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Recombinant Engineered Human Pancreatic RNase1 Efficiently Targets and Eliminates Prostate Cancerous Cells

Omid Mikaili[1], Zahra Kiadehi[1], Vida Khalatbari Limaki[1], Reza Goudarzi[1], Amin Ramezani[1], Nasrollah Erfani[2], Amir Maleksabet[1*]

[1]Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[2]Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

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***Corresponding Author:**
Dept. of Medical Biotechnology, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran

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ABSTRACT

Introduction: Targeted drug delivery has opened up a novel window for the specific delivery of anticancer therapeutics directly to tumor sites. Gonadotropin-releasing hormone (GnRH) is a decapeptide that has received attention for its potential use in targeted drug delivery due to its targeting properties. It exhibits a high affinity for its receptor and is not immunogenic in humans. Human pancreatic ribonuclease 1 (hpRNase1) has demonstrated anticancer properties when fused with targeting moieties such as growth hormones, antibodies, and their derivatives. The present study aimed to attach a GnRH-targeting peptide to the N-terminus of hpRNase1 to enhance its specificity for cells expressing the GnRH receptor (GnRH-R).

Methods and Materials: The coding gene was designed, synthesized, and cloned in the pET28a expression vector to produce the recombinant enzyme and subsequently expressed in *Escherichia coli* BL21 (DE3) bacteria. After induction of expression, the identity of the resulting protein was confirmed by SDS-PAGE and Western blot. Next, the recombinant protein was purified by affinity chromatography, and its cytotoxic effects on cancer cells expressing the GnRH-R were evaluated.

Results: The GnRH-hpRNase1 chimeric protein significantly inhibited the proliferation of PC-3 ($p = 0.021$), LNCaP ($p = 0.034$), and AD-Gn ($p = 0.041$) cells, while the growth of negative cells (AD-293) was not significantly affected ($p = 0.081$). GnRH-hpRNase1 decreased the IC_{50} values more than non-fused hpRNase1 by approximately 26.5-fold ($p = 0.036$) for PC-3 cells and exerted its growth inhibitory effects through apoptosis induction.

Conclusion and Discussion: Ribonucleases, particularly human pancreatic RNase1, have shown intriguing features for developing new therapeutics. However, they suffer from two main shortcomings: (1) being RI sensitive and (2) acting poorly specific to cancer cells. We showed that the engineered GnRH-hpRNase1 can specifically target the GnRH receptor-expressing cells and inhibit their proliferation through inducing apoptosis. Owing to its promising anti-tumor activity, the fusion enzyme can be further examined on GnRH-R-expressing tumor xenografts to evaluate its anti-tumor effects in vivo.

Keywords: Drug delivery systems, Gonadotropin-releasing hormone, Pancreatic ribonuclease 1