International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Flow Cytometry and Annexin V Marking Results

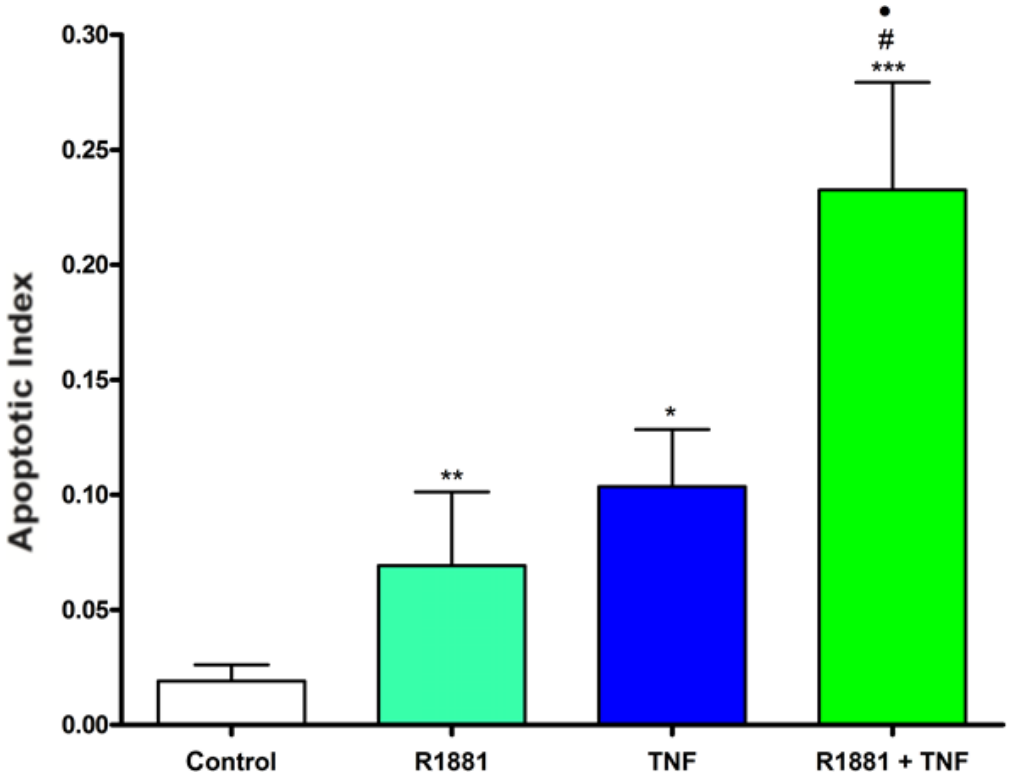
Ceren Gonen

PhD-Associate Professor, EGE University Pharmacy Faculty Dept. Pharmacologybornova Izmir Turkiye
Tel No: +905387377321
Fax No: +902323884687

Prostate cancer is an androgen-dependent cancer that initially develops. In this context, the first protocol of treatment consists of androgen deprivation therapy. Initially, cells that respond well to this treatment gain resistance after a while and begin to grow independently of androgen. The effects of androgens are mediated by the androgen receptor (AR). Therefore, studies focus on identification of AR regulated genes that are also highly expressed in the prostate. STAMP family genes STAMP1/ STEAP2 and STAMP2/STEAP4 are only expressed in androgen receptor positive cells Flow cytometer was used and annex in V signal were taken per sample.

Significance Statement
As you will see the Annex in V signal at all samples of the paper, flow cytometer is the ideal method for apoptosis identification.

Visual Abstract:



1. 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.

2. 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
- **Table name:** Unpaired Student's t-test
- After hydrogen peroxide induction, apoptonic index change was detected by flow cytometry via Annexin- V, and the index increased with the acquisition of STAMP genes of apoptotic index

	Control	TNF	R1881	R1881+ TNF
N	7	6	6	3
Average	0.01915	0.1036	0.06933	0.2326
Std. Deviation	0.01837	0.06088	0.0782	0.0811
Std. Error	0.006942	0.02485	0.03193	0.04682

Figure Legends

- Figure I: Apoptotic index responses taken by Annexin V induction at transfected with vector., ST1, ST2 and incubated with H2O2 DU145 cells by flow cytometer (n=3).
- **Figure 2:** Apoptotic index responses taken by Annexin V induction at transfected with vector.,ST1, ST2 and incubated with H2O2 PC3 cells by flow cytometer (n=3).
- **Figure 3:** Apoptotic index responses taken by Annexin V induction incubated with H2O2 LNCaP cells by flow cytometer (n=5).
- Figure 4, Apoptotic index responses taken by Annexin V induction incubated with TNF LNCaP cells by flow cytometer (n=5).mean ± S.E.M. * P < 0.05; Mann-Whitney-U test; control (n=5) vs TNF (n=5)
- **Figure 4c:** Apoptotic index responses taken by Annexin V induction incubated with synthetic androgen: R1881 and TNF LNCaP cells by flow cytometer (* - control vs other groups, # R1881 vs R1881+TNF, • TNF vs R1881+TNF)

Acknowledgement:
This study was supported by grants from the scientific and technological research council of Turkiye (TUBITAK) to CGK (Grant No. 106S295) and the Turkish Academy of Sciences (Tuba) to CGK (GEBIP- 2007)

Conflict of Interest: The author declares no conflict of interest regarding the publication of this paper.

Abstract: ***Flow-cytometry was used in the detection and evaluation of apoptosis in order to monitor a large number of cells, using FacsAria (BD Biosciences, USA), which was established in Ege University Faculty of Medicine Research Laboratory (AREL). For this purpose, the STAMP1 and 2 gene sequences (HisMax-STAMP1 and HisMax-STAMP2) inserted into the HisMax vector and the HisMax vector as a control were transfected into DU145 and PC3 cells. Since LNCaP cells already have these genes expression H2O2 and TNF-alpha induction were done and flow cytometry was performed with Annexin V labeling.***

Keywords: prostate cancer, apoptosis, flowcytometer, tnf-alpha, hydrogen perokside, androgen induction

3. Materials and Methods

Cell culture. *Du145 and PC3cell lines were cultured in DMEMF12 medium and* LNCaP cells were cultured in RPMI-1640 (Gibco-BRL, Gaithersburg, MD, USA) with 10% fetal bovine serum (FBS), 1% L- glutamine and 1 U/ml each of penicillin/ streptomycin. Cells were incubated at 37˚C with 5% CO_2 in a humidified atmosphere.

Plasmids HisMax-Vektör, HisMax-STAMP1 ve HisMax-STAMP2 genes were transfected with FUGENE. FuGENE HD: DNA ratio was 3:1.

Apoptosis induction: After H2O2 and TNF-alpha addition, flow cytometry was performed with Annexin V labeling.

Flow Cytometer

In order to see the apoptotic cell group formation we used FacsAria (BDBiosciences, USA) analyzed with FacsDiva 6.0 program. Cells were collected with 1 ml culture solution and suspended, after compensation values were arranged multicolor (five parameter) analysis with AnnexinV-FITC blotting had been done.

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.

4. Results

DU145 cells:

The STAMP1 and 2 gene sequences (HisMax-STAMP1 and HisMax-STAMP2) inserted into the HisMax vector and the HisMax vector as a control were transfected into DU145 cells. Apoptotic index was quite high by transfection. After H2O2 induction no change was shown at the index.

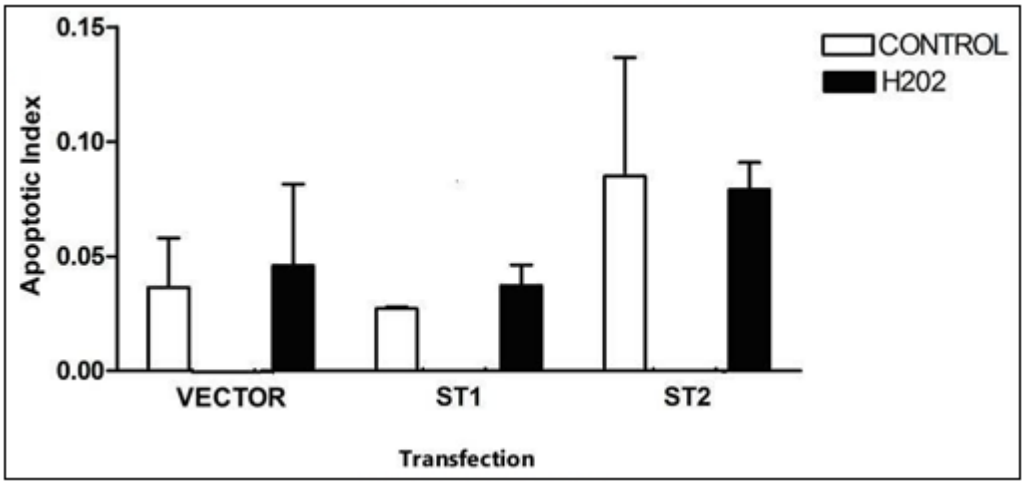


Figure I: Apoptotic index responses taken by Annexin V induction at transfected with vector.,ST1, ST2 and incubated with H2O2 DU145 cells by flow cytometer (n=3).

PC3 cells:

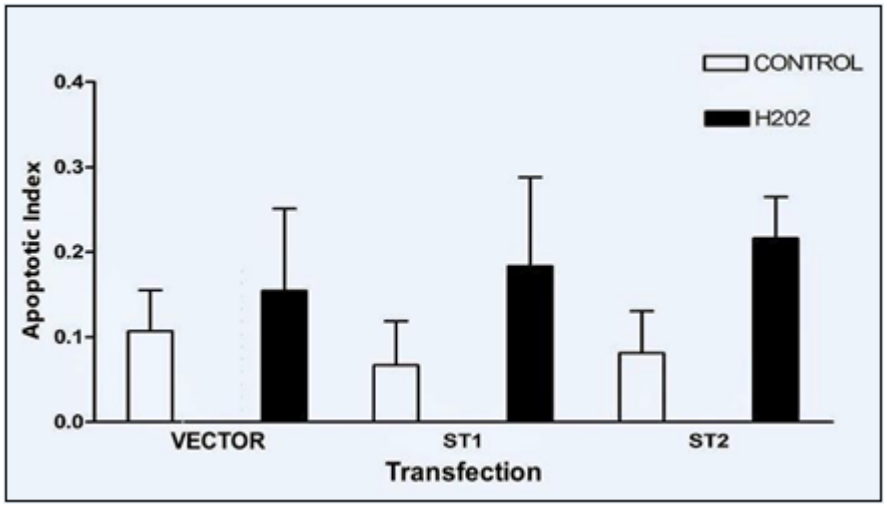


Figure 2: Apoptotic index responses taken by Annexin V induction at transfected with vector.,ST1, ST2 and incubated with H2O2 PC3 cells by flow cytometer (n=3).

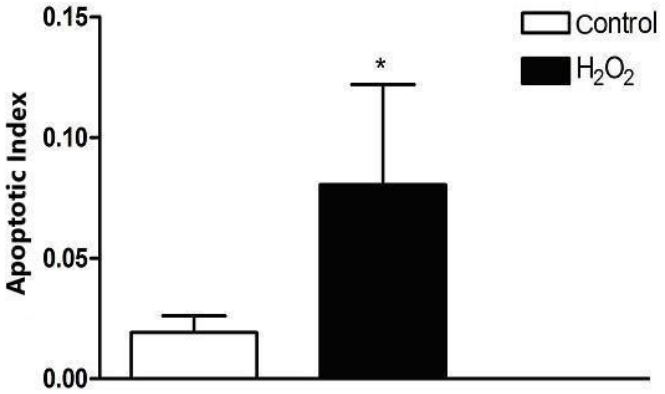
The STAMP1 and 2 gene sequences (HisMax-STAMP1 and HisMax-STAMP2) inserted into the HisMax vector and the HisMax vector as a control were transfected into PC3 cells. Apoptotic index was quite high by transfection. After H2O2 induction no change was shown at the index.

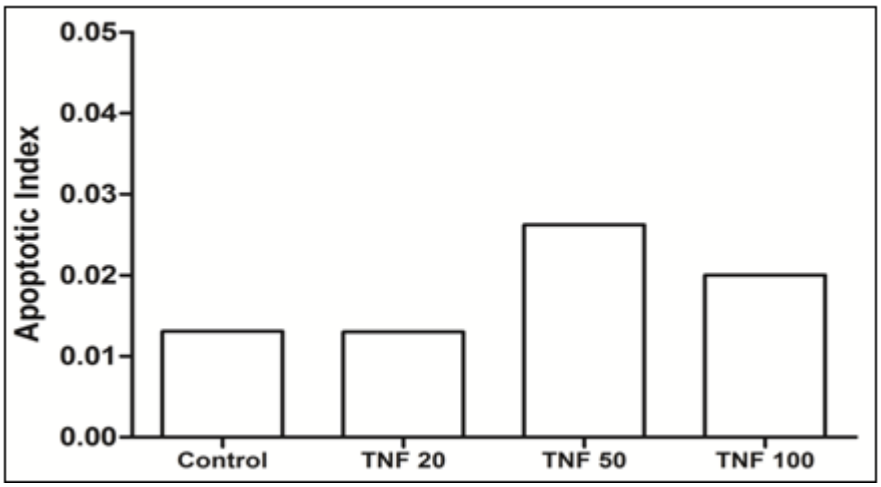
LNCaP cells

Apoptotic index responses taken by Annexin V induction incubated with H2O2 LNCaP cells by flow cytometer (n=5).

Figure 3: Apoptotic index responses taken by Annexin V induction incubated with H2O2 LNCaP cells by flow cytometer (n=5).

- **P*<0.05 control (*n*=7) versus H2O2 incubation (*n*=5); Mann-Whitney U testi.
- TNF-alpha responses (20, 50, 100 ng/ml) at LNCaP cells (Fiure 4a) were investigated and the highest responsive dose 50ng/ml dose was repeated (Figure 4b).





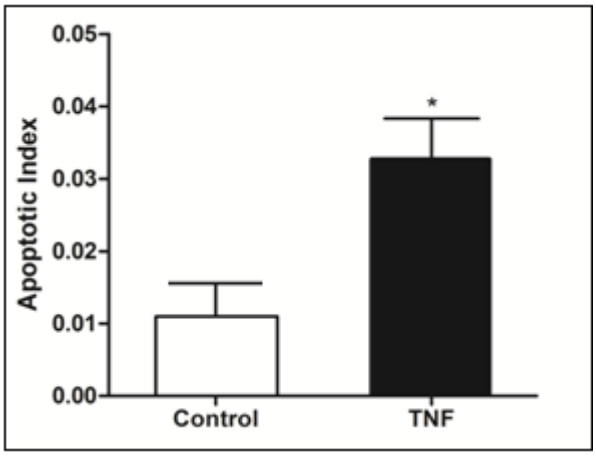


Figure 4: Apoptotic index responses taken by Annexin V induction incubated with TNF LNCaP cells by flow cytometer (n=5).mean ± S.E.M. * P < 0.05; Mann-Whitney-U test; control (n=5) vs TNF (n=5)

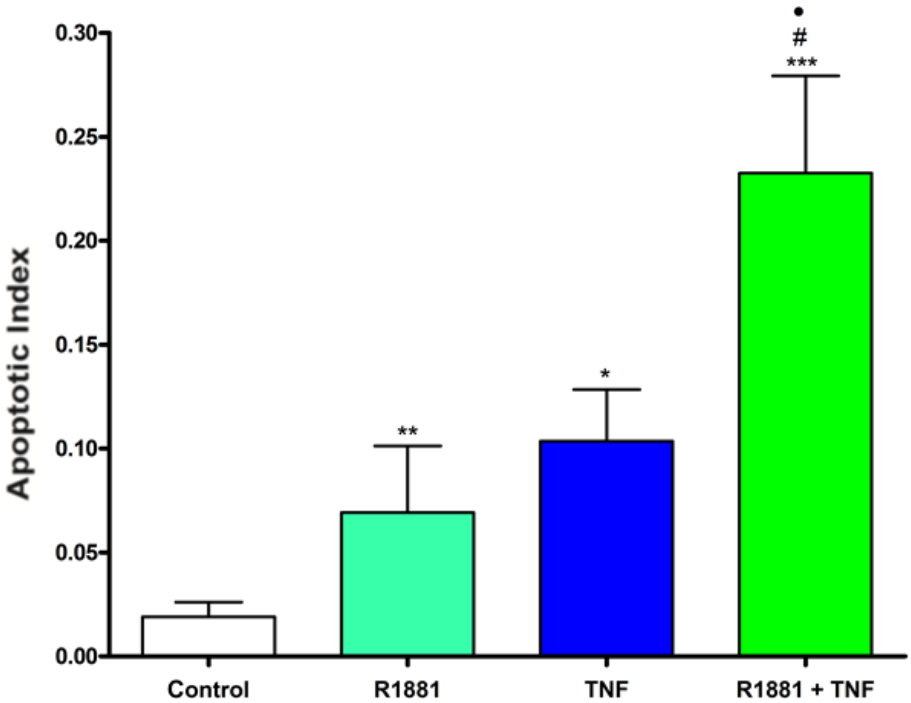


Figure 4 (c): Apoptotic index responses taken by Annexin V induction incubated with synthetic androgen: R1881 and TNF LNCaP cells by flow cytometer (* - control vs other groups, # R1881 vs R1881+TNF, · TNF vs R1881+TNF)

Apoptotic index responses taken by Annexin V induction incubated with synthetic androgen: R1881 and TNF-alpha at LNCaP cells and the last column showed a synergism.

This study was supported by grants from the Scientific and Technological Research Council of Türkiye (TUBITAK) to CGK (Grant no.: 106S295) and the Tukish Academy of Sciences (TUBA) to CGK (GEBIP-2007).

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

References

[1] BIEBERICH, C.J., Fujita, K., He, W.W. and Jay, G. Prostate-specific and androgen-dependent expression of a novel homeobox gene. *J Biol Chem*, 271, 31779-31782 (1996).
[2] BRUCKHEIMER, E. M. and Kyprianou, N. Apoptosis in prostate carcinogenesis. A growth regulator and a therapeutic target. *Cell Tissue Res*, 301(1), 153-62 (2000).
[3] CRAWFORD, E.D., Rosenblum, M., Ziada, A.M. and Lange, P.H. Hormone refractory prostate cancer. *Urology*, 54, 1-7 (1999).
[4] EVENTOFF W, Rossmann MG. The evolution of dehydrogenases and kinases. CRC Crit Rev Biochem. Aug;3(2):111-40(1975).
[5] FULDA S. Evasion of apoptosis as a cellular stress response in cancer. Int J Cell Biol. V 2010:370835. Epub Feb 18 (2010).
[6] HAASS, C. Presenile because of presenilin: the presenilin genes and early onset Alzheimer's disease. *Curr Opin Neurol*, 9, 254-259 (1996).
[7] HE, W.W., Sciavolino, P.J., Wing, J., Augustus, M., Hudson, P., Meissner, P.S., Curtis, R.T., Shell, B.K., Bostwick, D.G., Tindall, D.J., Gelmann, E.P., Abate-Shen, C. and Carter, K.C. A novel human prostate-specific, androgen-regulated homeobox gene (NKX3.1) that maps to 8p21, a region frequently deleted in prostate cancer. *Genomics*, 43, 69-77 (1997).
[8] HSING AW, Chu LW, Stanczyk FZ. Androgen and prostate cancer: is the hypothesis dead? Cancer Epidemiol Biomarkers Prev. 17(10):2525-30 (2008).
[9] HUBERT RS, Vivanco I, Chen E, Rastegar S, Leong K, Mitchell SC, Madraswala R, Zhou Y, Kuo J, Raitano AB, Jakobovits A, Saffran DC, Afar DE. STEAP: a prostate-specific cell-surface antigen highly expressed in human prostate tumors. Proc Natl Acad Sci U S A. 1999 Dec 7;96(25):14523-8(1999).
[10] HUGGINS, C. and Hodges, C.V. Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. 1941. *J Urol*, 167, 948-951; discussion 952 (2002).
[11] Gönen, C. (2023) Promoter Region Analysis of STAMP1/STEAP2 Gene-Silencing of STAMP1/STEAP2 Gene Triggers P53 Upregulation CellBio, 12,1-9. https://doi.org/10.4236/cellbio.2023.121001
[12] Gönen, C. (2022) Responses Taken by Silencing of NFkappaB, STAMP1 and STAMP2 Genes and Expression of NFkB, Act-1, p53 and p73 at -/+ TNFalpha Induced LNCaP Cells. *Journal of Cancer Therapy*, **13**, 685-700. doi: 10.4236/jct.2022.1312060
[13] KORKMAZ, C.G., Korkmaz, K.S., Kurys, P., Elbi, C., Klokk, T.I., Hammerstram, C., Svindland, A., Hager, G.L. and Saatcioglu, F. Molecular cloning and characterization of STAMP2, an androgen regulated six-transmembrane protein that is overexpressed in a subset of prostate cancers. Oncogene. 21; 24(31):4934-45 (2005).
[14] KORKMAZ, K.S., Elbi, C., Korkmaz, C.G., Loda, M., Hager, G.L. and Saatcioglu, F. Molecular cloning and characterization of STAMP1, a highly prostate-specific six transmembrane protein that is overexpressed in prostate cancer. *J Biol Chem*, 277, 36689-36696 (2002).
[15] KORKMAZ, K.S., Korkmaz, C.G., Pretlow, T.G. and Saatcioglu, F. Distinctly different gene structure of KLK4/KLK-L1/prostase/ARM1 compared with other members of the kallikrein family: intracellular localization, alternative cDNA forms, and Regulation by multiple hormones. *DNA Cell Biol*, 20, 435-445 (2001).
[16] KORKMAZ, K.S., Korkmaz, C.G., Ragnhildstveit, E., Pretlow, T.G. and Saatcioglu, F. An efficient procedure for cloning hormone-responsive genes from a specific tissue. *DNA Cell Biol*, 19, 499-506 (2000).
[17] MOLDES, M., Lasnier, F., Gauthereau, X., Klein, C., Pairault, J., Feve, B. and Chambaut-Guerin, A.M. Tumor necrosis factor-alpha-induced adipose-related protein (TIARP), a cell-surface protein that is highly induced by tumor necrosis factor-alpha and adipose conversion. *J Biol Chem*, 276, 33938-33946 (2001).
[18] NELSON, P.S., Gan, L., Ferguson, C., Moss, P., Gelinas, R., Hood, L. and Wang, K. Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate- restricted expression. *Proc Natl Acad Sci U S A*, 96, 3114-3119 (1999).
[19] NELSON, P.S., Clegg, N., Arnold, H., Ferguson, C., Bonham, M., White, J., Hood, L. and Lin, B. The program of androgen-responsive genes in neoplastic prostate epithelium. *Proc Natl Acad Sci U S A*, 99, 11890-11895 (2002).
[20] OHGAMI RS, Campagna DR, Greer EL, Antiochos B, McDonald A, Chen J, Sharp JJ, Fujiwara Y, Barker JE, Fleming MD. Identification of a ferrireductase required for efficient transferrin- dependent iron uptake in erythroid cells. Nat Genet.;37(11):1264-9 (2005).
[21] ORDOVAS, J.M. ABC1: the gene for Tangier disease and beyond. *Nutr Rev*, 58, 76-79 (2000). PASSER BJ, Nancy-Portebois V, Amzallag N, Prieur S, Cans C, Roborel de Climens A, Fiucci G, Bouvard V, Tuynder M, Susini L, Morchoisne S, Crible V, Lespagnol A, Dausset J,
[22] Oren M, Amson R, Telerman A. The p53-inducible TSAP6 gene product regulates apoptosis and the cell cycle and interacts with Nix and the Myt1 kinase. Proc Natl Acad Sci U S A.;100(5):2284-9. Epub 2003 Feb 26 (2003).
[23] POMMIER, Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nat Rev Cancer*, 6(10), 789- 802 (2006).
[24] RITTENHOUSE HG, Finlay JA, Mikolajczyk SD, Partin AW. Human Kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate. Crit Rev Clin Lab Sci. Aug;35(4):275-368(1998).
[25] ROOS WP, Kaina B. DNA damage-induced cell death

by apoptosis. *Trends Mol Med.* Sep;12(9):440- 50. Epub 2006 Aug 8. Review (2006).

[26] SRIKANTAN V, Zou Z, Petrovics G, Xu L, Augustus M, Davis L, Livezey JR, Connell T, Sesterhenn IA, Yoshino K, Buzard GS, Mostofi FK, McLeod DG, Moul JW, Srivastava S. PCGEM1, a prostate-specific gene, is overexpressed in prostate cancer Proc Natl Acad Sci U S A.;97(22):12216-21(2000).

[27] STEINER MS, Zhang X, Wang Y, Lu Y. Growth inhibition of prostate cancer by an adenovirus expressing a novel tumor suppressor gene, pHyde. Cancer Res.15;60(16):4419-25(2000).

[28] STEPHAN C, Jung K, Diamandis EP, Rittenhouse HG, Lein M, Loening SA.Prostate-specific antigen, its molecular forms, and other kallikrein markers for detection of prostate cancer.Urology.;59(1):2-8 (2002).

[29] STEPHENSON, S.A., Verity, K., Ashworth, L.K. and Clements, J.A. Localization of a new prostate- specific antigen-related serine protease gene, KLK4, is evidence for an expanded human kallikrein gene family cluster on chromosome 19q13.3-13.4. *J Biol Chem*, 274, 23210-23214 (1999).

[30] TAMURA T., Chıba J. (2009) STEAP4 regulates focal adhesion kinase activation and CpG motifs within STEAP4 promoter region are frequently methylated in DU145, human androgen- independent prostate cancer cells Int.J.Mol.Med. 24: 599- 604

[31] TUSNADY GE, Bakos E, Varadi A, Sarkadi B. Membrane topology distinguishes a subfamily of the ATP-binding cassette (ABC) transporters. FEBS Lett. Jan 27; 402(1):1-3(1997).

[32] XU, L.L., Su, Y.P., Labiche, R., Segawa, T., Shanmugam, N., McLeod, D.G., Moul, J.W. and Srivastava, S. Quantitative expression profile of androgen-regulated genes in prostate cancer cells and identification of prostate-specific genes. *Int J Cancer*, 92, 322-328 (2001).

[33] YOUSEF, G.M., Obiezu, C.V., Luo, L.Y., Black, M.H. and Diamandis, E.P. Prostase/KLK-L1 is a new member of the human kallikrein gene family, is expressed in prostate and breast tissues, and is hormonally regulated. *Cancer Res*, 59, 4252-4256 (1999).