

Rational Protein Design and Modular Functionalization for Targeted Drug Delivery in Advanced Prostate Cancer

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Background

While standard-of-care treatments offer control over tumor growth and initial metastases, the development of therapeutic resistance remains an unmet clinical need for patients battling advanced prostate cancer. Recent studies have shown that NSD2 inhibition can restore sensitivity to standard therapies in advanced prostate cancer by disrupting transcriptional pathways associated with androgen-independent signaling, highlighting the protein's promise as an avenue for resensitizing cancer cells to existing therapeutics. However, effective NSD2 targeting requires strategies that enhance tumor specificity, improve pharmacokinetics, and enable controlled intracellular delivery.

Methods and Results

While cisplatin can form covalent adducts with proteins, a knowledge gap remains in predicting its binding site on candidate protein carriers and the residue to which it reacts, limiting the rational design of protein-drug conjugates for targeted cisplatin delivery. This work addresses these challenges through a computational investigation of how a biologic nanocarrier, at both the amino acid and protein levels, directly supports active cisplatin transport, and a modular functionalization strategy that enables incorporation of diverse targeting and delivery moieties at the terminal handle of the NSD2 inhibitor. Using density functional theory, we quantify cisplatin binding to platinum-coordinating amino acids. We then rationally engineer a model MSH6 protein carrier and evaluate MSH6's ability to bind cisplatin via molecular docking simulations. Structure predictions of the engineered MSH6 show that inserting the cisplatin-binding site has a limited impact on the nearby protein architecture of MSH6. Our docking studies of engineered human MSH6 reveal preferential cisplatin binding to histidine, where the binding pocket contains cysteine, glutamate, and lysine residues as key neighbors of the cisplatin complex. These results are surprising compared with DFT calculations on amino acids, which showed stable, preferential sulfur-based platinum coordination with cysteine. These results provide a rational path from amino acid energetics to designing active sites within protein candidates for chemotherapy delivery. We also selected a prostate-specific membrane antigen (PSMA) targeting ligand to enhance tumor-specific localization in PSMA-expressing prostate cancer cells. This strategy leverages conditions in the prostate cancer tumor microenvironment, such as high glutathione levels, which facilitate reduction of cleavable linkers and enable controlled drug release. We therefore included an anti-albumin nanobody conjugate to enable albumin hitchhiking, taking advantage of endogenous serum albumin to improve circulation time and pharmacokinetic stability.

Conclusions

Given the various commercially available NSD2 small-molecule inhibitors, this alternative synthetic approach to NSD2 inhibition installs a highly versatile functional handle to which targeting moieties can be applied to tune the conjugate system for highly specific, accurate, and efficient drug delivery in late-stage prostate cancer.

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Conflicts of Interest

The authors declare no competing financial interests.

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