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高速原子間力顕微鏡と分子ドッキングの統合的アプローチによるCYP24A1へのDNAアプタマーの結合メカニズムの研究

Study of the binding mechanism of aptamer to CYP24A1 by an integrated approach of HS-AFM and molecular docking

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[Purpose] Overexpression of a mitochondrial enzyme CYP24A1 in cancer cells decreases the level of vitamin D3, which has the anti-proliferative effect. Therefore, inhibiting the CYP24A1 has been considered as a potential therapeutic target. We identified a DNA aptamer molecule (Apt-7) that could bind and inhibit the CYP24A1 specifically. However, to develop the aptamer as a lead candidate for targeted drug designs in cancer, a deeper insight into the conformational dynamics and the search of the most atomistic stable aptamer–target complexes are prerequisite. For this purpose, we utilized High-speed atomic force microscopy (HS-AFM) and cooperatively linked it with molecular docking method to directly visualize and analyze aptamer–target binding processes.[Methods] The binding between CYP24A1 and Apt-7 was directly visualized by using the HS-AFM platform. The CYP24A1 was loaded on mica surface whereas Apt-7 was injected into the imaging buffer chamber in HS-AFM set up. The sample was scanned, and real time images were captured. For molecular docking, the CYP24A1 crystal structure (PDB ID 3K9Y) and a computationally generated 3D aptamer structure obtained using the ZDOCK software were employed. [Results and Discussion] This study exploited a HS-AFM platform and correlated with molecular docking method to analyze the binding complex at molecular and atomistic levels. The molecular docking results showed that the top score model of Apt-7 combined steadily at active or allosteric ADX binding sites of CYP24A1 by strong hydrogen-bonds and electrostatic interaction. The dynamic interactions of Apt-7 and CYP24A1 were visualized by HS-AFM and the spatio-temporal analysis of captured images enabled us to characterize the binding interaction of Apt-7 and CYP24A1. Very interestingly, images from HS-AFM showed remarkably similar conformation with molecular docking results. [Conclusions] This study provided a more reasonable interpretation for high-affinity and specific binding of aptamer to CYP24A1. We could provide the foundation for further optimization and development of aptamers in molecular diagnostics and therapeutic applications.