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Development of PNA-Based Antisense Oligonucleotides Targeting XIAP mRNA for Sensitizing TRAIL-Resistant Cancer Cells

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Abstract

Peptide nucleic acids (PNAs) offer a chemically stable antisense platform for sequence-specific regulation of disease-associated RNA targets. Owing to their neutral pseudopeptide backbone, PNAs exhibit strong hybridization to complementary RNA sequences and high resistance to nuclease-mediated degradation. These properties make PNAs particularly suitable for targeting regulatory RNA elements that control the expression of disease-relevant proteins.

X-linked inhibitor of apoptosis protein (XIAP) is an anti-apoptotic factor frequently overexpressed in cancer and a key mediator of resistance to TRAIL-induced apoptosis, where it suppresses caspase activation downstream of death receptor signaling. XIAP translation is sustained under cellular stress through an internal ribosome entry site (IRES), making this regulatory RNA element an attractive target for selective downregulation of XIAP in apoptosis-resistant cancer cells.

Here, we developed cell-penetrating peptide (CPP)-conjugated PNAs directed against the XIAP IRES to enable both sequence-specific translational repression and efficient intracellular delivery. The XIAP-targeting CPP-PNAs effectively reduced XIAP protein levels in multiple cancer cell lines, including TRAIL-resistant cells. In the TRAIL-resistant context, co-treatment of the CPP-PNAs with TRAIL produced a synergistic effect that restored caspase activation and substantially enhanced apoptotic cell death compared with either treatment alone. These findings demonstrate that PNA-based antisense targeting of the XIAP IRES can overcome XIAP-mediated apoptosis resistance by re-engaging the caspase cascade, and highlight CPP-conjugated PNAs as a promising modality for RNA-directed regulation of oncogenic survival pathways.

Introduction

1. XIAP/XIAP IRES

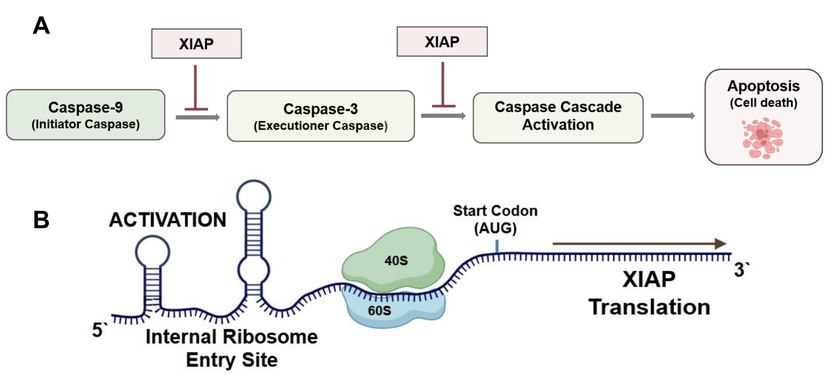


Fig. 1 A. XIAP regulates caspase process **B.** Translation controlled by IRES

2. TRAIL/XIAP Signaling

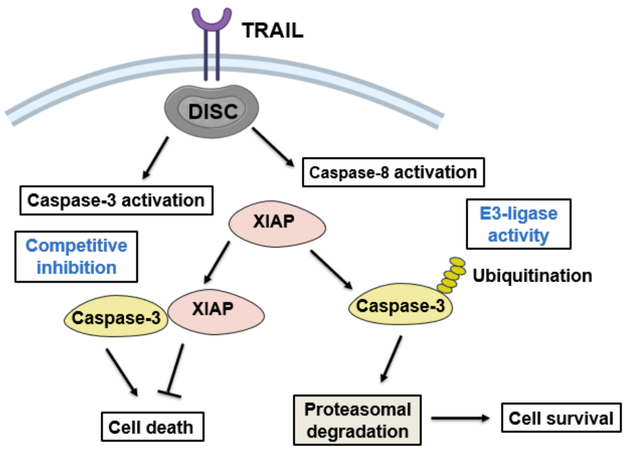


Fig. 2 Molecular mechanism of TRAIL, caspase, and XIAP

3. Types of ASO(Antisense Oligonucleotide) in project

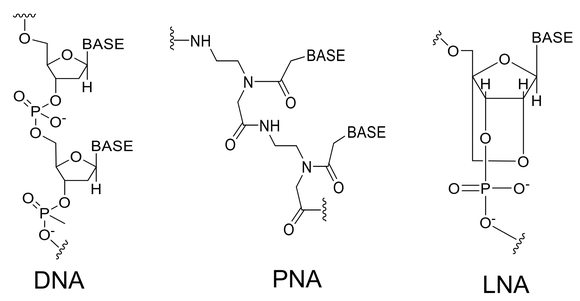


Fig. 3 The structure of DNA, PNA, and LNA

Identification of the Targeting Site of XIAP IRES

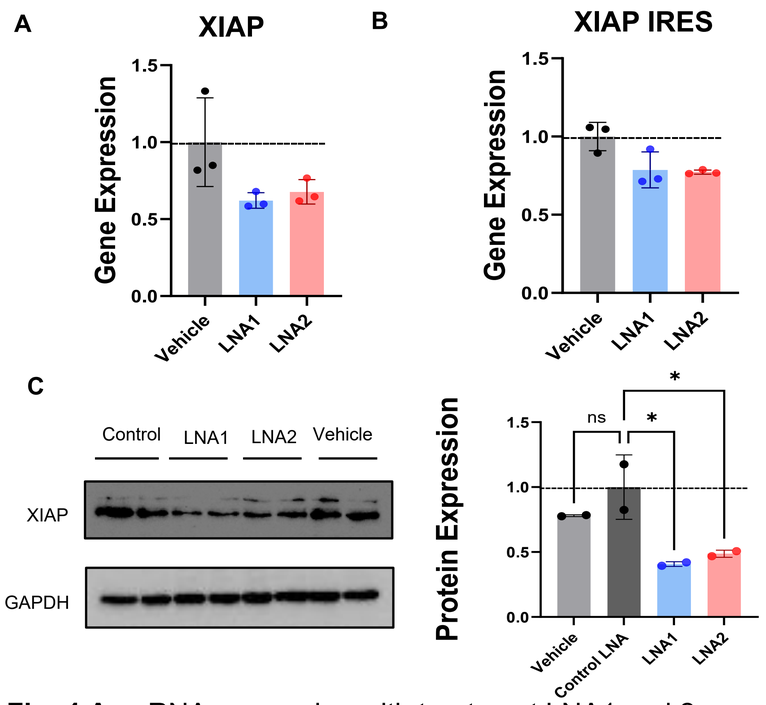
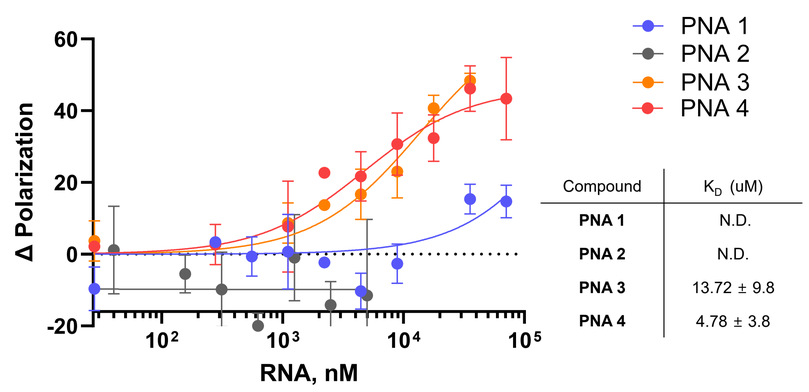


Fig. 4 A. mRNA expression with treatment LNA1 and 2 targeting XIAP **B.** mRNA expression with treatment LNA1 and 2 targeting XIAP IRES **C.** Western blot analysis of treatment Control LNA, LNA1, and 2 in HeLa cells

Binding Assay



Compound	K_D (uM)
PNA 1	N.D.
PNA 2	N.D.
PNA 3	13.72 ± 9.8
PNA 4	4.78 ± 3.8

Fig. 5 Fluorescence polarization assay result. N.D. Not detected.

Reporter Assay

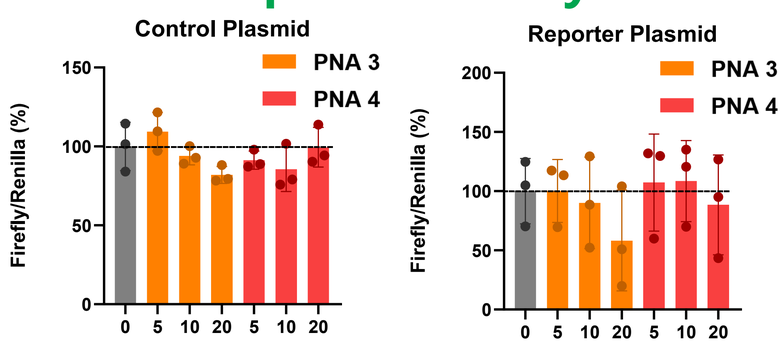


Fig. 6 Reporter assay result with treatment **PNA 3** and **4**

Western Blot Analysis

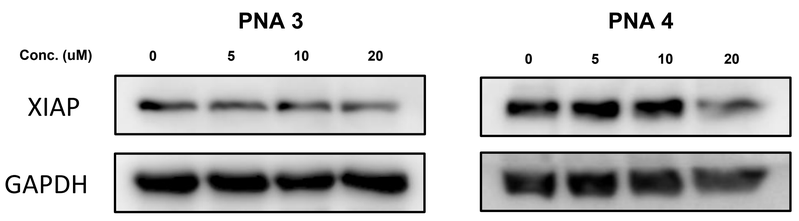


Fig. 7 Western blot analysis of treatment **PNA 3** and **4** in HeLa cells using XIAP Antibody

Effect of PNA Inhibitors in Apoptosis

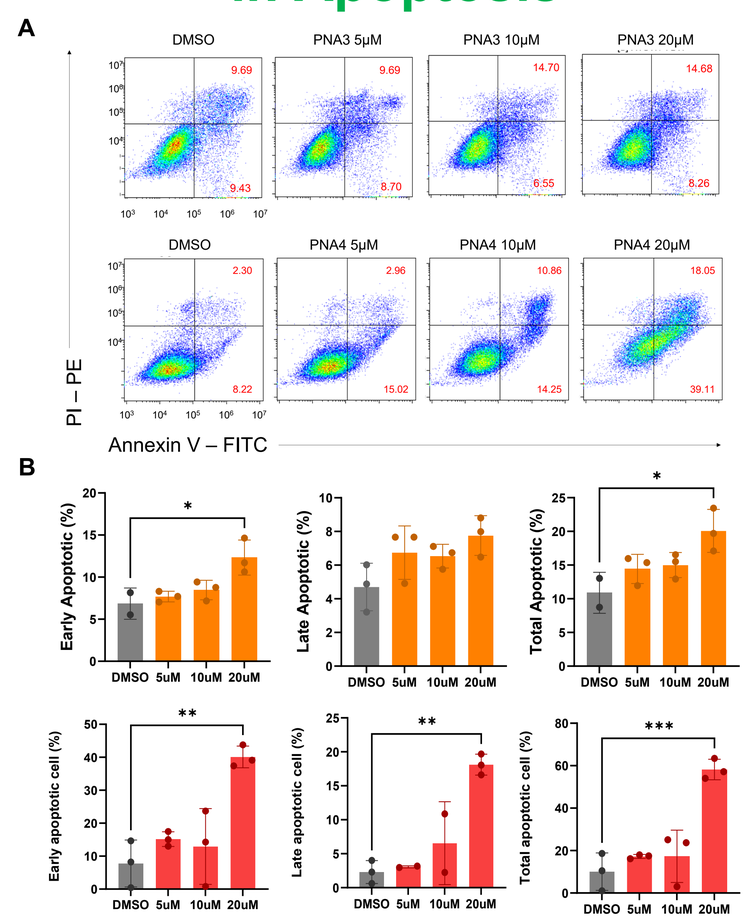


Fig. 8 A. FACS analysis with treatment **PNA 3** and **4** **B.** Apoptotic cell quantification graph with treatment **PNA 3** and **4**

Combination Treatment

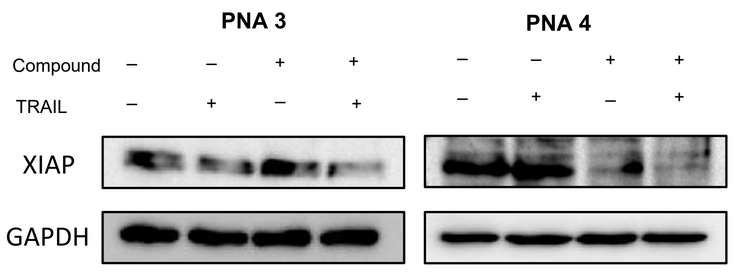


Fig. 10 Western blot analysis of combination treatment **PNA 3** or **4** with TRAIL in AsPC-1 cells

Caspase Activation

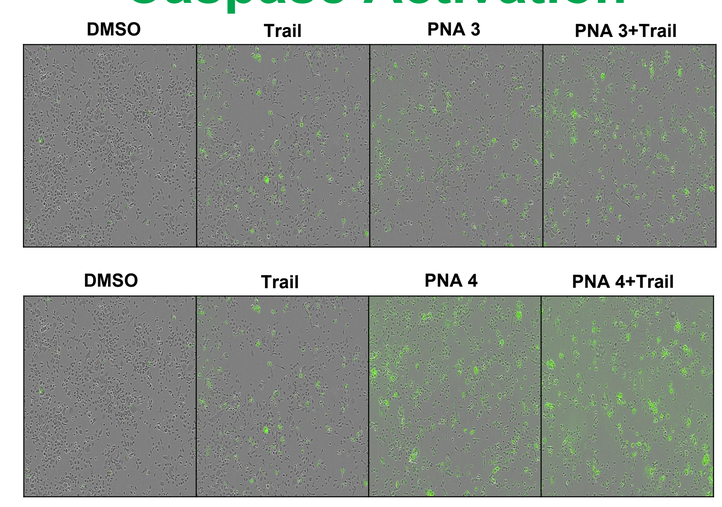


Fig. 9 Caspase activation analysis of combination treatment **PNA 3** or **4** with TRAIL in AsPC-1 cells

Synergetic Effect of TRAIL and PNA Inhibitor

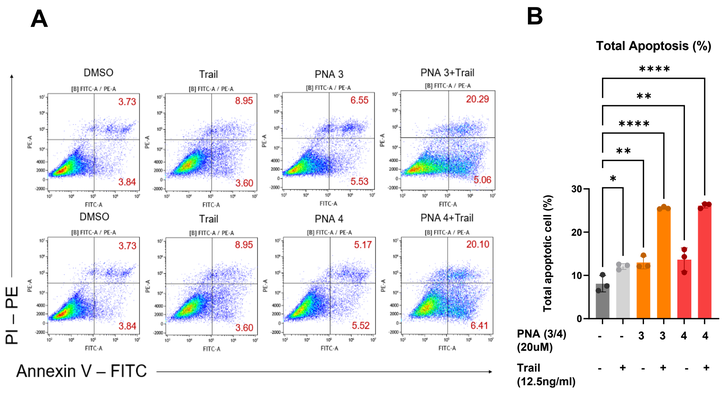


Fig. 11 A. FACS analysis with treatment **PNA 3** and **4** **B.** Apoptotic cell quantification graph with treatment **PNA 3** and **4**

Conclusions

We developed PNA compounds specifically targeting the XIAP IRES and confirmed theirz strong RNA binding affinity and effective suppression of XIAP expression at both mRNA and protein levels. In TRAIL-resistant AsPC-1 cells, co-treatment with TRAIL significantly enhanced apoptotic cell death, demonstrating that XIAP IRES-targeting PNAs can sensitize resistant cancer cells to TRAIL. These findings suggest that PNAs targeting XIAP IRES represent a promising strategy to overcome apoptosis resistance and may serve as potent candidates for combination cancer therapy against TRAIL-resistant cancers.

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