学 位 論 文 の 要 旨

論文題目　　Study on the effects of Diindolylmethane on autophagy and apoptosis in fission yeast (ジインドリルメタンの分裂酵母オートファジーやアポトーシスへの効果の研究)

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Apoptosis is a process to kill the damaged and unrepairable cells to maintain health in multicellular organisms. Apoptosis is a promising target for cancer therapy because it induces cell death in cancer cells. Autophagy recycles the cellular components and can be induced by stress or nutrient starvation. Autophagy can also suppress the growth of cancer cells, therefore, autophagy induction could be useful in cancer therapy. A study of the drugs that induce apoptosis or autophagy would contribute to developing new anti-cancer drugs. 3,3'-Diindolylmethane (DIM) is one of the promising anti-cancer drugs that can kill the cancer cell trough the apoptosis induction. DIM is a compound derived from the digestion of indole-3-carbinol, found in the broccoli family. DIM induces apoptosis and autophagy in various types of human cancer. DIM extends lifespan in stationary-phase cells in the fission yeast *Schizosaccharomyces pombe*, which is a useful model organism. However, the mechanisms by which DIM induces apoptosis and autophagy in humans are not fully understood. The acute effects of DIM on log-phase cells in fission yeast are still unknown.

Here, by studying the early effects of DIM on fission yeast cells, I found that DIM possibly induces apoptosis and causes autophagy in log-phase cells, which is dose-dependent in fission yeast. I showed that a 10-min treatment is enough to kill the cells and trigger the possible apoptosis in cells. My results suggested that 10 min treatment with a high concentration of DIM (20μg/ml) possibly disrupts the nuclear envelope (NE) structure and induces chromosome condensation dramatically. Because mitochondrial ATPase is one of the target candidates of DIM in human cells, thus, the timing for ATP level reduction was compared to the timing for NE disruption by DIM (20μg/ml) in fission yeast. I found that NE disruption happens earlier than ATP level reduction. It implied that NE, not mitochondrial ATPase may be the earlier target of DIM.

In contrast to the high DIM concentration, a low concentration of DIM (5μg/ml) did not disrupt the structure of NE. My results showed that DIM (5μg/ml) induces autophagy in log-phase cells. DIM (5μg/ml) might mimic nitrogen starvation or sulfur depletion to induce autophagy, or unidentified pathway(s) may cause autophagy by DIM (5μg/ml) in fission yeast. I found that a mutant defective in autophagy (*atg7Δ*) is more sensitive to a low concentration of DIM (5μg/ml) than wild-type cells, demonstrating that the autophagic pathway contributes to the survival of cells against DIM.

Moreover, my results showed that the *lem2Δ* mutant is more sensitive to DIM. NE in the *lem2Δ* mutant is disrupted even at the low concentration of DIM (5μg/ml). There are some possibilities for the sensitivity of NE in *lem2Δ* cells to low DIM concentration. It may be due to the change in the fatty acid composition of NE in *lem2Δ* cells. In fission yeast, some fatty acids such as C24:0 fatty acid are required for membrane integrity and cell survival. In the absence of Lem2 and Bqt4 (nuclear membrane protein), some of the required fatty acids for membrane integrity are decreased. It is possible that even in *lem2Δ* single mutant, some of the required fatty acids are reduced, which may make cells more sensitive to DIM (5μg/ml). It is known that Lem2 acts as a barrier to membrane flow between the NE and other parts of the cellular membrane system. It seems that without Lem2 as a barrier, even low DIM concentration may dramatically affect the membrane flow between NE and ER membrane, therefore *lem2Δ* cells are sensitive to DIM. Another possibility is related to the potential role of Lem2 protein in the sealing process of NE by ESCRT-III complex. If it is assumed that DIM makes holes in NE at a low DIM concentration, the wild-type cells may repair the holes. However, cells without Lem2 have defects in the sealing of NE, thus, *lem2Δ* cells are more sensitive to the low DIM concentration.

Finally, my results demonstrated that the autophagic pathway and NE integrity are important to maintain the viability of log-phase cells in the presence of a low concentration of DIM. The mechanisms of apoptosis and autophagy induction by DIM might be conserved in fission yeast and humans. Further studies will contribute to the understanding of the mechanism of apoptosis and autophagy by DIM in fission yeast and humans.