Summary of the Research work

Title: Synergistic Discovery of Novel Telomerase Inhibitors: From Structure-Based Pharmacophore Screening to Machine Learning Validation.

The focus of this research is on the discovery of novel telomerase inhibitors through a combined approach of structure-based drug discovery and machine learning. Telomerase, an enzyme responsible for maintaining telomere length, plays a critical role in cellular aging, cancer progression, and regenerative medicine. Despite its significance, developing small-molecule inhibitors targeting telomerase has been challenging due to the enzyme's complex structure and the absence of approved inhibitors. This study addresses these challenges by exploring new binding sites within the telomerase enzyme, specifically targeting the thumb domain, which is crucial for ribonucleoprotein assembly and enzymatic activity.

We initiated our research by leveraging the first reported structure of the human telomerase reverse transcriptase (hTERT) thumb domain (PDB ID: 5UGW) to identify potential binding sites. Inspired by the binding characteristics of BIBR1532, a known selective telomerase inhibitor, we focused on the FVYL pocket within the thumb domain. Our molecular dynamics simulations uncovered a novel binding site, termed site II, distinct from the previously known site I. Binding free energy calculations using MM-PBSA revealed that BIBR1532 exhibits a more favorable binding energy at site II, suggesting a possible shift from site I to site II during binding.

To validate these findings, we developed pharmacophore models based on both identified sites and screened curated small molecule libraries from ChemDiv, Otava, and the Binding Database. The screening yielded ten promising lead compounds, which were further refined through molecular docking studies, molecular dynamics simulations, and binding free energy calculations. Five of these compounds demonstrated significant potential as telomerase inhibitors.

Parallel to our structure-based approach, we developed a machine learning (ML) model using 12 molecular fingerprints and 6 different algorithms like Random Forest, XGBoost, AdaBoost, Support Vector Classifier, LightGBM and CART. The best model based on evaluation metrics like Accuracy, AUC-ROC, 5-Fold-CV. The Klekota-Roth-SVC model performed the best and was chosen for external validation on active enriched decoy set. The Enrichment factor of 21 indicated 21-fold enrichment of true actives within the top 1% of ranked test compound. The selected ML model accurately identified three of the top five leads from the pharmacophore-based screening, showcasing the robustness of our approach. Additionally, the model successfully screened compounds from the Binding Database, highlighting the consistency and reliability of our findings. Moreover, the ML model for telomerase inhibitors prediction will soon be made available on a public database, providing the entire research community with a valuable tool for screening potential telomerase inhibitors.

The convergence of results from both computational and machine learning methods underscores the potential of the three identified leads i.e compounds 26295 and 138187 from the ChemDiv anticancer database, and compound 77574 from the Otava drug-like lead collection as strong candidates for further experimental validation. Notably, compounds 834 and 637 emerged as leading candidates with high probabilities of telomerase inhibition,

validated across both methodologies. This innovative approach of integrating molecular dynamics, pharmacophore modeling, and machine learning has not only identified novel telomerase inhibitors but has also introduced a new perspective on telomerase drug targeting, particularly through the discovery of a new binding site in hTERT.

In conclusion, this research represents a significant advancement in the field of telomerase inhibitor development, offering promising new candidates for therapeutic intervention against cancer. The proposed leads, validated through rigorous computational methods, show significant potential for further in vitro and in vivo studies, which are already underway in our lab using various in-vitro and ex-vivo assays. Successful results will ultimately be contributing to the development of effective anti-cancer therapies.

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