Screening for small molecules interacting with telomerase RNA by NMR

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Prostate cancer is the most prevalent cause of cancer in men, and there is no available cure for patients with advanced disease. There are a variety of potential targets for treatment. Among the viable targets is telomerase, since telomerase activity has been shown in several studies to be correlated with cell immortality, and chromosome stability in cancer cells. These facts lead to the general supposition of telomerase as a potential target for cancer diagnosis and therapy. Telomerase is a eukaryotic ribonucleoprotein enzyme essential for telomere length maintenance by adding telomeric sequence onto chromosome ends. The core enzyme contains the protein TERT and the RNA component TER. The RNA moiety contains one highly conserved putative pseudoknot which is required for telomerase activity and assembly RNP (Fig. 1). Our goal is to develop a method to discover novel non-peptide, non-nucleotide compounds that interact with high affinity and specificity with a new target: the structured P2b hairpin in telomerase RNA in order to inhibit telomerase activity. These compounds also represent promising scaffolds for subsequent chemical modification that would enhance their pharmaceutical properties. To find such lead compounds we tested two different approximations. First we use rigid docking and second flexible docking with a new program called MORDOR (MOlecular Recognition with a Driven dynamics OptimazeR). MORDOR allows flexibility not only in the ligand but also in the receptor. This characteristic is essential in nucleic acids because it is known that structures of drugs bound to nucleic acids show that drugs displace bases, provoking a local reorganization of the nucleic acid. 200 compounds with the highest scores were assayed experimentally for binding using saturation transfer difference NMR. This technique is sensitive to binding over the broad range of 10^{-8}-10^{-3} M and also reveals the precise binding site on RNA. By traditional docking, we identified 42% binders by NMR STD and we experimentally found 54% binders with the new program MORDOR. Most of the compounds bind to the U bulge in the P2b hairpin. Specifity of the binders to hTR RNA was also tested by STD NMR using HIV-1 TAR and the 16 S aminoacyl-tRNA site (A-SITE) of ribosomal RNA. Results show that binder compounds from traditional docking are not specific since they also bind to TAR and/or A-SITE RNA. However, we found 14 binders specific to hTR RNA from MORDOR program. This preliminary data shows that the new program MORDOR obtained scaffolds that bind to a unique tertiary RNA structure with the required specificity; binding affinity of telomerase RNA is responsive to ligand characteristics and can be modulated by addiction or deletion of certain chemical groups. In the relatively new field of targeting RNA with small molecules, it remains to be seen if one will be able to generate a drug of high affinity and specificity from a low affinity lead compound as has been accomplished for protein target.

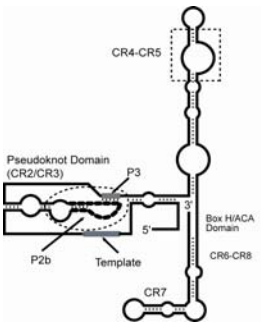

Fig.1. Vertebrate telomerase RNA secondary structure.