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Proceeding Paper

pMyc and pMax Peptides Nanosystems and Potential Treatment of Prostate Cancer, In Vitro Assays †

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Abstract: The Myc transcription factor and its associated Max protein have an essential role in developing several types of cancers, including prostate cancer. They dimerize into a Myc-Max heterodimer and bind to DNA sequences known as enhancer box (E-box). Therefore, disrupting the binding of this E-boxes to derange transcription is a promising strategy to treat cancer. Using computational biology tools, we designed pMyc and pMax peptides from Myc and Max reference sequences and evaluated their ability to bind to E-boxes through an electrophoretic mobility shift assay (EMSA). We then coupled them to AuNPs and evaluated the hemocompatibility and the cytotoxic effect in three different prostate adenocarcinoma cell lines and a non-cancerous cell line The EMSA results suggest that the pMyc-pMax dimers bind to CMEs. The hemolysis test showed little hemolytic activity for the nanosystems (NS) at the three concentrations evaluated. Cell viability assays showed mixed results depending on which cell line was being evaluated. Overall, results suggest that NS with pMyc and pMax peptides might be suitable for further research regarding Myc-driven prostate adenocarcinomas.

Keywords: Myc; max; nanosystem; AuNPs; prostate cancer

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1. Introduction

The Myc transcription factor is the protein encoded by the oncogene with the same name. It regulates several essential cell processes that can lead to a cancerous phenotype if the Myc protein is unregulated. In its sequence, Myc contains a basic helix-loop-helix domain and a leucine zipper domain allowing many dimerization interactions with different proteins and resulting in different gene expression patterns [1].

The Myc-associated factor X, also known as Max, is Myc's primary binding partner; the Myc:Max dimer is responsible for the transcription initiation of its target genes. Myc:Max dimer binds to a DNA sequence known as enhancer box (E-box); this sequence is located in the promoters of the target genes and has the following sequence: CACGTG (CME); the dimer's affinity is higher for this sequence than other degenerated sequences known as non-canonical E-box [2].

The *Myc* oncogene is overexpressed in prostate cancer (PCa), which has been determined to be the second most common cancer in men worldwide [3,4]. With this in mind, is has been suggested that disrupting the Myc:Max dimer at oncogenic levels, or its binding into E-boxes could be a promising strategy for treating cancer [5]. Designing peptides through computational biology tools to target Myc could lead to new therapeutic approaches for PCa and other Myc-related cancer types. However, the primary limitation of this approach is the difficulty in delivering these peptides into the cell's nucleus. Several strategies can be used to achieve this, and one of them is the nanotechnology. This work aimed to construct pMyc:pMax:AuNPs nanosystems (NS) using Myc and Max-derived peptides and gold nanoparticles, and to evaluate their effect in PCa cell lines.

2. Materials and Methods

2.1. Peptide Design and Synthesis

We used the protein reference sequences for Myc (NP_002458.2) and Max (NP_660087.1) obtained from Genbank available at the NCBI; we also used Swiss-Model for protein homology modeling [6], Cn3D for visualizing the macromolecular structure [7], and ScooP for predicting thermal stability [8]. The pMyc and pMax peptides were chemically synthesized using Accura's Custom Peptide Synthesis service (Accura, Monterrey, Nuevo Leon, Mexico).

2.2. Electrophoretic Mobility Shift Assay

To avoid undesired oligonucleotides mismatches in the electrophoretic mobility shift assay (EMSA), we designed ssDNA oligonucleotides with similar melting point temperatures using the OligoAnalyzer tool. Oligonucleotides were synthesized using IDT's Custom DNA oligos service (IDT, Coralville, Iowa, USA) (Table 1).

Table 1. Designed oligonucleotides for the EMSA carrying E-box and non E-box sequences.

IC	Sequence
CME Allevato F	5′ CCG GC**C ACG TGC ACG TG**T TAA TAG CTC AGA CTA CTG TGT CGA CG 3′
CME Allevato R	5′ CGT CGA CAC AGT AGT CTG AGC TAT TAA **CAC GTG CAC GTG** GCC GG 3′
CME F	5′ AGA TCT CGA GCT GCA TGC TGT A**CA CGT G**AT GTC GTA CGT CGA GCT CTA GT 3′
CME R	5′ ACT AGA GCT CGA CGT ACG ACA T**CA CGT G**TA CAG CAT GCA GCT CGA GAT CT 3′
NE F	5′ AGA TCT CGA GCT GCA TGC TGT AAA CGT TAT GTC GTA CGT CGA GCT CTA GT 3′
NE R	5′ ACT AGA GCT CGA CGT ACG ACA TAA CGT TTA CAG CAT GCA GCT CGA GAT CT 3′
CTRL F	5′ AGA TCT CGA GCT GCA TGC TGT ATT AGC AAT GTC GTT ATC AGA GCT CTA GT 3′
CTRL R	5′ ACT AGA GCT CTG ATA ACG ACA TTG CTA ATA CAG CAT GCA GCT CGA GAT CT 3′

IC, oligonucleotide identification code; CME, canonical E-box; NE, non-E-box element; CTRL, control. Bold, target E-box sequence; Underline, non-CME sequence; F, forward sequence; R, reverse sequence.

2.3. Nanosystem Construction

Three different NS were constructed as depicted in Figure 1: pMyc:AuNPs with only the pMyc peptide, pMax:AuNPs with only the pMax peptide, and pMyc:pMax:AuNPs with both peptides. We used Maleimide 5 nm Gold Nanoparticle Conjugation kits (cat.

No 900458-1EA Cytodiagnostics, Burlington ON, CAN) for constructing the NS. All AuNPs have polyethylene glycol (PEG) and maleimide for peptide conjugation.

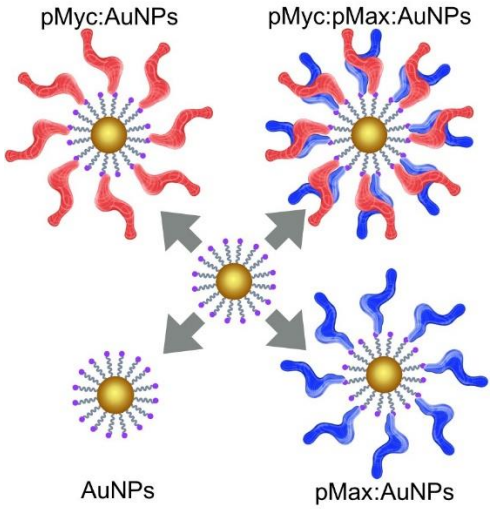

Figure 1. Schematic depiction of the constructed nanosystems with either individual pMyc and pMax homodimers or pMyc:pMax heterodimer.

2.4. Hemolysis Test

We extracted 2 mL of blood from a volunteer; the sample was centrifuged, and the pellet washed to obtain an erythrocyte solution. Finally, we read the absorbance spectra with an ND-1000 Nanodrop at 415 nm to obtain the hemolysis percentage.

2.5. Cell Viability Assays

All cells were obtained from the American Type Culture Collection (ATCC, Manassas,VA). PC-3 and Vero CCL-81 cells were cultured in DMEM (Thermofisher, 11965092), and LNCaP and DU145 were cultured in RPMI 1640 (Gibco, 72400047). Cell viability was determined following the Cell Proliferation Kit I (MTT) (Roche, 11465007001) protocol. The formazan crystals were solubilized with isopropanol (pH = 3) and read in a BioTek Cytation3 Imaging reader at 570 nm and 651 nm.

2.6. Statistical Analyzes

Two-way ANOVA was used along with the Bonferroni correction for this work's statistical analysis. GraphPad Prism 5 was used for statistical analysis and figures.

3. Results and Discussion

The Cn3D rendering showed that the structure predicted for the pMyc:pMax heterodimer conserved the recognition and binding site to the CACGTG canonical E-box sequence Furthermore, ScooP modeling shows high thermal stability in the heterodimer up to 76.6 °C. ScooP's results can be seen in Table 2.

Table 2. Thermal stability prediction for the designed peptides.

Peptide	T_m (°C)	ΔG (kcal/mol)
pMyc	80.1	−8.4
pMax	81.4	−6
pMyc:pMax dimer	76.6	−4.2

The Figure 2 shows a shift or retardation in the electrophoretic mobility when the designed oligonucleotides containing the CACGTG sequence where incubated with the pMyc:pMax heterodimer (lanes 4 and 8). These results suggest the binding of the peptides to the e-box. On the other hand, this shift was not observed with the oligonucleotides carrying the non-e-box element.

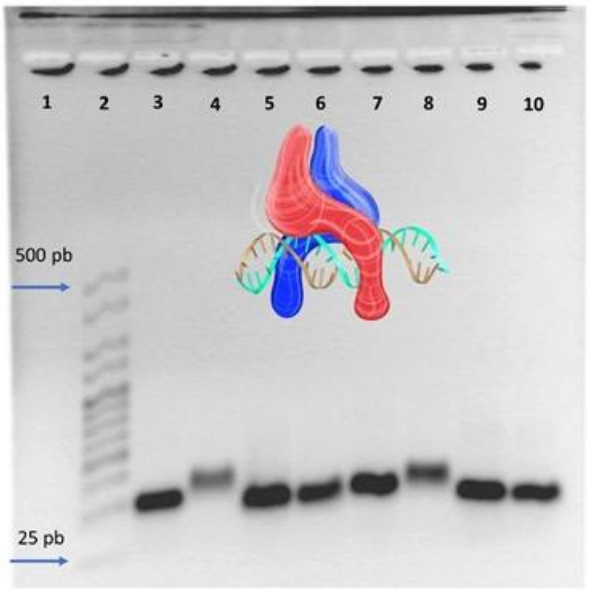

Figure 2. Resolved EMSAs with pMyc:pMax. Lanes were filled and resolved as the following: 1, empty. 2, Bioline Hypperladder 25 base pairs; 3, CME; 4, CME and peptides; 5, Ctrl; 6, Ctrl and peptides; 7, CME-Allevato; 8, CME-Allevato and peptides; 9, NE; 10, NE and peptides.

According to the ASTM-F756-17 standard practice for assessing hemolysis in materials, all NS were considered materials with no hemolysis at the lowest concentration (Figure 3). pMyc:AuNPs at 5 ng/µL have low hemolytic properties under the ASTM-F756-17 norm. Other peptide conjugates that have been reported also showed hemolysis < 10% [9], whereas another peptide-AuNPs NS reported a <1% hemolysis [10].

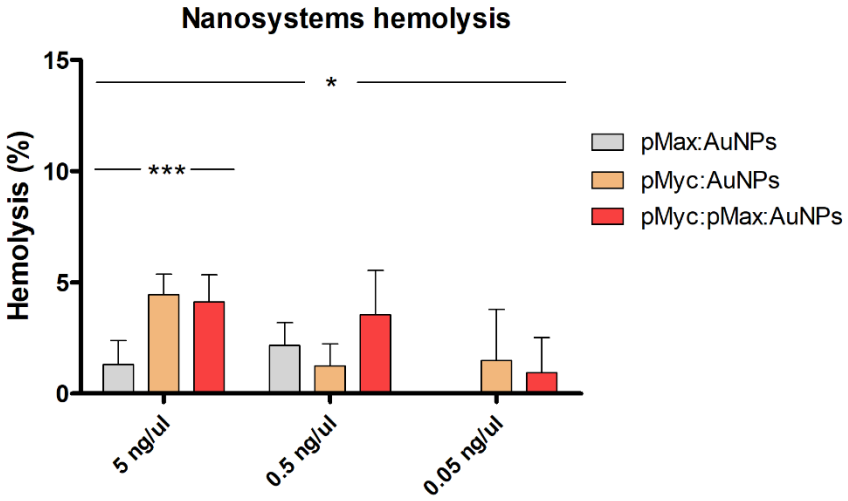

Figure 3. Hemolytic properties of the different nanosystems at three different concentrations. * $p < 0.05$; *** $p < 0.001$.

Figure 4 shows the results of the cell viability assays. We obtained mixed results depending on the NS used and the cell line evaluated. In Vero CCL-81, pMyc:pMax.AuNPs showed little to no cytotoxic effect. In LNCaP, pMyc:AuNPs had the highest cytotoxic effect. With different pMyc:pMax disruption compounds Carabet and colleagues obtained a reduced LNCaP's cell viability of <20%. Whereas Holmes and colleagues used a small-molecule Myc inhibitor (MYCi975) and obtained a cell viability reduction of 28.4%, however, their assays involved treatments in the order of micromolar concentrations [11,12]. These results show that different compounds can affect cell viability in different proportions. In PC-3 cells, little cytotoxicity can be seen; only pMyc:pMax:AuNPs had a cytotoxic effect in these cells. Finally, in DU145 cells, the most significant cytotoxic effect was shown by the pMax:AuNPs, whereas pMyc:AuNPs had a mild effect.

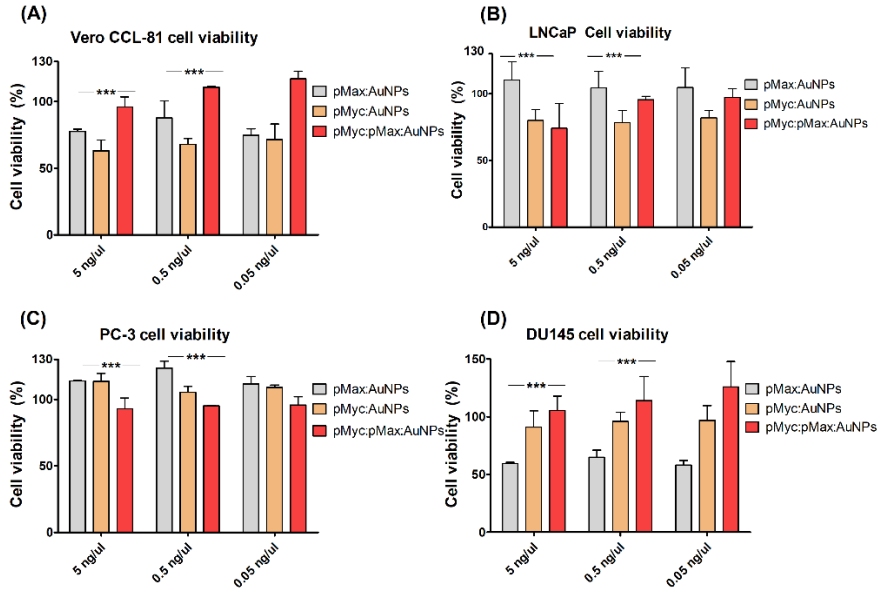

Figure 4. Cell viability assays for the different nanosystems in the four different cell lines. * $p < 0.05$; *** $p < 0.001$. (**A**) Cell viability of Vero CCL-81 cells with the three NS. (**B**) Cell viability of LNCaP cells with three NS. (**C**) Cell viability of PC-3 cells with three NS. (**D**) Cell viability of DU145 cells with three NS.

4. Conclusions

The pMyc and pMax peptides have predicted thermal stability well over 37 °C and have been shown to recognize and bind to canonical e-boxes in an EMSA assay. The hemolytic properties allow us to determine the concentrations for cell viability assay evaluation. All NS that we tested are considered to have low hemolytic properties at the three different concentrations evaluated. Cell viability was affected at different levels depending on the cell line evaluated. Our results suggest that pMyc:pMAx:AuNPs potentially could have a cytotoxic effect by binding to e-boxes in cell's nucleus reducing cell viability.

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Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Conacci-Sorrell, M.; McFerrin, L.; Eisenman, R.N. An Overview of MYC and Its Interactome. *Cold Spring Harb. Perspect. Med.* **2014**, *4*, a014357. https://doi.org/10.1101/cshperspect.a014357.
2. Allevato, M.; Bolotin, E.; Grossman, M.; Mane-Padros, D.; Sladek, F.M.; Martinez, E. Sequence-Specific DNA Binding by MYC/MAX to Low-Affinity Non-E-Box Motifs. *PLoS ONE* **2017**, *12*, e0180147. https://doi.org/10.1371/journal.pone.0180147.
3. Barsouk, A.; Padala, S.A.; Vakiti, A.; Mohammed, A.; Saginala, K.; Thandra, K.C.; Rawla, P.; Barsouk, A. Epidemiology, Staging and Management of Prostate Cancer. *Med. Sci.* **2020**, *8*, 28. https://doi.org/10.3390/medsci8030028.
4. Gandaglia, G.; Leni, R.; Bray, F.; Fleshner, N.; Freedland, S.J.; Kibel, A.; Stattin, P.; Van Poppel, H.; La Vecchia, C. Epidemiology and Prevention of Prostate Cancer. *Eur. Urol. Oncol.* **2021**, *4*, 877–892. https://doi.org/10.1016/j.euo.2021.09.006.
5. Chen, H.; Liu, H.; Qing, G. Targeting Oncogenic Myc as a Strategy for Cancer Treatment. *Signal Transduct. Target. Ther.* **2018**, *3*, 5. https://doi.org/10.1038/s41392-018-0008-7.
6. Waterhouse, A.; Bertoni, M.; Bienert, S.; Studer, G.; Tauriello, G.; Gumienny, R.; Heer, F.T.; De Beer, T.A.P.; Rempfer, C.; Bordoli, L.; et al. SWISS-MODEL: Homology Modelling of Protein Structures and Complexes. *Nucleic Acids Res.* **2018**, *46*, W296–W303. https://doi.org/10.1093/nar/gky427.
7. Wang, Y.; Geer, L.Y.; Chappey, C.; Kans, J.A.; Bryant, S.H. Cn3D: Sequence and Structure Views for Entrez. *Trends Biochem. Sci.* **2000**, *25*, 300–302. https://doi.org/10.1016/S0968-0004(00)01561-9.
8. Pucci, F.; Kwasigroch, J.M.; Rooman, M. SCooP: An Accurate and Fast Predictor of Protein Stability Curves as a Function of Temperature. *Bioinformatics* **2017**, *33*, 3415–3422. https://doi.org/10.1093/bioinformatics/btx417.
9. Jabir, M.S.; Taha, A.A.; Sahib, U.I.; Taqi, Z.J.; Al-Shammari, A.M.; Salman, A.S. Novel of Nano Delivery System for Linalool Loaded on Gold Nanoparticles Conjugated with CALNN Peptide for Application in Drug Uptake and Induction of Cell Death on Breast Cancer Cell Line. *Mater. Sci. Eng. C* **2019**, *94*, 949–964. https://doi.org/10.1016/j.msec.2018.10.014.
10. Verimli, N.; Demiral, A.; Yılmaz, H.; Çulha, M.; Erdem, S.S. Design of Dense Brush Conformation Bearing Gold Nanoparticles as Theranostic Agent for Cancer. *Appl. Biochem. Biotechnol.* **2019**, *189*, 709–728. https://doi.org/10.1007/s12010-019-03151-6.
11. Carabet, L.A.; Lallous, N.; Leblanc, E.; Ban, F.; Morin, H.; Lawn, S.; Ghaidi, F.; Lee, J.; Mills, I.G.; Gleave, M.E.; et al. Computer-Aided Drug Discovery of Myc-Max Inhibitors as Potential Therapeutics for Prostate Cancer. *Eur. J. Med. Chem.* **2018**, *160*, 108–119. https://doi.org/10.1016/j.ejmech.2018.09.023.
12. Holmes, A.G.; Parker, J.B.; Sagar, V.; Truica, M.I.; Soni, P.N.; Han, H.; Schiltz, G.E.; Abdulkadir, S.A.; Chakravarti, D. A MYC Inhibitor Selectively Alters the MYC and MAX Cistromes and Modulates the Epigenomic Landscape to Regulate Target Gene Expression. *Sci. Adv.* **2022**, *8*, eabh3635. https://doi.org/10.1126/sciadv.abh3635.