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学 位 論 文 要 旨
Dissertation Summary

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論　文　名: Studies on Caspase-dependent Signaling Mechanism
(Dissertation Title)

Life of an individual is organized under strict control of the balance between proliferation, differentiation and cell death. Among them, apoptosis, a form of programmed cell death, is indispensable for multicellular organisms, because the fundamental cellular response of apoptosis plays a crucial role in shaping developing bodies and in regulating tissue homeostasis by eliminating unwanted cells. Therefore, defects in the apoptotic program are implicated in a variety of diseases, such as maldevelopment, tumorigenesis, neurodegeneration disease and autoimmune disease. The complex process of apoptosis is orchestrated by caspase, a family of cysteine proteases with a unique specificity for cleaving their substrates after aspartic acid residues in target proteins. The apoptotic pathway is finely tuned by multiple signaling events, including direct phosphorylation of caspases, whereas protein kinases are often substrates of active caspases. In healthy cells, caspases are turned off by phosphorylation-mediated suppression in addition to other mechanisms. However, once cells receive a substantial stimulus that overcomes this suppression, caspases are turned on to cleave kinases. The caspase-mediated cleavage of kinases can terminate prosurvival signaling (e.g. loss of Akt kinase activity) or generate proapoptotic protein (e.g. cleaved ROCK1 and cleaved MST1). In this way, it seems that cross-regulation of caspases and kinases allows for fine-tuning of the apoptotic threshold. Thus, taken together, understanding how the balance between cell survival and cell death can be regulated through the crosstalk would be of great biological and medical importance.

To understand the big picture of caspase-dependent signaling, identification of caspase substrates is absolutely essential. Comprehensive characterization of protease substrates in

complex biological samples is limited by conventional proteomic methods such as mass spectrometry. It is difficult to detect a small amount of intracellular substrates and to identify specific pairs between proteases and substrates because numerous cleavage events occur simultaneously in cells. For this reason, an *in vitro* approach that could complement cell-based approaches is required. Prior to this study, we have developed a powerful screening method for substrate identification using protein array produced by a cell-free system and luminescence-based inter/intra molecular interactions detection technology. This method allowed me to identify 30 protein kinases as novel substrates for caspase-3 out of 304 kinases. In this study, I investigated the regulatory mechanism of apoptosis and cell differentiation underlying caspase-mediated cleavage of the protein kinases.

[CHAPTER I]

Pseudokinase TRB3 is a stress-inducible nuclear protein, which has recently been shown to be involved in ER stress-induced apoptosis. However, it remains unclear how TRB3 contributes to the process. We recently demonstrated that TRB3 was cleaved by caspase-3 (CASP3) *in vitro* and also in apoptosis-induced cells. In CHAPTER I, I thus investigated the role of TRB3 cleavage in the apoptotic process to address the above question. Overexpression studies revealed that the cleavage of TRB3 promoted CASP3/7 activation and apoptosis. In contrast, the anti-apoptotic effects were found under TRB3 non-cleavable conditions, such as ER stress, and also when the CASP3/7 activation was enhanced by knockdown of endogenous TRB3 expression. Interestingly, nuclear translocation of procaspase-3 (proCASP3) was observed in cells either overexpressing TRB3 or under tunicamycin-induced ER stress. Although forced cytoplasmic expression of proCASP3 enhanced apoptosis significantly, its nuclear expression did not produce any pro-apoptotic effect, suggesting that nuclear distribution of proCASP3 is not critical for the execution of apoptosis. Thus, TRB3 might prevent cytoplasmic activation of CASP3 by promoting proCASP3 entry into the nucleus, and thereby inhibit apoptosis. Taken together, my results suggest that TRB3, through its own cleavage, functions as a molecular switch between the cell survival and apoptotic pathways under stressful conditions.

[CHAPTER II]

Accumulating evidence suggests that caspase-3-mediated cleavage of protein kinase could be a key event to regulate cell differentiation as well as apoptosis. In CHAPTER II, I described the role of Nek5 kinase, identified as a novel substrate for caspase-3, in skeletal muscle differentiation. Up-regulation of Nek5 mRNA expression was accompanied by cell differentiation. The myotube formation was promoted in Nek5 expressing cells, and conversely, which was inhibited in Nek5 knockdown cells. Furthermore, I found that caspase-3 activity, an important factor for myogenic differentiation, was enhanced by Nek5 cleavage. Although caspase-3-cleaved Nek5 partially exerted promyogenic effect, it tended to induce apoptotic cell death. In summary, my findings suggest that Nek5 promotes myogenic differentiation through up-regulation of caspase activity.