Prostate tumor specific peptide–peptoid hybrid prodrugs

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A B S T R A C T

Inspired by naturally occurring host defense peptides, cationic amphipathic peptoids provide a promising scaffold for anti-cancer therapeutics. Herein, we report a library of peptide–peptoid hybrid prodrugs that can be selectively activated by prostate cancer cells. We have identified several compounds demonstrating potent anti-cancer activity with good to moderate selectivity. We believe that these prodrugs can provide a useful design principle for next generation peptide–peptoid hybrid prodrugs.

Antimicrobial peptides (AMPs) have long served as an effective defense for virtually every living organism and are indispensable parts of the innate immunity. Most AMPs are relatively short (10–50 amino acids), highly cationic (+2 to +9), and also contain a significant proportion of hydrophobic residues (over 30%).

These properties allow AMPs to form amphipathic structures that can interact with the negatively charged outer membrane of microbes and lead to membrane permeation and disruption. Because the membranes of normal eukaryotic cells consist of lipids with no net charge (i.e., phosphatidylcholine), most cationic AMPs are selectively effective against prokaryotic cells. Interestingly, it has also been reported that AMPs exhibit anti-cancer activity possibly due to higher content of negatively charged phosphatidylserine on the outer membrane of rapidly dividing cancer cells. This potent and selective cytotoxicity against cancer cells makes AMPs an excellent alternative that can overcome the issues with drug resistance and therapeutic range.

Despite the advantages of AMPs over conventional therapeutic agents, AMPs have not yet been used widely in the clinic due to several reasons including rapid proteolytic degradation, potential immunogenicity, and systemic toxicity. Various synthetic non-natural analogs of AMPs have been developed to overcome these limitations. Among these peptidomimetics, oligo-N-substituted glycines (or peptoids) are thought to be a marked candidate because they are highly resistant to proteolysis and indicate lack of immunogenicity while maintaining structural and functional characteristics of peptides.

These unique features of peptoids come from having a peptide backbone with side chains attached to the amide nitrogen rather than to the α-carbon. Another advantage of peptoids over other peptidomimetics is that peptoids can be readily prepared using the solid-phase submonomer synthesis protocol in a sequence-specific manner.

Recently, we have demonstrated that a library of cationic amphipathic peptoids exhibited a broad spectrum of cytotoxicity against various cancer cell lines including cells with multidrug resistance. Notably, these peptoids effectively inhibited tumor growth in a mouse model of breast cancer suggesting that amphipathic peptoids are a promising alternative for anti-cancer AMPs; however, it was also apparent that the issues with overall selectivity and systemic toxicity should be addressed for further development. Therefore, as a continuing effort to develop amphipathic peptoids for anti-cancer therapy, we have designed peptide–peptoid hybrid prodrugs for the treatment of prostate cancer.

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Abstract

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successfully, patients with advanced and metastatic prostate cancers mainly rely on hormonal therapy, to which patients eventually become unresponsive due to the development of hormone-refractory prostate cancer. Recently, it has been reported that patient-specific vaccines and second and third-generation taxanes appear to extend overall survival of patients suggesting that highly selective cytotoxic agents with less chance of acquiring drug resistance can provide an improved treatment option for hormone-refractory prostate cancer. We believe that anti-cancer peptoids that can be selectively activated by prostate cancer cells perfectly meet these criteria; therefore, we designed our peptide–peptoid hybrid prodrugs (Fig. 1).

To achieve high selectivity, we conjugated the peptides targeting prostate specific antigen (PSA) and prostate specific membrane antigen (PSMA) to parent peptoid (1). Both PSA and PSMA are overexpressed in prostate cancer and exert catalytic activity. More specifically, PSA is an endopeptidase that is active in the local environment near prostate cancer cells, and PSMA is a carboxypeptidase that cleaves terminal \( \gamma \)-linked glutamic acids. It has been reported that the amino acid sequences of His-Ser-Ser-Lys-Leu-Gln (HSSKLQ), and a non-natural amino acid derivative, 4-hydroxyprolyl-Ser-Ser-cyclohexylglycyl-Gln-Ser-Ser-Pro (HypSSChgQSSP) can be efficiently hydrolyzed by PSA. Another recent work demonstrated that the crown ether modified peptides with \( \gamma \)-linked glutamic acids can selectively inhibit growth of PSMA over-expressed LNCaP cells. Therefore, we decided to incorporate these specific peptide sequences and amino acids into our parent peptoid (1) to generate a series of prodrugs and investigate their anti-cancer activity. As shown in Table 1, we designed PSA-targeted prodrugs (2–3 and 7–8), PSMA-targeted prodrugs (4–6), and PSA-PSMA dual targeting prodrugs (9–14). Notably, the pro-moieties of 7 and 8 are attached at the \( \epsilon \)-amine of the NLys side chain rather than at the N-terminus of the prodrug sequence. All compounds except 7 and 8 were synthesized on an automated peptide synthesizer according to the peptoid submonomer protocol and standard Fmoc/tBu solid-phase peptide synthesis (SPPS) method. Synthesis of peptide–peptoid hybrids 7 and 8 was carried out manually on a solid-phase resin, and detailed synthetic procedures and HPLC purification conditions are provided in the Supplementary data.

To assess anti-cancer activity of the hybrid prodrugs, we carried out MTS assays of each compound on PSA/PSMA-producing prostate cancer cells (LNCaP) and non PSA/PSMA-producing prostate cancer cells (PC-3) as described in Table 2. We found that our initial PSA-targeting compounds, 2 and 3, exhibited moderate cytotoxicity and slightly better selectivity against PSA-producing LNCaP cells compared to the parent peptoid (1). It was also observed that the HSSKLQ moiety (compound 3) seemed to be cleaved more efficiently than the non-natural sequence, HypSSChgQSSP (compound 2). On the other hand, the PSMA-targeting compounds (4–6) showed comparable cytotoxicity with LC50 values ranging from 9.5 to 15 \( \mu \)M against LNCaP cells; however, they also exhibited similar degree of cytotoxicity against PC-3 cells, indicating that the \( \gamma \)-glutamate group alone might not be a suitable pro-moiety. Although previously reported work showed that the \( \gamma \)-glutamate groups at the N-termini seemed to be hydrolyzed more efficiently than the same groups at the C-termini, it should be noted that PSMA is an exopeptidase catalyzing the hydrolytic cleavage of glutamates at the C-terminus. Overall, compounds in this group (2–6) appeared to be less cytotoxic than compound 1 suggesting that the pro-moiety on the N-terminus could not be cleaved effectively possibly due to steric hindrance.
The anti-cancer activity of the parent peptoid (1) comes from its spatially well-defined cationic, amphiphatic structure enabling the compound to disrupt negatively charged surface of cancer cells. Therefore, we believed that, in order to improve overall selectivity, it was necessary to mask a cationic side chain with the pro-moiety that could be removed by PSA. We also expected that having the pro-moiety on side chains rather than on the N-terminus would facilitate the cleavage of the pro-moiety. To test this hypothesis, we directly attached the pro-moiety to the N-Lys residues of the parent peptoid instead of the N-terminal secondary amine, hence we obtained compounds 7 and 8 (Fig. 1 and Table 1). As we expected, direct modification of the N-Lys groups provided significantly improved cytotoxicity and selectivity. Compared to compound 1, compounds 7 and 8 maintained similar cytotoxicity against LNCaP cells (LC50 = 5.7 and 5.9 μM) with much less toxicity against PC-3 cells (LC50 = 16.9 and 19.1 μM). In addition, compound 7 and 8 showed similar cytotoxicity and selectivity, suggesting that this minor difference in the pro-moiety placement results in a minimal effect on the cytotoxicity. Next, we tried another strategy to improve the selectivity by attaching dual-targeting groups as demonstrated in compounds 9–14. These compounds have both the PSA targeting group (HSSKLQ) and the PSMA targeting group (γ-glutamate) that can be subsequently or simultaneously cleaved in prostate cancer cells. Furthermore, we believed that the anionic γ-glutamate groups could act as a charge quencher for the cationic anti-cancer peptoid, and inserting the PSA cleavable sequence between these moieties would further enhance overall selectivity of the prodrugs. Unfortunately, all the compounds (9–14) in this series exhibited less cytotoxicity and low selectivity compared to the compounds with only one targeting groups. The γ-glutamate groups possibly masked the positive charge of the parent drug, rendering the prodrug ineffective against cancer cells; but, they also seemed to interfere with selective cleavage by PSA. Compounds with a greater number of γ-glutamate groups (compounds 9–11) exhibited less cytotoxicity against non-PSA/PSMA producing cells (PC-3) supporting our hypothesis. Furthermore, the N-acetylated analogs of the poly γ-glutamate derivatives (compounds 12–14) demonstrated much lower cytotoxicity and virtually no selectivity compared to their free amine counterparts, again, suggesting that masking the free amine blocked the hydrolysis of the γ-glutamate groups even further.

In conclusion, we have designed and synthesized peptide–peptoid prodrugs targeting PSA and PSMA for the treatment of prostate cancer. We conjugated an anti-cancer peptoid with the PSA and PSMA targeting peptide sequences that can be selectively hydrolyzed in prostate cancer cells. We found that the prodrugs targeting PSA were more selective than the prodrugs targeting PSMA. When we directly placed the PSA targeting pro-moiety on N-Lys, these prodrugs demonstrated much improved selectivity suggesting that masking positive charges on the cationic anti-cancer peptoid enhanced selectivity. We also observed that the dual-targeting prodrugs were much less toxic than the parent drug, indicating that the γ-glutamate groups successfully masked the cationic charges on the parent drug, however, they also appeared to interfere with selective hydrolysis by PSA. Therefore, future research
endeavors would include inserting a spacer group or attaching different targeting sequences that are readily cleaveable to improve selective hydrolysis. Molecular modeling studies can also provide insights into more efficient hydrolysis of pro-moieties. In addition, we believe that, as it was demonstrated in compounds 7 and 8, direct attachment of the γ-glutamate groups to the NLYs side chain may improve selectivity of the PSMA targeted prodrugs.

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Supplementary data

Supplementary data (experimental procedures and characterization of representative compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.04.092.

References and notes