Systems biology research on mitochondria apoptotic pathway in mammalian cells

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Cell apoptosis is a biological process that eliminates the damaged and useless cells in order to maintain inner balance in organisms. It is responsible for many vital processes such as development, morphogenesis, homeostasis and deletion of dangerous cells. Aberrant apoptosis can cause a lot of diseases such as cancer. As such a vital process, apoptosis is regulated by a complex network and it can be divided into 2 pathways: extrinsic and intrinsic pathway. We'd like to study the characteristics of intrinsic apoptosis network, which will give instructions to drug design for related diseases in future.

***Keywords* — systems biology, apoptosis, mitochondria, mammalian cells**

I. Purpose

APOPTOSIS removes superfluous or damaged cells from the body of multicellular organisms. Enhanced or repressed apoptotic cell death contributes to developmental defects, autoimmune diseases, cancer and neurological disorders[1]. Activation of effector caspases is a central and ultimate step in many apoptosis pathways. In the intrinsic pathway, the activation of effector caspases is triggered by mitochondrial outer membrane permeabilisation (MOMP) and the subsequent release of proapoptotic proteins into the cytosol. The release of cytochrome c (cyt-c) triggers the formation of the apoptosome, a multiprotein complex comprising apoptotic protease activating factor 1 (Apaf-1), procaspase 9, dATP/ATP and cyt-c. Apoptosome-bound caspase-9 subsequently activates effector caspases 3 and 7 that are responsible for most of the morphological and biochemical changes occurring during this type of active cell death. The signalling network gains complexity by additional caspase 3 dependent feedbacks[2].

What I am interested in is that the characteristics of the whole intrinsic apoptosis network. Firstly, as there are so many feedbacks in it, we'd like to apply the techniques of systems biology to find out their functions in apoptosis, which can make important guidance for co-drugging on cancer[3].

Secondly, as we all know, caspase 3 seems to be a decision maker in cell apoptosis. There exists a obvious characteristic in casapse 3' s behavior: all or none[4]. So maybe bistability is a property of caspase 3 while nobody knows it. That is what I want to demonstrate too.

Cancer, as a terrible and complex disease, is mostly caused by the mutation of apoptosis network. What's more, cancer cells always exhibit big variability on the execution of apoptosis. So the third interesting question is why variability exists and how to reduce it.

II. Results

In this work we examine the characteristics of intrinsic apoptosis network. On one hand we have constructed FRET reporter for caspase 3 activation and BiFC reporter for caspase 9 dimerization. On the other hand, the inducible caspase 9 dimerization system has been constructed well and all the mutated type and wild type of caspase 9 have been aquired successfully.

We have done preliminary experiments on these mutated loops. In the WT network, there is a sharp decrease in the Venus/CFP ratio, which implies a rapid increase in caspase3 activation. While in the mutation network, the increase is slower than that in the WT network. It is worth noting that in M2 network, there isn't any V/C decrease but appears a rapid increase there. In all the inducible dimerization system, it seems not all the cells undergo apoptosis. This result may account for a large number of untransfected cells or inactivated caspase 9.

III. Conclusion

Hela cells exhibit different behaviors on apoptosis when we disturb its natural apoptotic networks. Further experiments remained to be done to get more specific and quantitative results.

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References

[1] Meier P. et al. Apoptosis in development (2000). Nature 407: 796–801

[2] Slee EA. et al. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner (1999). J Cell Biol 144: 281-292

[3] Micheal JL. et al. Sequential app;ication of anticancer drugs enhances cell death by rewiring apoptotic signaling networks (2012). Cell 149: 780-794

[4] John G. Albeck et al. Modeling a snap-action, variable delay switch controlling extrinsic cell death (2008). PLoS Biology 6 (12): e29