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Abstract of thesis (EN)

Anthracyclines are the most effective and important chemical compounds used in cancer treatment. Among them, doxorubicin hydrochloride (DOX), daunorubicin hydrochloride (DNR), epirubicin hydrochloride (EPR), and idarubicin hydrochloride (IDR) are the most widely used ones. They induce the apoptosis of cancer cells by binding with deoxyribonucleic acid (DNA). In novel anticancer drug discoveries or cancer treatment, scientists are always seeking an anticancer drug with a tight binding with DNA. The equilibrium constants (K) of anthracycline-DNA interactions are determined to quantify how strong the anthracyclines can bind with DNA. Association constant (K_a) and dissociation constant (K_d) are two special cases of K. K_a is the inverse of K_d. The larger the K_a value, the smaller the K_d value, the stronger the drug binding, and vice versa. However, currently, there are few issues to determine the K accurately.

First of all, the K values for reported anthracyclines vary from 10^4 M^{-1} to 10^8 M^{-1}. As a result, a 4-order of magnitude error occurs in determining the effective anthracycline concentration to form anthracycline–DNA complexes. The second issue is the detection limit. Most of the experiments are conducted with an anthracycline concentration at the micromolar level. However, anthracyclines may aggregate with each other at such a high concentration. The self-aggregation of anthracyclines can compete with anthracycline-DNA interactions, leading the equilibrium of anthracycline-DNA interactions to be shifted. Consequently, the K values of anthracycline-DNA interactions are altered. Thirdly, the action mechanisms of anthracyclines are still full of debates. Researchers have widely accepted that anthracyclines react with DNA through intercalation. However, my recent reports have shown an additional reaction occurring between anthracyclines and anthracycline-DNA complexes. The reaction is dominated when the amount of anthracyclines is in large excess to DNA binding sites. The K value in such a

reaction is 2-order smaller than that in the intercalation. Fourthly, the macromolecular crowding effect on *K* values. The previous reported K values are obtained in aqueous solution. However, the cell nucleus, where anthracycline-DNA interactions take place, is packed with macromolecules. The macromolecular crowding can shift the equilibrium of anthracycline-DNA interactions and further change the *K* values. Accordingly, the *K* values determined in aqueous solution may differ significantly from their values from *in vivo* experiments. The last issue is that there are fewer reports concerning the *K* values for DOX analogs: DNR, EPR, and IDR. Therefore, we addressed these issues by studying the fluorescence property changes of anthracyclines in interactions.

At the beginning of my research, I cleared the self-aggregation issue of anthracyclines. By using UV-Visible (Uv-Vis) spectroscopic techniques, I found anthracyclines had no self-aggregations at the micromolar level (see chapter 2). Next, I determined the *K* for anthracycline-DNA interactions at the nanomolar scale with fluorescence correlation spectroscopy (FCS) and single-molecule brightness (MB) analysis methods. Moreover, with the single-MB analysis method, I confirmed the formation of DOX-(DOX-DNA) complexes and determined the K for DOX analogs (see chapter 3). Lastly, I developed a method based on the photobleaching of DOX to determine the *K* of DOX-DNA interaction in single cells, and I discussed the possible reasons for the smaller *K* determined in cells (see chapter 4). In chapter 5, I concluded for the whole study. In chapter 6, I showed the future work to do to have a more accurate *K* determination in cells.

Overall, the thesis provides the vision of anthracycline-DNA interactions at the single molecular level, and gives a clue in new drug development and cancer treatment.