Equilibrium constant measurement for doxorubicin-DNA interation in situ

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Doxorubicin (DOX) is an anti-cancer drug used in the clinic, it can disturb the growth of cells by binding with DNA directly. However, cellular toxicity and drug resistance problem have limited the usage of DOX in recent years [1]. Thus, it is of great importance to make clear the DOX-DNA interaction pattern. Though a lot of researches have calculated the equilibrium constant for DOX-DNA interaction, they were conducted in vitro [2]. Considering the complexity of DNA architecture inside nucleus [3], we try to measure the equilibrium constant of DOX-DNA interaction in a living cell directly, which can better reflect the real DOX-DNA interaction environment. Further, we hope to utilize the equilibrium constant acquired from different cell lines to help the clinicians to use DOX more effectively. Moreover, to understand the drug resistant mechanism in cancer treatment.

In our research, fluorescence correlation spectroscopy (FCS) is the principal research tool. As DOX can emit fluorescence after excitation, no more external fluorescent tags are required. On the other hand, DOX can enter into the living cells by passive diffusion. Thus, the whole progress is to treat cells with DOX for a period (30 minutes, for instance) and place the nuclei part of a single cell in the confocal. In FCS, the fluctuation of fluorescent signal inside the confocal is recorded and an autocorrelation curve is yielded. By fitting the autocorrelation curve with proper model, the diffusion time and fraction of free and bounded DOX can be fit, The rate constants and equilibrium constant can be determined [4].

References

1. Wijdeven RH, Pang B, Assaraf YG, Neefjes J. Old drugs, novel ways out: Drug resistance toward cytotoxic chemotherapeutics. Drug Resist Updat. 2016;28:65-81.2. Pèrez-Arnaiz C, Busto N, Leal JM, García B. New insights into the mechanism of the DNA/doxorubicin interaction. J Phys Chem B. 2014;118(5):1288-1295.3. Zink D, Fischer AH, Nickerson JA. Nuclear structure in cancer cells. Nat Rev Cancer. 2004;4(9):677-687.

4. Zhang X, Poniewierski A, Jelińska A, Zagożdżon A, Wisniewska A, Hou S, Hołyst R. Determination of equilibrium and rate constants for complex formation by fluorescence correlation spectroscopy

supplemented by dynamic light scattering and Taylor dispersion analysis. Soft Matter. 2016;12(39):8186-8194.

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