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

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Introduction

- Myc is a overexpressed transcription factor in several cancer types, including prostate cancer (PCa) (1, 2).
- Myc dimerize with its associated protein Max; the dimer regulates several essential cell processess such as ribosome and mitochondria biogenesis, DNA synthesis, and glycolisis.
- The dimer regulates this proccesses by recognizing and binding to specific DNA sequences known as E-boxes. The dimer has a higher affinity to the canonical e-box (CME) with a sequence of CACGTG, however there exists degenerated sequences to which the dimer have a lower affinity (NE).
- Disrupting the Myc:Max dimer, its recognition of its target DNA or both is a promising strategy to treat cancer (3).

Methods

- pMyc and pMax synthetic peptides were designed, synthesized and then coupled to AuNPs as depicted in Figure 1
- An Electrophoretic Mobility Shift Assay (EMSA) was carried out in an agarose gel to evaluate the peptide's ability to recognize synthetic DNA E-boxes. We designed four different types of double-stranded oligonucleotides. Two oligos had the CME, one had a NE and the last one was a control without a e-box sequence.
- We determined the hemolysis percentage of each different nanosystem (NS) through a Hemolysis test.
- MTT cell viability assays were carried out in four different cell lines: a non-cancerous one (Vero-CCL81) and three prostate cancer ones (PC-3, DU145, and LNCaP).

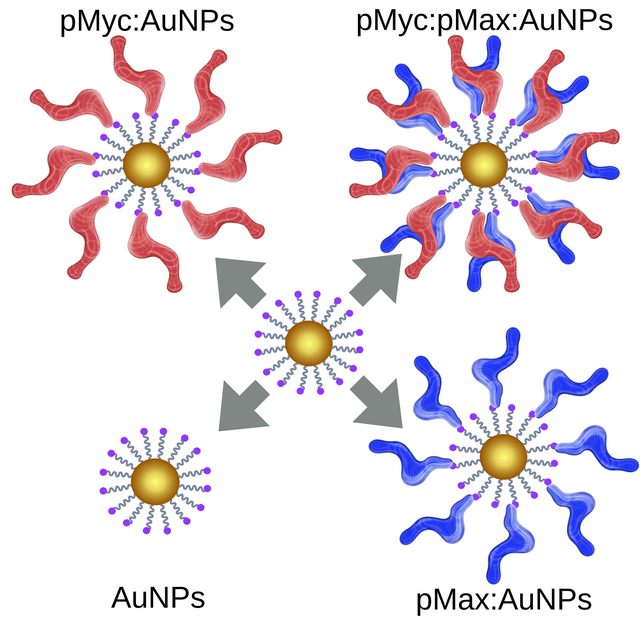
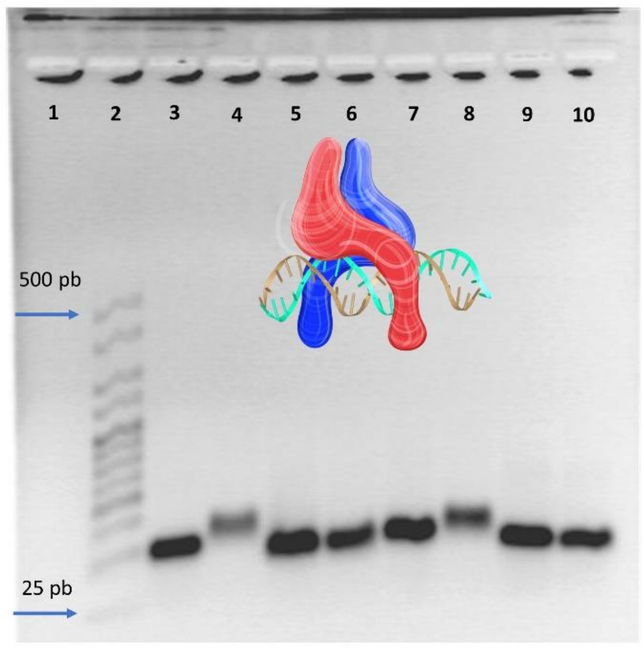


Figure 1. Schematic depiction of the constructed nanosystems with either individual pMyc and pMax homodimers or pMyc:pMax heterodimer

Results and discussion

EMSA



- A shift or retardation in the electrophoretic mobility can be seen in the designed oligonucleotides containing the CME sequence when they were incubated with the pMyc:pMax heterodimer (lanes 4 and 8); whereas this shift is non-existent in the oligonucleotides without a CACGTG sequence.
- These results suggest that the pMyc and pMax peptide can recognize in a specific manner CMEs, but don't recognize NE sequences.

Figure 2. Resolved EMSA 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.

Cell viability assay

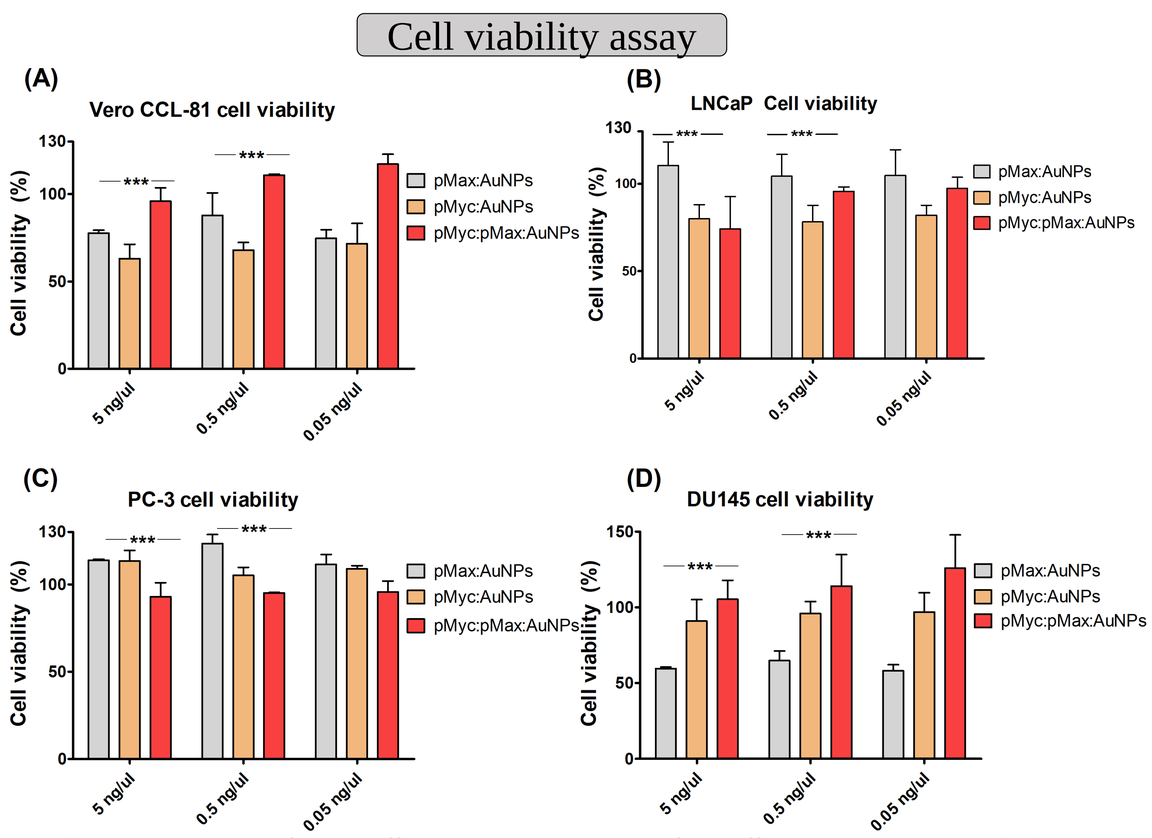


Figure 3. Cell viability assays for the different nanosystems in the four different cell lines. * $p < 0.05$; *** $p < 0.001$ (A) Cell viability of Vero CCL-81 cells with the three NS. (B) Cell viability of LNCaP cells with three NS. (C) Cell viability of PC-3 cells with three NS. (D) Cell viability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

- In Vero CCL-81, the pMyc:pMax:AuNPs showed little to no cytotoxic effect. In LNCaP, pMyc:AuNPs had the highest cytotoxicity.
- In PC-3 cells little cytotoxicity can be seen; only pMyc:pMax:AuNPs showed a cytotoxic effect in these cells.
- Finally, in DU145 cells the most significant cytotoxic effect was shown by the pMax:AuNPs whereas pMyc:AuNPs have a mild effect.

Hemolysis assay

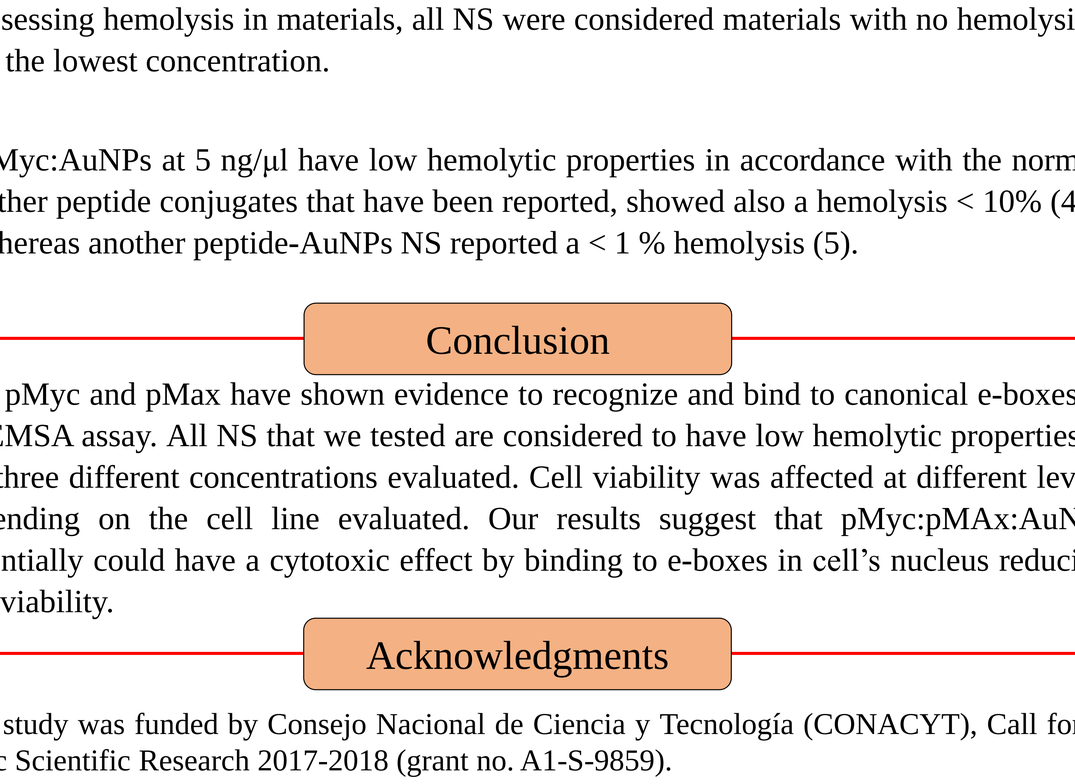


Figure 4. Hemolytic properties of the different nanosystems at three different concentrations. * $p < 0.05$; *** $p < 0.001$

- According to the ASTM-F756-17 norm, which states the standard practice for assessing hemolysis in materials, all NS were considered materials with no hemolysis at the lowest concentration.
- pMyc:AuNPs at 5 ng/µl have low hemolytic properties in accordance with the norm. Other peptide conjugates that have been reported, showed also a hemolysis < 10% (4) whereas another peptide-AuNPs NS reported a < 1 % hemolysis (5).

Conclusion

The pMyc and pMax have shown evidence to recognize and bind to canonical e-boxes in an EMSA assay. 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.

Acknowledgments

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References

1. 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, doi:10.3390/medsci8030028.

2. 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, doi:10.1016/j.euo.2021.09.006.

3. Conacci-Sorrell, M.; McFerrin, L.; Eisenman, R.N. An Overview of MYC and Its Interactome. *Cold Spring Harb. Perspect. Med.* **2014**, *4*, 1–24, doi:10.1101/cshperspect.a014357.

4. 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, doi:10.1016/j.msec.2018.10.014.

5. 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, doi:10.1007/s12010-019-03151-6.