A technique to distinguish two modes of immune cell killing on single cell level

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Death of cells in the body is involved in many diseases or injuries, but also occurs during development under physiological conditions. The killer cells of the immune system like cytotoxic T lymphocytes (CTL) or natural killer (NK) cells can eliminate malignant cancer cells or virus-infected cells by inducing cell death in their targets. Cell death can occur as a highly organized process during apoptosis or by plasma membrane disruption during necrosis. We have generated cell lines expressing a genetically-encoded FRET-sensor to quantify both necrosis and apoptosis in single living target cells by time-lapse fluorescent microscopy. We have observed that NK cells induce both types of cell death in a clonal population of target cells. Interestingly, individual NK cells can switch from necrosis to apoptosis during serial target cell killing. We postulate that the relative contribution of apoptosis and necrosis is important in regulating the immune response towards cancer and infection. We have also established a high-content protocol for this assay on an automated microscope and an analysis in a three-dimensional collagen matrix using light sheet fluorescence microscopy. This will enable us to use the assay for screening purposes and under conditions as physiological as possible.