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Selective and Random Patterning of Programmed Cell Death in Zebrafish Embryonic Development

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Programmed cell death (PCD) is thought as a well-controlled process by which unwanted cells are selectively eliminated. During the last decade many researches have elucidated molecules and their interactions involved in cell death by using largely *in vitro* induction of cell death or survival signals in a more defined manner. While these critical information and novel findings provide us with clearer understanding of mechanisms underlying cell death, it does by no means explain how PCD occurs and which cells or tissues are affected during normal embryonic development *in vivo*. In this study, we used zebrafish to examine whether the PCD is occurring selectively or randomly in developing embryos by whole mount *in situ* TUNEL analysis with specific markers for neural cells. The result revealed that the degree and distribution of TUNEL staining varied considerably throughout gastrulation stage, and there was also a number of TUNEL-negative embryos. Most of TUNEL-positive cells were scattered randomly throughout the blastoderm. During the gastrulation stage about 75 % of the embryos analyzed exhibited more than 5 TUNEL-positive cells. As the dorsal epiblast begins to thicken rather abruptly near the end of gastrulation, TUNEL-positive cells were mainly located along the dorsal side. Although there were some variations in TUNEL staining during segmentation and pharyngeal stages, TUNEL staining continued to be localized to the central nervous system, and was also detected in the sensory organs, trigeminal ganglions, and the primary sensory neurons. High levels of the cell death in developing brain between 20-somite and prim-6 stages are thought to play a role in the morphogenesis and organization of the brain. At prim-16 stage, cell death is considerably reduced in the brain region. Dying cells are mainly localized to the prospective brain region where ectodermal cells are about to initiate neurogenesis. As development progressed, high levels and more reproducible patterns of cell death were observed in the developing nervous system. Intensive TUNEL staining was restricted to the trigeminal ganglions, the primary sensory neurons, and sensory organs, such as olfactory pits and otic vesicles. Thus, PCD patterning in zebrafish embryos occurs randomly at early stages and becomes restricted to certain region of the embryos. The spatio-temporal pattern of PCD during the early embryonic development in zebrafish will provide basic information for further studies to elucidate genes involved in regulation of PCD largely unknown *in vivo* during vertebrate embryogenesis.