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SERVICES FOR SCIENCE AND EDUCATION

Cell Kinetics and siSTEAP4 Responses

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ABSTRACT

STAMP family genes STAMP1/STEAP2 and STAMP2/STEAP4 have only expressed in androgen receptor-positive cells, the role of AR in STAMP family gene expression is an important question.

Keywords: STAMP2, STEAP4, LNCaP, Tyripan blue.

Highlights:

1. Transfection of STEAP genes to DU145 and PC3 cells.
2. Androgen induction to LNCaP cells
3. Gene silencing of STEAP4
4. Amplification of apoptosis related genes.

INTRODUCTION

Prostate cancer is cancer of the prostate. The prostate is a gland in the male reproductive system that surrounds the urethra just below the bladder. Most prostate cancers are slow growing. Cancerous cells may spread to other areas of the body, particularly the bones and lymph nodes. Globally, it is the second-most common cancer. It is the fifth-leading cause of cancer-related death in men.] In 2018, it was diagnosed in 1.2 million and caused 359,000 deaths. It was the most common cancer in males in 84 countries, occurring more commonly in the developed world. Rates have been increasing in the developing world. Detection increased significantly in the 1980s and 1990s in many areas due to increased PSA testing. One study reported prostate cancer in 30% to 70% of Russian and Japanese men over age 60 who had died of unrelated causes. Scientists have established prostate cancer cell lines to investigate disease progression. LNCaP, PC-3 (PC3), and DU-145 (DU145) are commonly used prostate cancer cell lines. The LNCaP cancer cell line was established from a human lymph node metastatic lesion of prostatic adenocarcinoma. PC-3 and DU-145 cells were established from human prostatic adenocarcinoma metastatic to bone and to brain, respectively. LNCaP cells express AR, but PC-3 and DU-145 cells express very little or no AR. The proliferation of LNCaP cells is androgen-dependent but the proliferation of PC-3 and DU-145 cells is androgen-insensitive. We searched for prostate-specific genes expressed in the early stages of prostate cancer. In one project we came across a gene with six transmembrane domains at its C-terminus (six transmembrane protein of prostate1, STAMP1) (Korkmaz KS., 2002) and later STAMP2 (Korkmaz CG., 2005) and STAMP3 were identified.

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RESULTS

The apoptotic activity that was investigated following the transfer of STAMP genes; typical morphological changes, cell death/cell growth were followed by a standard method, Trypan Blue/Trypan Blue counting. The growth kinetics of DU145 and PC3 cells transfected with FuGENE HD with HisMax-vector, HisMax-STAMP1, HisMax-STAMP2 and HisMax-STAMP3 plasmids were determined by trypsinizing the cells after 48 hours of transfection, seeding them at 10,000 cells/ml and counting them with Trypan Blue for 12 days. Cells were seeded in 1ml in 12-well plates and on the following 2nd, 4th, 6th, 8th, 10th and 12th days, cells were examined under a microscope and cell counts were made with the addition of Trypan Blue.

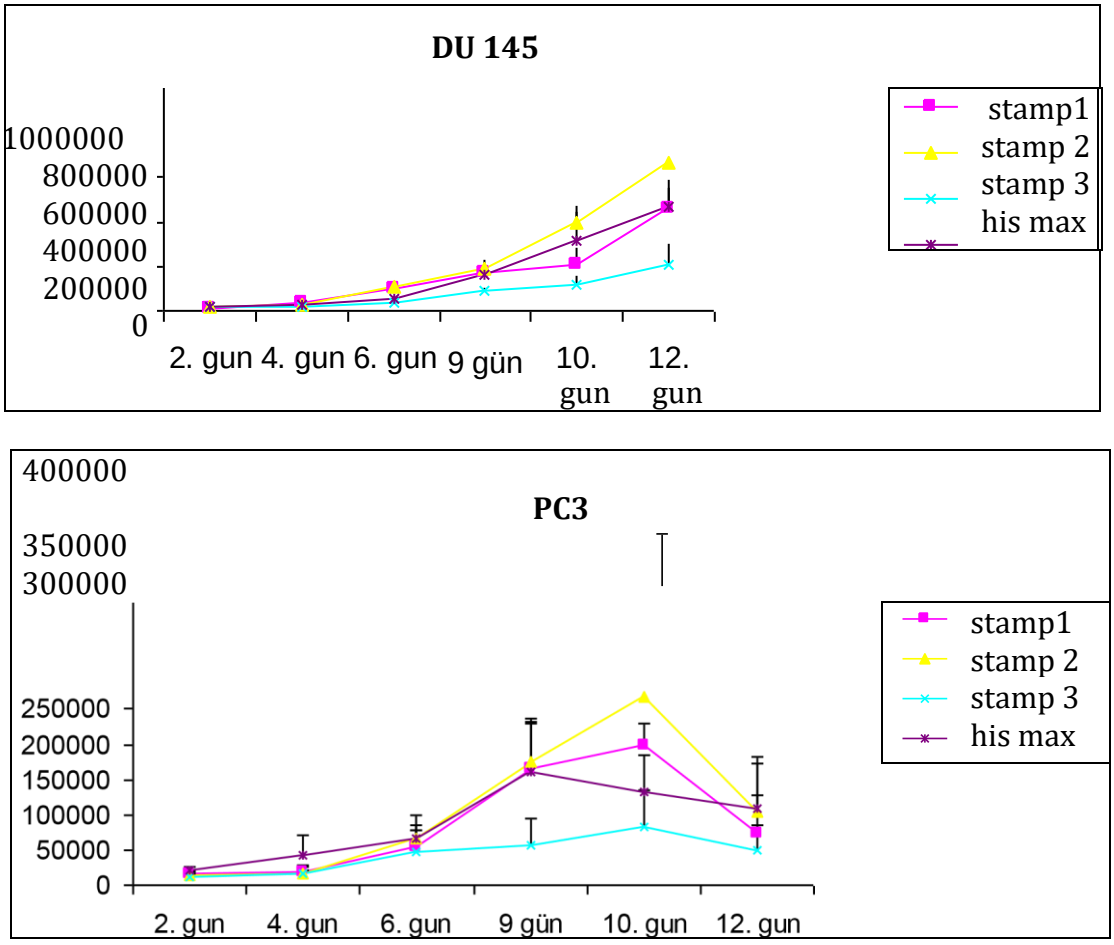

Figure 1 shows the kinetics of DU145 and PC3 cells transfected with HisMax-STAMP1, HisMax-STAMP2 and HisMax-STAMP3.

As shown in the figure, HisMax-STAMP3 transfected cells in both cell lines showed lower growth kinetics compared to HisMax-vector. HisMax-STAMP1 transfection showed a similar picture to HisMax-vector transfection in DU145 cells, while an increased proliferation was detected in PC3 cells on day 10. Both HisMax-STAMP2 transfected cell lines showed a trend

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toward increased expression compared to HisMax-vector transfected cells. Amplification of STAMP2-siRNA (h):sc-89820 Santa Cruz Biotechnology Inc. (Bergheimer, Germany) in LNCaP cells: For STAMP2, the efficiency of silencing was examined in day 1, 2, and 5 samples with specific primers (Figure 2)

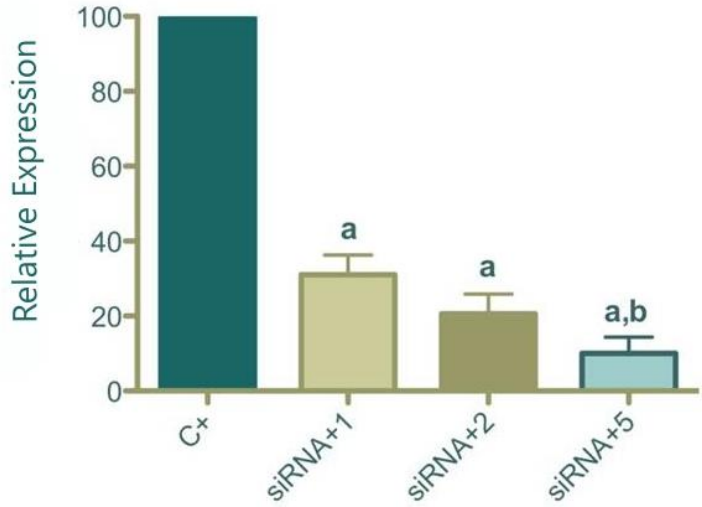


Figure 2: LNCaP cells STAMP2-siRNA treatment. ANOVA followed by Tukey's Multiple Comparison Test C+ vs siRNA+1 aP < 0.001, C+ vs siRNA+2 aP < 0.001, C+ vs siRNA+5 aP < 0.001, siRNA+1st day vs siRNA+5th day bP < 0.05

In the amplifications performed using specific primers for STAMP2 1st, 2nd and 5th day siRNA samples against the control, the 5th day was accepted as the day with the most ideal silencing, For STAMP2, androgen-R1881 induction was performed (Figure 3).

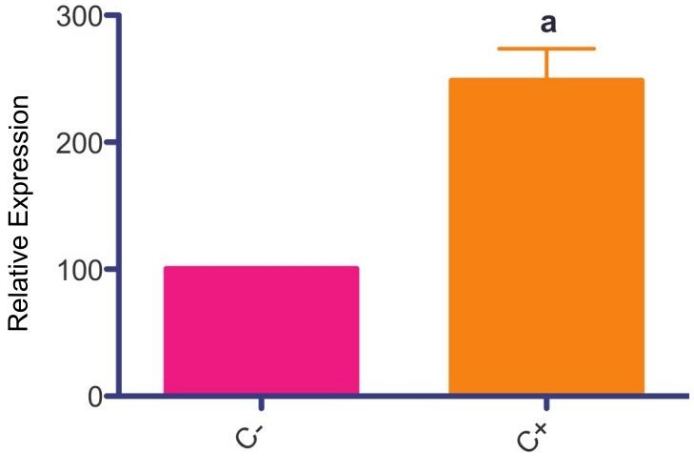
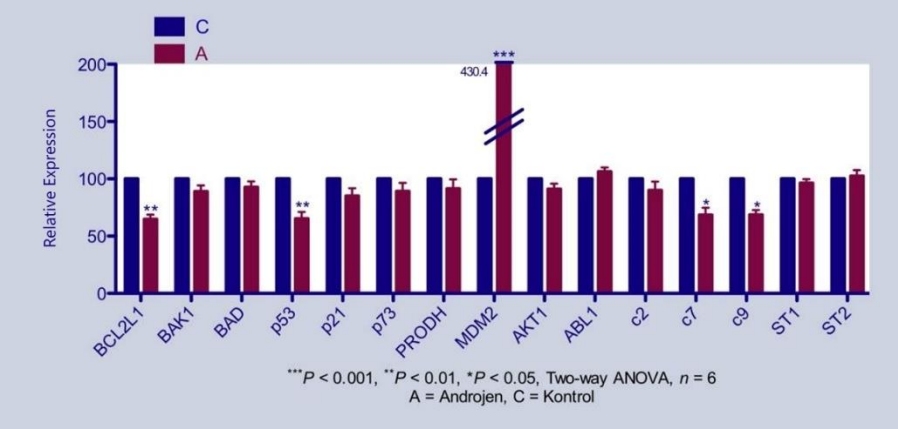
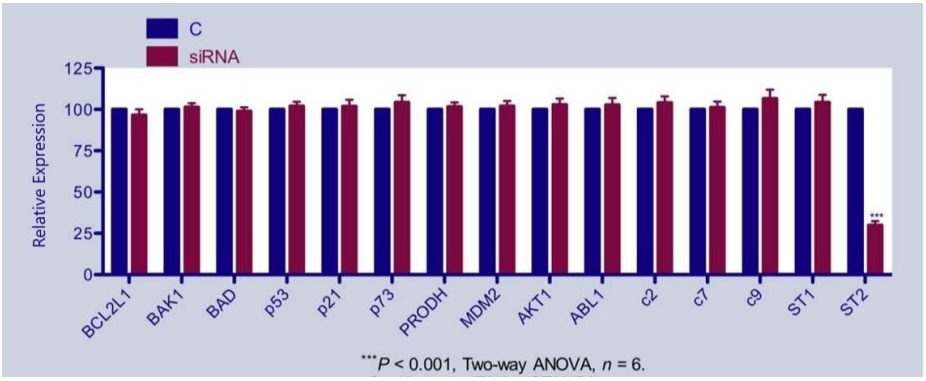


Figure 3: LNCaP cells androgen- R1881 induction.

Paired Samples T Test: C- vs C+, aP < 0.05, n = 3 Increased STAMP2 expression was shown with androgen induction in cells.



Apoptosis panel was applied in -/+ androgen-R1881 induction, control and siRNA groups (Figure 4).



(B)

Figure 4: LNCaP cells in control and R1881 induction (A) and STAMP2-siRNA (B) application

In Figure 4-A, application of synthetic androgen-R1881 to LNCaP cells decreased the expression of androgen proapoptotic and apoptotic genes, but the p53 inhibitor caused a 4.3-fold increase in MDM2. However, STAMP2-siRNA application in Figure 4-B did not cause any change in the apoptosis panel. Androgen induction and STAMP2-siRNA androgen comparison were performed (Figure 5).

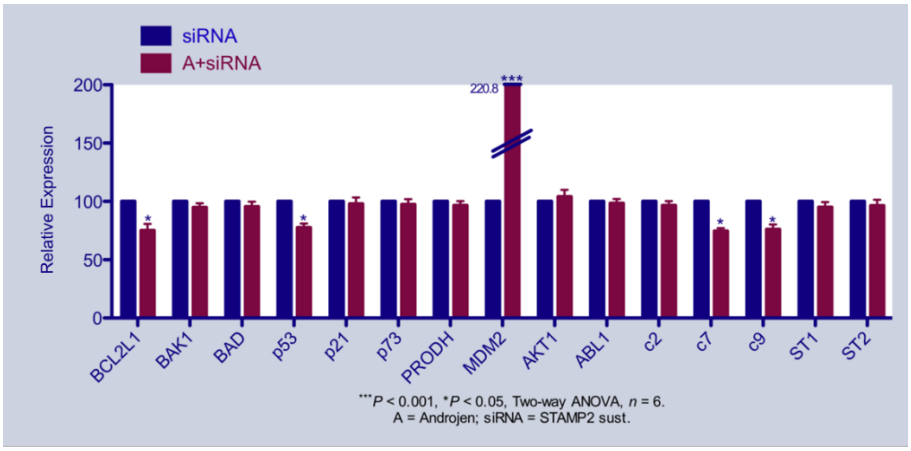


Figure5: LNCaP cells Androgen induction and STAMP2-siRNA+ androgen comparison STAMP2-siRNA group was compared with R1881 + STAMP2-siRNA group (Figure 5).

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Although no change in response was observed with siRNA application, androgen application and synthetic androgen-R1881 caused a decrease in androgen proapoptotic and apoptotic gene expressions, but the p53 inhibitor caused an increase in MDM2 response. At this point, androgen regulation of STAMP2 plays an important role.

DISCUSSION

Prostate cancer is the second most common type of cancer in men worldwide today. Despite advances in diagnosis, follow-up and treatment, prostate cancer is a highly heterogeneous disease. By regulating some genes involved in the cell cycle, STAMP1 causes cycle arrest in the G0 - G1 phase. The proliferative activities of STAMP1 appear to be related to the ERK (extracellular signal-regulated kinase) pathway. Other members of the STAMP family include pHyde, a rat homologue that has been implicated in the apoptosis of prostate cancer cells, and its human homologue TSAP6 (also known as STEAP3), a p53-inducible gene involved in apoptosis and the cell cycle in prostate cancer and HeLa cells. • Studies reported that STAMP family members have metalloreductase activities associated with iron and copper uptake into HEK-293T cells though mentioned activities have been shown for prostate cells

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper

Statistical Analysis

All results represent one of at least three independent experiments with similar outcomes. All data are expressed as the mean ± standard error of mean. One-way analysis of variance (ANOVA) and Tukey post hoc test were used to compare groups of data. $P \leq 0.05$ was considered to indicate a statistically significant result. GraphPad Software, Version 4.03 (San Diego, CA, USA) was used for the statistical analysis.

Abbrevations

MDM2	E3 ubiquitin ligase
STEAP	six transmembrane epithelial antigens of prostate
STAMP	six transmembrane protein of prostate
TUBA	Tukish Academy of Sciences
TUBITAK	The Scientific and Technological Research Council of Türkiye

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Table 1: Genes and primers used as an apoptosis panel for quantitative polymerase chain reaction (qPCR) analysis

GenBank/Symbol	Description	Gene name	Primer sequence
NM_005163/AKT1	V-akt murine thymoma viral oncogene homolog 1	PKB/PRKBA	Forward: TCCCCCTCAGATGATCTCTCCA Reverse: CGGAAAGGTTAAGCGTCGAAAA
NM_001227/CASP7	Caspase 7, apoptosis-related cysteine peptidase	CMH-1/ICE-LAP3	Forward: AAGTGAGGAAGAGTTTATGGCAAA Reverse: CCATCTTGAAAACAAAGTGCCAAA
NM_001229/CASP9	Caspase 9, apoptosis-related cysteine peptidase	APAF-3/APAF3	Forward: TCCTGAGTGGTGCCAAACAAAA Reverse: AGTGGTTGTCAGGCGAGGAAAG
NM_005427/TP73	Tumor protein p73	P73	Forward: AGCAGCCCATCAAGGAGGAGTT Reverse: TCCTGAGGCAGTTTTGGACACA
NM_000546/TP53	Tumor protein p53 (Li-Fraumeni syndrome)	CYS51STOP/P53	Forward: AGATGGGGTCTCACAGTGTTGC Reverse: ATGTTGACCCTTCCAGCTCCAC
NM_002392/MDM2	MDM2 proto-oncogene, E3 ubiquitin ligase	HDMX/MGC71221	Forward: GGGTTCGCACCATTCTCCTG Reverse: GGCAGATGACTGTAGGCCAAGC
NM_152999.3/STAMP1	STEAP family member 2, metalloreductase (STEAP2),	STEAP2/STAMP1	Forward: ATAGGAAGTGGGGATTTTGC Reverse: AGATGTCTCAGGTCCCACAA

	transcript variant 1		
NM_024636.3/STAMP2	STEAP family member 4 (STEAP4), transcript variant 1	STEAP4/STAMP2	Forward: GCACTTACACTGCTTGC Reverse: CAGTGGTCAAGCCAGTC
NM_002046/GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	G3PD, GAPD	Forward: CATTGCCCTCAACGACCACTTT Reverse: GGTGGTCCAGGGGTCTTACTCC

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