Quantitative Analysis on Mitochondrial Apoptosis Pathway

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***Short Abstract* — Apoptosis is a biological process that eliminates the damaged or useless cells in order to maintain inner balance in organisms. It's responsible for many vital processes such as development, morphogenesis, homeostasis and deletion of dangerous cells. Escaping apoptosis can cause a lot of diseases such as cancer. As a vital process, apoptosis is regulated by a complex network. It can be divided into 2 pathways: extrinsic and intrinsic apoptosis network, which will give instructions to drug design for related diseases in future.**

***Keywords* — Apoptosis, Caspase 3, Caspase 9, XIAP**

I. Intruoduction

THE apoptotic mode of cell death is an active and defined process which plays an important role in the development of multicellular organisms and in the regulation and maintenance of the cell populations in tissues upon physiological and pathological conditions.[1]Apoptotic processes are of widespread biological significance, being involved in development, differentiation, proliferation, regulation and function of the immune system and in the removal of defect and therefore harmful cells. As a vital process, apoptosis is regulated by a complex network. [2][3][4] `In the vision of systems biology, the most important factor is the dynamics of Caspase3 activity in the mitochondria apoptotic pathway. The dynamics of Caspase3 mainly depends on the concentration of Caspase9, proteasome and XIAP. The questions I take most interest in are the network consists of these four nodes, in other words, I concerned about the dynamics of Caspase3 activity and apoptosis percentage which are caused by these three feedback loops. Based on experiments have been taken, I would like to take three measures to address my questions, the small molecule inhibitors, knocking down the expression of Caspase9 and XIAP, and knocking out those genes in the means of CRISPR.

In order to monitor the dynamics of Caspase3, Goldstein et.al designed FRET reporter[5] which its principle is measuring the extent of fluorescence resonance energy transfer within a recombinant substrate containing cyan fluorescent protein (CFP) linked by a short peptide possessing the Caspase3 cleavage sequence, DEVD, to yellow fluorescent protein (YFP). When Caspase3 was not activated, we can see the yellow fluorescent (YFP) if it was given the excitation spectrum of CFP. While we can see the CFP when Caspase3 was activated because DEVD was cleaved by Caspase3. Rehm et.al monitored the dynamics of Casp3 using FRET report.[6] They found that Casp3 is always activated quickly and absolutely no matter what was the apoptotic inducing signal. We concerned about the dynamics of Caspase3 activity and apoptosis percentage which are caused by these three feedback loops.

II. Results

I used siRNAs to knock down the expression of Casp9 and XIAP. By the results of quantitative western blot, I successfully knocked down the expression of Caspase9 and XIAP with approximately 60% and 40% efficiency each. Then we will monitor the dynamics of Casp3 by the imaging analysis.

III. Conclusion

I successfully knocked down the expression of Caspase9 and XIAP with approximately 60% and 40% efficiency each.

In the following two years, I plan to knock out Caspase9 and induce exogenous Caspase9 dimerization in HeLa cells. By this way, I can work out the influence of Caspase9 dimerization on the three-node network consists of Caspase3, XIAP and Caspase9 that play a vital role in the mitochondria apoptotic pathway

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