Pre-twisting for improved genome modification and miRNA targeting

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Two reports by Dhuri et al. and Oyaghire et al., respectively, show that, through installing chiral centers at the backbone of the artificial nucleic acid, peptide nucleic acid (PNA), enhanced miRNA targeting and genome modification can be achieved, with important implications in fighting cancers and β-thalassemia.

Regulatory tools precisely targeting DNA and RNA sequences are needed as new therapeutic modalities. Initially developed in 1991 by Nielsen and coworkers, PNA (Figure 1A, B) [1], shows great potential in targeting disease-related DNA and RNA sequences. PNAs can pair with natural DNA/RNA sequences forming PNA-DNA and PNA-RNA duplexes. Compared with the sugar ring moiety of DNA/RNA with three or four chiral centers, canonical PNA has no ring structure or chiral center. The relatively flexible and charge-neutral backbone makes PNA advantageous in the recognition of DNA/RNA sequences through Watson-Crick base pairing with the backbone and nucleobase, has resulted in new promising PNA-based molecular platforms in targeted DNA repair and recombination processes. For example, functionalizations at α, β, and γ carbon atoms, generating chiral centers, in the backbone of PNA give rise to pre-twisted right-handed structure in PNA (Figure 1A, B) and thus enhanced hybridization strength with DNA/RNA targets [2]. γ-PNAs with a diethylene glycol (mini poly ethylene glycol, mp) moiety (with an R-configuration of the γ carbon, designated as MPγ-PNA, Figure 1A) are relatively more biocompatible with improved solubility and biological activity [3]. Interestingly, a simple hydroxymethyl group attachment (serine modification γPNA (syPNA), Figure 1A) also improves the biological activity in targeting RNA [3]. Various γPNAs have shown potential in biomedical applications such as gene editing and anti-miRNA technology (Figure 1C, D) [2–4, 6, 7], though it was not clear how the chemistry and bulkiness of the newly installed functional groups in the PNA backbone affect the binding and biological activities. Recently, two head-to-head comparison studies of MPγ-PNA and syPNA assembled with PLGA nanoparticles for DNA correction in mouse bone marrow cells [2]. Interestingly, compared with MPγ-PNA, syPNA has an enhanced binding and a higher frequency (1.5-fold) in stimulating gene modification of a mutation that causes β-thalassemia.

Oncogenic miRNA targeting

miRNAs overexpressed in cancer cells may be targeted through miRNA-PNA duplex formation. Conjugating pHLP and γPNA (pHLP-γPNA) allows selective targeting of miRNAs in the acidic tumor microenvironment but not the normal cells (Figure 1D) [4]. Dhuri et al. reported that a pHLP-syPNA conjugate is more advantageous compared with pHLP-MPγ-PNA conjugate in targeting the seed region of miR-155 for restricting the U2932 lymphoma cell proliferation in vitro and in a mouse model [4].

These two studies on syPNA on genome modification and miRNA targeting [2,4] suggest that there is significant room for improving the physical and binding properties and biological activities of PNAs by carrying out interdisciplinary medicinal chemistry campaigns. A close inspection orientated of the 3D structure of PNA-
DNA duplexes shows that the installed groups on α and β/γ carbons are orientated toward the major and minor grooves, respectively (Figure 1A). Compared with a relatively small hydroxymethyl group, the bulky and hydrophilic diethylene glycol moiety exposed on the minor groove side may disrupt the hydration layer of the PNA-DNA and PNA-RNA duplexes. Clearly, optimization of the chemistry and the size of the installed group on the γ position would generate further advanced PNAs.

In the two recent studies [2,4], only the PNA segment forming Watson-Crick pairs contains γ position modifications. It would be interesting to see how y position modifications on the strand forming Hoogsteen pairs may affect the binding and activity [5,9]. It is conceivable that careful structure-based engineering of the chiralities of α and β as well as other positions (Figure 1A) may offer rich opportunities in tuning PNAs for improved targeting of DNA and RNA. Furthermore, the Hoogsteen pair-forming strand may be engineered with base modifications to enhance the binding strength and specificity [9]. In addition, efforts on engineering the delivery vectors including PLGA nanoparticles and pH-LIP may also be worthwhile as vectors may have complex interactions in biological environments [10].

In summary, the two reports by Dhuri et al. and Oyaghire et al. [2,4] have shown that advanced modular synthesis techniques combined with biophysical and biological activity studies may allow precise molecular engineering of PNAs for enhanced reprogramming of the functions of DNA and RNA in diseased cells for fighting β-thalassemia, cancers, and many other diseases.

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Declaration of interests
The authors declare no competing interests.

DNA/RNA, canonical PNA, and γPNA. PNA has an aminomethylglycine building block and a methylene carbonyl linker replacing the standard phosphate-sugar backbone of DNA/RNA. Both the backbones of DNA/RNA and PNA contain six atoms with a two-atom linker connecting the nucleobases. A chiral center on the γ carbon is generated by attaching miniPEG (mpyPNA) or hydroxymethyl group (sγPNA). (B) 3D structure of a helical PNA strand extracted from a crystal structure of PNA-DNA duplex (PDB: 1PNN). The hydrogen atoms on the γ carbons to be replaced by functional groups for pre-twisting the PNA strand into a right-handed structure are shown in magenta. The carbon, nitrogen, and oxygen atoms are shown in white, blue, and red, respectively. (C) Schematic of γPNA invasion of DNA for inducing DNA editing. Formation of a PNA-DNA-PNA structure results in correction of a disease-causing mutation through a donor DNA. The Watson-Crick and Hoogsteen pairs are indicated by - and △, respectively. (D) Schematic of γPNA targeting the seed sequence of a miRNA. Acidic microenvironment of tumors facilitates the formation of α-helical structure for the pH-low insertion peptide (pH-LIP) for targeted delivery of γPNA into cancer cells. The γPNA is released by the cleavage of the disulfide bond with a reducing cancer cell environment.

Figure 1. Peptide nucleic acid (PNA) structures and potential applications. (A) Chemical structures of DNA/RNA, canonical PNA, and γPNA. PNA has an aminomethylglycine building block and a methylene carbonyl linker replacing the standard phosphate-sugar backbone of DNA/RNA. Both the backbones of DNA/RNA and PNA contain six atoms with a two-atom linker connecting the nucleobases. A chiral center on the γ carbon is generated by attaching miniPEG (mpyPNA) or hydroxymethyl group (sγPNA). (B) 3D structure of a helical PNA strand extracted from a crystal structure of PNA-DNA duplex (PDB: 1PNN). The hydrogen atoms on the γ carbons to be replaced by functional groups for pre-twisting the PNA strand into a right-handed structure are shown in magenta. The carbon, nitrogen, and oxygen atoms are shown in white, blue, and red, respectively. (C) Schematic of γPNA invasion of DNA for inducing DNA editing. Formation of a PNA-DNA-PNA structure results in correction of a disease-causing mutation through a donor DNA. The Watson-Crick and Hoogsteen pairs are indicated by - and △, respectively. (D) Schematic of γPNA targeting the seed sequence of a miRNA. Acidic microenvironment of tumors facilitates the formation of α-helical structure for the pH-low insertion peptide (pH-LIP) for targeted delivery of γPNA into cancer cells. The γPNA is released by the cleavage of the disulfide bond with a reducing cancer cell environment.

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