Targeting the Human DEAD-Box RNA Helicase, DDX3, as a Novel Drug Target for Aggressive Breast Cancer Cells

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Aggressive breast cancer cells are insensitive to many of the present drugs, and new therapeutic agents that halt aggressive breast cancer cells' metastasis are much needed. DDX3 is an ideal drug target to halt aggressive breast cancer metastasis because DDX3 is not essential for healthy cells' metabolism, yet it is an important regulator of aggressive breast cancer cell motility; hence, inhibiting the DDX3 function is not expected to lead to side effects in a therapeutic context. The separated catalytic core of the DDX3 protein possesses no ATPase or helicase activity, and DDX3 N- and C-terminal domains are required for DDX3 function. We are investigating the mechanism employed by DDX3 auxiliary domain to modulate DDX3 function with the ultimate goal of using this under-studied family of small molecule inhibitors of the DDX3 protein. The small molecule inhibitors will serve as lead compound for drugs that stop aggressive breast cancer metastasis. Our experimental results show that the C-terminal of DDX3 is involved in tethering of the DDX3 protein to RNA, and DDX3 dimer formation. Experiments are in progress to identify the C-terminal amino acid residues involved in RNA tethering, dimer formation and the role of the dimerization for the DDX3 physiological function. Moreover, by screening a library of 2000 natural products we were able to find three specific inhibitors of DDX3 ATPase activity. We are currently investigating these inhibitors exact mode of action.

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Interaction of Ribonuclease III with the Regulatory Macromolecular Protein YmdB Analyzed by Docking Calculations and SPR Experiments


YmdB is a bacterial endonuclease that cleaves double-stranded DNA. It is essential in RNA maturation and decay pathways. RNase III is subject to multiple levels of regulation, allowing fine-tuning of its catalytic activity depending on the cellular physiological state. The regulatory macromodian protein YmdB interacts with RNase III, and an increase in YmdB levels correlates with a decrease in RNase III activity in vivo. However, the molecular details of the YmdB-RNase III interaction are not yet known. Here, docking calculations and computationally-driven mutagenesis were combined with surface plasmon resonance (SPR) experiments to identify energetically important determinants of the YmdB-RNase III interaction. The computational results reveal two alternative YmdB binding sites in RNase III: one located in the N-terminal nuclease domain (RNIID) and a novel site in the C-terminal dsRNA-binding domain (dsRBD). The binding site in the RNIID is composed of a cluster of negatively charged residues that interact with a conserved arginine in YmdB, and the importance of this interaction is confirmed by SPR analysis of the YmdB Arg to Ala mutation. These results suggest a mechanism of RNase III regulation in which YmdB can bind separate sites in a concentration-dependent manner, leading to inhibition of catalytic activity.

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Targeting the Human DEAD-Box RNA Helicase, DDX3, as a Novel Strategy to Inhibit Aggressive Breast Cancer Metastasis

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DDX3 is a human DEAD-box RNA helicase that is expressed at a high level in aggressive breast cancer cells. The increased expression of the DDX3 protein downregulates cadherin expression, which results in an increase in the aggressive breast cancer cells' metastatic properties. Aggressive breast cancer cells are insensitive to many of the present drugs, and new therapeutic agents...