apoptosis is a stable feature of cell populations.

I will describe the use of biochemical reconstitution, single-cell measurement, genome engineering and computational modeling to: (i) determine precisely how death inducing signaling complexes control initiator caspase activity in single cells (ii) dissect the regulation of MOMP by Bcl2 family members (iii) understand the origins and significance of non-genetic variability in determining fractional killing. I will describe two thresholds that control apoptosis – one operating at the level of receptor assembly (the DISC) and one at mitochondrial outer membrane permeabilization (MOMP) – and show how intrinsic fluctuation in these thresholds explains the poor potency of therapeutic agonists of TRAIL receptors such as Apomab (which failed in Phase II), and propose ways to design improved therapeutic molecules

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