Department of Nanobio Drug Discovery

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Research Projects:

Apoptosis, or programmed cell death, plays an important role in many biological processes, including embryogenesis, development of immune system, maintenance of tissue homeostasis, and elimination of virus-infected and tumor cells. We found cell surface Fas antigen (Fas), which can directly mediate apoptosis-inducing signals into cells by stimulation with agonistic anti-Fas monoclonal antibodies or Fas ligand. Our main research project is to understand the intracellular signal transduction mechanism of cell death including apoptosis and caspase-independent novel types of cell death, and the biological significance/physiological role of cell death and cell death-regulating molecules. In conjunction with these studies, we have been trying to identify other cell death-related molecules that play a key role in embryogenesis, tumorigenesis or immune system. Investigations of molecular mechanisms and physiological roles of cell death and cell death-related molecules are important for a better understanding of mammalian embryogenesis, tumorigenesis and immune system.

1. Interferon-γ (IFN-γ)-induced programmed necrosis/ necroptosis

We found that not only tumor necrosis factor (TNF) but also IFN-γ could induce necroptosis when caspase-8-dependent apoptotic pathway was inhibited. Although necroptosis has been considered to be induced by the function of RIPK1 and RIPK3 to activate MLKL which executes necrosis by inducing plasma membrane rupture (Figure 1), we found that RIPK1 is not necessary, but RIPK3 and MLKL are necessary, to induce necroptosis in the absence of caspase-8 protein. Protease activity of caspase-8 suppresses RIPK1 and RIPK3 to inhibit necroptosis, but adaptor activity of caspase-8 is necessary for RIPK1 to activate RIPK3 and MLKL.

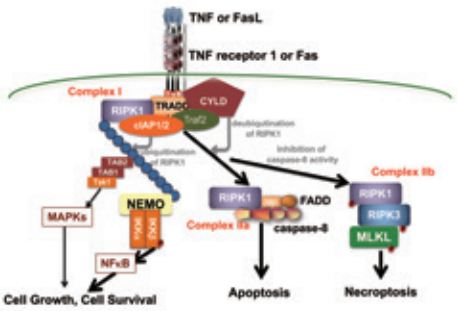

Figure 1. Cell death and survival signals mediated by Death Receptors

Phosphatidylserine (PS), an anionic phospholipid enriched in the inner leaflet of the plasma membrane, is exposed to the outer leaflet during apoptosis and the cell surface PS plays an essential role in apoptotic cells to be engulfed by macrophages as "eat me" signal. PS exposure was recently shown to be induced during TNF-induced necroptosis. We found that PS exposure mediated by RIPK3-activated MLKL was induced by a treatment with IFN-γ for more than 10 hours before the induction of necroptosis by membrane rupture. We suppose that the main activity of IFN-γ may be the induction of PS exposure, not necroptosis.

2. Retinoic acid (RA) signaling is regulated by the signaling molecules of necroptosis.

We found that knockdown of *Caspase-8* expression in embryoid bodies derived from ES cells markedly enhances RA-induced cell differentiation and necroptosis, both of which are dependent on *Ripk1* and *Ripk3*; however, the enhancement of RA-induced cell differentiation is independent of *Mlkl*. RA treatment obviously enhanced the expression of RA-specific target genes having the RA response element in their promoters to induce cell differentiation, and induced or enhanced the expression of RIPK1, RIPK3 and MLKL to stimulate necroptosis. *Caspase-8* knockdown induced RIPK1 and RIPK3 to translocate into the nucleus and to form a complex with RA receptor (RAR), and RAR interacting with RIPK1 and RIPK3 showed much stronger binding activity to RA response element than RA receptor without RIPK1 or RIPK3. In *Caspase-8*-deficient as well as *Caspase-8*- and *Mlkl*-deficient mouse embryos, the expression of RA-specific target genes and the expression of RIPK1, RIPK3 and MLKL were obviously enhanced. Thus, Caspase-8, RIPK1, and RIPK3 coordinately regulate RA-induced cell differentiation and necroptosis both *in vitro* and *in vivo* (Figure 2).

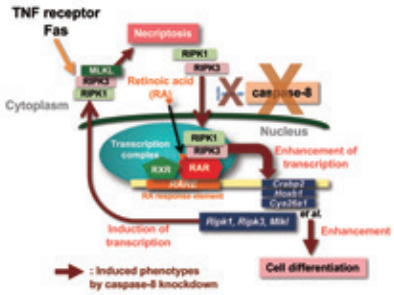

Figure 2. A model of the retinoic acid signaling in the presence and absence of Caspase-8.

3. A novel type of cell death specifically induced in carcinoma-derived cells.

By utilizing a tetracycline/doxycycline (Dox)-inducible short hairpin RNA (shRNA) expression (Tet-On) system, we established various cell lines from untransformed and transformed cells, in which knockdown of specific target genes could be induced by the treatment with Dox. Among these cells, induced knockdown of the expression of SMC2 or CAPRIN1 was shown to induce cell death specifically in transformed cells derived from human carcinoma. In case of knockdown of SMC2, a component of the condensin complex, a couple types of cell death including apoptosis and nonapoptotic cell death were observed in carcinoma-derived tetraploid cells, while a senescence-like phenotype associated with cell growth retardation was induced in untransformed diploid cells. In case of CAPRIN1 knockdown, cell growth retardation was observed in all the cells; however, nonapoptotic cell death was observed specifically in carcinoma-derived cells. The cell death with comparatively normal nucleus is not known types of cell death dependent on caspase or RIPKs, and is associated with vigorous membrane raffling and enforced detachment (abruptio) from the substrate and neighboring cells (Figure 3), both of which are dependent on a small G protein, Rac1. We named the cell death to be "Abruptosis". We are now analyzing the molecular mechanism and physiological roles of these novel types of cell death.

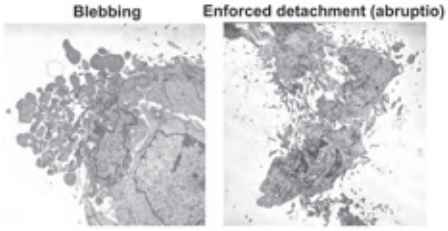

Figure 3. Analysis of Abruptosis under transmission electron microscope

Recent publications

- Shibata *et al.* 2017. Protein-driven RNA nanostructured devices that function in vitro and control mammalian cell fate. *Nat Commun*, **8**, 540, 2017.
- Chalabi-Dchar *et al.* Loss of somatostatin receptor subtype 2 promotes growth of KRAS-induced pancreatic tumors in mice by activating PI3K signaling and overexpression of CXCL16. *Gastroenterology*, **148**, 1452, 2015.
- Nakanishi *et al.* Dclk1 distinguishes between tumor and normal stem cells in the intestine. *Nat Genet*, **45**, 98, 2013.
- Fukuoka *et al.* 2013. Identification of a novel type 2 innate immunocyte with ability to enhance IgE production. *Int Immunol*, **25**, 373, 2013.