AD_____

Award Number: W81XWH-11-1-0685

TITLE: Adipose Stem Cell-Based Therapeutic Targeting of Residual Androgens in African Americans With Bone-Metastatic Prostate Cancer

PRINCIPAL INVESTIGATOR: Asim B. Abdel-Mageed, D.V.M., MS, Ph.D.

CONTRACTING ORGANIZATION: Tulane University School of Medicine

New Orleans, LA 70112

REPORT DATE: September 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

D			Form Approved				
Rublic reporting burden for this			OMB No. 0704-0188				
data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions of reducing the time of reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currer valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS .							
1. REPORT DATE (DD September 2014	HMM-YYYY)	2. REPORT TYPE Ann	ual	3. D/ 1 S	ATES COVERED (From - To) Sep 2013 - 31 Aug 2014		
4. TITLE AND SUBTIT Adipose Stem	LE Cell-Based Th	erapeutic Targe	ting of Residua	.1 5a	. CONTRACT NUMBER		
Androgens in A Cancer	ans With Bone-M	etastatic Prost	ate				
					Grant Number B1XWH-11-1-0685		
6. AUTHOR(S)				5d.	PROJECT NUMBER		
Asim B. Abdel-Mag	jeed			5e.	TASK NUMBER		
email: amageed@tulane.edu							
					I. WORK UNIT NUMBER		
7. PERFORMING ORG	AND ADDRESS(ES)		8. F N	PERFORMING ORGANIZATION REPORT			
Tulane University	School of Medicine	9					
1430 Tulane Ave	1099, SL-42						
New Orleans, LA 7	0112						
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS			S(ES)	10.	SPONSOR/MONITOR'S ACRONYM(S)		
Command Fort Detrick, MD 21702	-5015						
				11.	SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / A Approved for public	VAILABILITY STATE	MENT					
13. SUPPLEMENTAR	NOTES						
14. ABSTRACT	he disproportiona	te incidence and m	ortality of prostate	cancer (CaP)) among African Americans (AA) in		
comparison to Caucasian American (CA) are not well understood. It is believed that high circulating androgens reported in AA men may account for such racial disparities. It has been shown that metastatic tumors maintain functional androgen receptor							
signaling, suggest	ing that local (intr	acrine) androgens n	nay contribute to th	e outgrowth c	of 'castration-adapted' tumors under		
androgen deprivation therapy (ADT). Evidence exists for direct correlation between increased obesity and body-mass-index (BMI), which is significantly higher in AA-men, and the risk for accreasive CaP. Active steroidogenic pathways are active in							
adipocytes and adipose-derived mesenchymal stem cells (ASCs) are often recruited to tumor-stroma. Our goal will be to							
exploit the tumor-tropism of normal ASCs to deliver androgen inactivating genes to tumor microenvironments and enable an							
errective treatment strategy against CRPC. This will be achieved by: (a) investigate it "intracrine" production of testosterone by osteotropic ASC ^{AA} modulates growth and metastatic potential of CaP cells under ADT in vitro and in vivo: (b) determine if g							
HSD-expressing osteotropic ASC ^{Cont} will nullify the ADMSCAA-mediated CaP cell growth and metastasis in vitro; and (c)							
examine the efficacy of therapeutically engineered ASC ²⁰¹¹ to target and inhibit CaP tumor growth under CRPC in vivo. The							
androgens. Considering the aggressive nature CaP, the outcome of our study is expected to have a positive impact on establishing preventive and/or therapeutic intervention strategies to reduce or circumvent PC, especially among AA men							
15. SUBJECT TERMS			<u> </u>		,,		
Prostate cancer, health disparity, stem cells, hormone inactivating enzymes, CRPC, steroidogenic enzymes, African Americans.							
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON		
a. REPORT	b. ABSTRACT	c. THIS PAGE		12	19b. TELEPHONE NUMBER (include area code)		

Standard	Form	298	(Rev.	8-98
Prescribed b	y ANSI	Std. Z	39.18	

Table of Contents

<u>Page</u>

Table of Contents	3
Introduction	4
Body	4
Discussion	9
Key Research Accomplishments	9
Reportable Outcomes	9
Conclusion	10
References	12

Introduction:

Race, a Risk Factor for Prostate Cancer (CaP). In the US, African Americans have the highest annual incidence of CaP, at 272 new cases per 100,000 men [1]. In 2009, 230,000 men were diagnosed with CaP, and more than 38,000 afflicted men are expected to die [2]. Although the etiology remains largely unknown, racial make-up has been identified as one of several risk factors for CaP. African-American (AA) men bear a disproportionately heavy burden from this disease with incidence and mortality rates over 50% higher than Caucasian American (CA) men [3, 4]. Furthermore, AA men are more likely to develop CaP at an earlier age, have higher rate of Gleason-7, aggressive tumors, and metastasis, and exhibit a poorer survival rate than CA males [3-7]. Socioeconomic and environmental factors, such as diet, access to care, and screening, have been cited as factors contributing to the more clinically aggressive CaP in AA patients [8, 9]. Family history accounts for 5-10% of total CaP cases [8, 9], and it does not differ among AA, Asian Americans, and CA men [10, 11]. A more biologically aggressive CaP has been proposed as one possible explanation for the younger age at presentation and disease progression in AA men compared with CA men [12, 13].

Obesity and BMI as Risk Factors for CaP progression in AA men. Prostate cancer incidence and mortality rates correlate well with the average intake of fats, including polyunsaturated fats [14]. *In vivo* and *in vitro* models have demonstrated a decreased rate of proliferation of prostate tumors with reduced fat intake [15-17]. One meta-analysis showed a 5% excess risk of developing prostate cancer for each 5 kg/m² increment of BMI [18]. When disease stage was considered, the analysis showed a rate ratio for advanced cancer of 1.12 per 5 kg/m² increment. An analysis from the CaP Prevention Trial noted that compared with men with a BMI below 25 kg/m², those with a BMI above 30 kg/m² had an 18% decrease in the risk of low-grade cancer, but a 29% increase in the risk of high-grade cancer [19]. In addition, obesity has been linked to aggressive CaP [20] and increased BMI has also shown a positive correlation to Gleason score and positive surgical margins [21]. The latter is critical as it can be an indicator for disease relapse. However, the mechanisms linking obesity to CaP development and progression are not fully understood. Since the prevalence of obesity is significantly high in AA men [22], accounting for 37.3%, unraveling such mechanisms is of paramount significance.

"Intracrine androgens" and CaP progression. Androgen-deprivation therapy (ADT) has been the mainstay treatment for patients with metastatic CaP [23]. Although initially effective, hormonal therapy is marked by progression to castration-resistant prostate cancer (CRPC) over a period of 18-20 months. with median survival of 1-2 years. Importantly, large body of evidence indicate that in the setting of 'castrate' serum testosterone levels, prostatic androgen concentrations remain at approximately 10-25% of the levels found in untreated patients [24-26] well within the range capable of mediating continued androgen-receptor (AR) signaling and gene expression [27]. Moreover, residual intra-prostatic androgens are implicated in nearly every mechanism whereby AR-mediated signaling leads to the development of castration-resistant disease [28]. The increased expression of androgen-metabolizing genes within castration-resistant metastatic tumors [29] strongly suggests that up-regulated activity of endogenous steroidogenic pathways is driving the outgrowth of 'castration-adapted' tumors. The source of residual androgens within the prostate tumors of castrate men has not been fully elucidated, but has been attributed to the uptake and conversion of circulating adrenal androgens [30]. Whether the de novo biosynthesis of androgens from cholesterol or earlier precursors occurs within castration-resistant metastases is not known [28] but has significant implications for treatment strategies targeting sources of androgens exogenous to the prostate versus 'intracrine' sources active within the actual metastatic tumor microenvironments.

Body

Initially, we confirmed Data generated last year in Aim-2. To this end, the optimal dose, growth inhibitory effects, and ability of recombinant α -HSD to degrade DHT and suppress AR activity in LNCaP cells were examined in vitro. Briefly, the conditioned media (CM) of <u>normal ADMSCs</u> transduced with either pLVX-IL-2SS-Akr1c14-IRES-GFP or the control LV constructs were collected and protein concentrations were measured by BCA assays. Figure 1 (next page) depicts high expression of Akr1c14-

IRES-GFP (α -hydroxysteroid dehydrogenase, α -HSD) and GFP in tumor-tropic ADMSCs. The enzyme gene expression and the release of its recombinant protein product were measured by PCR and immunoblot analysis (Fig. 1).

Next, we examined the effect of the enzyme in the CM on growth of the androgen dependent LNCaP cells. Briefly, cells were cultured overnight in

a charcoal stripped medium supplemented with 0.1 nM DHT in 96-well plates in triplicates and then subjected to various protein concentrations of CM derived from control or 3αHSDexpressing ADMSCs for an additional 24 hrs. As shown in Figure 2, the enzyme inhibited growth of the LNCaP cells in a dose dependent manner in presence, but not in absence, of NAD, which is required for enzyme activity. The growth inhibition/cell death was monitored with MTT (Fig 2A) and LDH (Fig 2B).

To verify if the growth inhibitory and induced cell death is mediated through degradation of androgens, DHT levels were measured by EIA assay in LNCaP treated cells with rhAKR1C4 or CM derived from control or 3aHSD-expressing ADMSCs. As shown in Figure 3, both rhAKR1C4 and r3αHSD degraded DHT in presence NAD compared to controls (Fig 3A). Additionally, the degradation of in the medium was coupled with suppression of AR activity in LNCaP cells (Fig 3 B). The





Fig.1: AKR1C14 EXPRESSES & RELEASES TYPE 13αHSD IN CULTURE MEDIA

results suggested that the enzyme-induced growth inhibition/cell death in LNCaP cells was attributed to degradation of DHT and suppression of AR activation in the androgen-dependent cells.

In another set of *in vitro* experiments, we tested the hypothesis that tumor-tropic CaP patientderived, but not normal, contributes to production of residual androgens upon recruitment and interaction with prostate tumor cells in primary or metastatic microenvironment. To achieve this, we examined and compared if conditioned media (CM) of prostate cancer (CaP) cells induces expression of androgen metabolizing enzymes (AMEs) in tumor-tropic enriched ADMSCs procured from Gleason score-matched (Gleason 6) African Americans (AA) and Caucasian (CA) prostate cancer patients. Briefly, normal and AAderived ADMSCs were cultured in DMEM F-12 with 10% charcoal stripped FBS and at 70% confluence, and then treated with CM of LNCaP, C4-2B or normal prostate epithelial cells (RWPE1) for 72 hours. Thereafter. AME genes expression was quantitated through gRT-PCR and the results were normalized to normal stem cells. The results shown in Figure 4. demonstrate that the CaP cell CM-treated ADMSCs from AA-men, but not normal ADMSCs, induces



steroidogenic gene expression of 17- β -hydroxysteroid dehydrogenase (17beta-HSD) as well as SRD5A1 and 2 transcripts, which encode for 5 α -reductase subtype 1 and 2 required for conversion of T into dihydrotestosterone (DHT) in response CM of LNCaP and C4-2B cells, and a decrease in androgen hydrolyzing enzyme 3 α HSD1 compared to CM of normal prostate epithelial cells (RWPE1). The results suggest that tumor-tropic ADMSCs contributes to production of residual androgens in the tumor microenvironments (primary or metastatic) in prostate cancer patients, especially among AA-men. Our initial analysis of AME expression in 5 ADMSCs derived from CA men (*data not shown*) demonstrate lesser expression of AMEs compared to ADMSCs derived from AA men, further attest to racial differences in production of residual androgens in the tumor microenvironment.

We further examined if there are differences in androgen dependent prostate tumor growth *in vivo* due to racial disparity of residual androgen production by CaP patient-derived ADMSCs from AA and CA men. Briefly, LNCaP cells (2×10^6) were mixed in Matrigel with ADMSCs (5×10^5) procured from AA (n=5) or CA (n=5) with CaP and inoculated s.c. on the right and left flank of athymic nude mice, respectively. Tumor growth was monitored for 6 weeks. As shown in Fig. 4B, LNCaP tumor formation was larger (2 to 3 fold) when co-inoculated with ADMSCs from AA (right flanks) compared to ADMSCs from CA men with CaP. The results further affirm that production of residual androgens by ADMSCs in the tumor micro environment may contribute to disease progression among AA men. Current studies are focused on immune-staining of key AME enzymes by IHC on tumor sections. A manuscript under preparation.

Figure: 4B



In addition to the above experiments, we attempted to complete Specific Aim 3 in the 3rd year of the project as described below:

<u>Specific Aim-3</u>:- Examine the efficacy of the therapeutically engineered ADMSC^{Cont} to target and inhibit CaP tumor cell growth under hormone deprived conditions *in vivo*. This aim tests the *working hypothesis* that therapeutic targeting by osteotropic α -HSD-expressing ADMSC^{Cont} suppresses CaP cell metastatic lesions when co-transplanted in bone with ADMSC^{AA} in castrated male mice.

<u>Task-3-1</u>: Compare the ability of ADMSC^{Cont} and ADMSC^{Sel} cells to colocalize to bone tumor xenografts *in vivo*. 24-30

<u>3.1.1</u> Inject CaP cells, alone or with ADMSC^{AA}, at intraosseous (femur) sites in nude-mice and monitor tumor development by tumor (~3-5 wks) by palpating and by serum PSA levels.

Unlike soft tissue, current imaging technologies are limited in their ability to detect tumor formation in bones. To overcome such a limitation, we employed IRDye® 800CW BoneTagTM Optical Probe (LI-COR); calcium-chelating compounds have been used effectively for the detection of bone mineralization, to produce a near infrared (NIR) optical bone marker for small animal imaging. To perform the *in vivo* testing, LNCaP cells (2 x 10⁶) were re-suspended in Matrigel and injected intratibially in in 3 nu/nu mice. After 4 to 6 weeks, the IRDye IRDye 800 BoneTag optical probe (reconstituted in 0.5 ml of sterile 1X PBS for a final concentration of 0.02 nmol/µL) was injected at 2 nmol (100 µL) intravenously



via the tail vein. Imaging was performed 48 hrs later to allow unretained agent to clear from the animal's circulation. I imaging was performed on sedated mice using Odyssey CLX at 800 channel by placing the animal on the imaging bed with the tumor side facing down for optimal signal. As shown in Figure 5 (previous page), tumors successfully developed in the left tibia (white arrow) in 2 out of 3 animals tested and their images was captured by Odyssey system. However, a limitation of this system is that the tumor size cannot be quantitatively measured. To overcome this problem, we plan to use RDye 800CW 2-DG Optical Probe (Catalog# 926-08946), which specifically stain tumor cells green, or the Y-27 Optical Probe specifically designed to target prostate specific membrane antigen (PSMA) on tumor cells. These studies are currently pursued (see below).

3.1.2. Tail vein injection of labeled-ADMSCs (either ADMSC^{Cont} or ADMSC^{Sel} cells from same batch) and monitor whether the enriched ADMSCs cells preferentially engraft within the bone tumor foci. These experiments are meant to monitor the ability of LV-transduced ADMSC^{Sel} cells to suppress the CaP cell growth promoting effects of ADMSC^{AA} cells in tumor xenografts *in vivo*. The completion of these experiments is contingent upon successful transplantation of LNCaP in bone (Aim 3.1.1).

3.1.3. Investigate the effects of coinjected ADMSC^{Sel} cells producing α -HSD on tumor size in male nude-mice subcutaneously injected with CaP cells.

In a pilot study, we examined the efficacy of dual labeling (green for tumor and red for bone) in nude mice. To achieve this goal, three nude mice were injected s.c. and intra-tibially with LNCaP cells as shown above. This was followed by iv injection of IRDye 680RD Optical Probe, which stained tumors with green color, and IRDye 680RD, which stain bone with red color. As shown in Fig 6, the LNCaP tumors stained green and the bone stained red. While s.c tumors are well developed, the intra-tibial tumors are weekly stained with green (yellow circle). Additionally, the guts stained non-specifically with the bone dye due to presence of minerals in the diet (red arrow). Due to poor intra-osseous tumor growth, the results

suggest that the s.c. model will be suitable to trace engraftment and expression of the transgene by the tumor-tropic ADMSCs at the tumor sites in vivo (Fig 6).

We requested a one-year extension to use the developed models to (a) determine the effects of engineered ADMSC^{Sel} cells injected via tail vein on growth of bone tumor xenografts containing CaP cells, alone or with ADMSC^{AA}; (b) determine ADMSCs (mCherry) engraftment and local α -HSD expression in tumor bearing mice injected with either ADMSC^{Cont}, ADMSC^{Sel} or engineered ADMSC^{Sel} cells; (c) carry out similar experiments in castrated mice, to demonstrate that the tumor-homing of engineered ADMSC^{Sel} cells can target local androgens and suppress tumor growth, even under ADT.



Discussion

Emerging evidence suggests that "intracrine" androgens, produced de novo in the tumor microenvironment, pose challenge in the clinical management of prostate cancer. A number of studies demonstrated that the levels residual androgens at metastatic sites of castrated patients are sufficient to restore AR signaling and support growth of prostate tumor cells. To overcome, the therapeutic limitation of the androgen ablation therapy, we proposed to examine the efficacy of androgen catabolizing enzyme, 3αHSD, in degrading/suppressing DHT activity in the tumor microenvironment. In studies presented herein, we were able to generate stable normal ADMSCs expressing GFP using a lentivirus construct. Additionally, normal ADMSCs transduced with a pLVX-IRES-ZsGreen construct successfully expressed secretable IL-2SS-Akr1C4 gene product. The secreted Akr1C4 successfully inhibited growth of androgendependent LNCaP cells in vitro. Subsequent functional assays demonstrate that 3aHSD (whether recombinant or produced in CM) is effective in degrading DHT in vitro. The suppression of DHT activity is coupled by inhibition of AR transactivation and proliferation and induction of cytotoxicity of the androgendependent LNCaP cells, Additionally, after several attempts, we also demonstrated using bone tag probes that LNCaP tumors could be successfully developed in long bone through intra-tibial injections. However, current strategies are focused on use of dual fluorescent color system to make osseous tumor growth quantifiable. Additionally, a model based on s.c. transplantation was also established to quantify targeting of ADMSCs and tumor growth in vivo. In another set of experiments, we demonstrate that, unlike normal ADMSCs, patient derived ADMSCs express AMEs in response to the CM of prostate cancer cells. Moreover, ADMSCs from African Americans (AA-men) have higher AME expression than those procured from Caucasian Americans CA). Co-transplantation of ADMSCs from AA-men with LNCaP cells triggered 2 to 3 fold higher tumor growth in comparison to CA-derived ADMSCs in vivo. Together, our findings propose new concepts for targeted residual androgens within the tumor microenvironment to circumvent clonal expansion at primary and metastatic sites. Our observations also suggest that a multi-targeted hormonal therapeutic modality designed to ablate 'intracrine' androgens in tumor environment is required to enable effective and optimal treatment response in patients with castration-resistant PC (CRPC), especially among AA-men.

Key Research Accomplishments

The following experiments were completed:

- a. The recombinant 3αHSD (whether recombinant or produced in CM) is effective in degrading DHT only in presence of NAD *in vitro* (ELISA assays).
- b. The recombinant, whether recombinant or produced in CM, was able to inhibit growth and AR transactivation in androgen-dependent LNCaP cells in a dose dependent manner.
- c. Patient-derived ADMSCs, especially from AA-men, express AMEs and DHT when triggered by CM of prostate cancer cells compared to CA-men.
- d. The patient-derived ADMSCs from AA-men trigger prostate tumor growth by 2 to 3 fold when cotransplanted with LNCaP cells compared to CA-derived ADMSCs.
- e. LNCaP tumors successfully developed intraosseously. The tumors were detected by Li-COR using bone tag probes. Dual fluorescent probes are currently tested to quantify engraftment of ADMSCs and inhibition of tumor growth by 3αHSD-expressing ADMSCs.
- f. We developed a s.c. LNCaP tumor xenografts as alternative strategy to bone xenografts.

Reportable Outcomes

A manuscript will be submitted for publication.

Conclusions

African Americans (AA) have twice the incidence and mortality of prostate (PC) than Caucasian Americans (CA). While the disproportionate burden was partially explained by genetic, socioeconomic, and environmental factors, racial variation in the biology of prostate tumors was not investigated. Emerging evidence suggests that unknown factors promote tumor growth via unknown mechanisms. Recently, "Intracrine" and rogens have been consistently implicated in the outgrowth of castration-adapted prostate tumors through activation of functional androgen-signaling cascade under androgen deprivation therapy (ADT). To overcome, the therapeutic limitation of the androgen ablation therapy, we proposed to examine the efficacy of androgen catabolizing enzyme, 3αHSD, in degrading/suppressing DHT activity in the tumor microenvironment. In studies presented herein, we were able to generate stable normal ADMSCs expressing GFP using a lentivirus construct. Additionally, normal ADMSCs transduced with a pLVX-IRES-ZsGreen construct successfully expressed secretable IL-2SS-Akr1C4 gene product. The secreted Akr1C4 successfully inhibited growth of androgen-dependent LNCaP cells in vitro. Subsequent functional assays demonstrate that 3α HSD (whether recombinant or produced in CM) is effective in degrading DHT in vitro. The suppression of DHT activity is coupled by inhibition of AR transactivation and proliferation and induction of cytotoxicity of the androgen-dependent LNCaP cells. Additionally, after several attempts, we also demonstrated using bone tag probes that LNCaP tumors could be successfully developed in long bone through intra-tibial injections. However, current strategies are focused on use of dual fluorescent color system to make osseous tumor growth guantifiable. Additionally, a model based on s.c. transplantation was also established to quantify targeting of ADMSCs and tumor growth in vivo. In another set of experiments, we demonstrate that, unlike normal ADMSCs, patient derived ADMSCs express AMEs in response to the CM of prostate cancer cells. Moreover, ADMSCs from African Americans (AA-men) have higher AME expression than those procured from Caucasian Americans CA). Co-transplantation of ADMSCs from AA-men with LNCaP cells triggered 2 to 3 fold higher tumor growth in comparison to CA-derived ADMSCs in vivo. Together, our findings propose new concepts for targeted residual androgens within the tumor microenvironment to circumvent clonal expansion at primary and metastatic sites. Our observations also suggest that a multi-targeted hormonal therapeutic modality designed to ablate 'intracrine' and rogens by patient derived ADMSCs is required to enable effective and optimal treatment response in patients with castration-resistant PC (CRPC), especially among AA-men.

Bibliography

- 1. Quinn M and Babb P (2002) Patterns and trends in prostate cancer incidence, survival, prevalence and mortality. Part II: individual countries. *BJU Int* **90**: 174–184.
- 2. American Cancer Society: Cancer Facts & Figures, 2009.
- 3. Zaridze, D.G., Boyle, P., and Smans, M. International trends in prostate cancer. Int. J. Cancer. 33: 223, 1984.
- 4. de Vere White, R.W., Deitch, A.D., and Jackson, A.G. radical differences in clinically localized prostate cancers of black and white men. J. Urol., 159:1979-83, 1998.
- 5. Jones, G.W., Mettlin, C., Murphy G.P., et al. Patterns of care of carcinoma of the prostate gland, results of a national survey of 1984 and 1990. J. Amer. Coll. Surg., 180: 545, 1995.
- 6. Ndubuisi, S.C., Kofie, V.Y., Andoh, J.Y., and Schwartz, M. Black-white differences in the stage at presentation of prostate cancer in the District of Columbia. Urology, 46:71, 1995.
- 7. Kim, J.A., Kuban, D.A., el-Mahdi, A.M., and Schellhammer, P.E. Carcinoma of the prostate, race as a prognostic indicator in definitive radiation therapy. Radiology, 194:545, 1995.
- 8. Ries, L, Esner, M., Kosary, CL, et al. SEER cancer statistiAC review, 1973-96. National Cancer institute, Bethesda, MD, 1999.
- 9. Lu-Yao, G. L., McLerran, D., Wasson, J. and Wennberg, J. E. An assessment of radical prostatectomy. Time trends, geographic variation, and outcomes. The Prostate Patient Outcomes Research Team. (1993) *J. Am. Med. Assoc.* 269:2633-2636.
- 10. Whittemore, A.S., Wu, A. H., Kolonel, L. N., et al. Family history and prostate cancer risk in black, white, and Asian men in the United States and Canada. Amer. J. Epidemiol., 141:732, 1995.
- 11. Carter BS, Bova GS, Beaty TH, Steinberg GD, Childs B, IsaaAC WB, Walsh PC. Hereditary prostate cancer: epidemiologic and clinical features. J Urol., 150(3):797-802, 1993.
- 12. Boring, C. C. Cancer StatistiAC. (1991) CA Cancer J. Clin. 41:19-36.
- 13. Epstein, J. I., Pizov, G. and Walsh, P. C. Correlation of pathologic findings with progression after radical retropubic prostatectomy. (1993) *Cancer* 71:3582-3593.
- 14. Bostwick DG et al. (2004) Human prostate cancer risk factors. Cancer 101: 2371–2490
- 15. Clinton SK *et al.* (1988) The combined effects of dietary protein and fat intake during the promotion phase of 7,12-dimethylbenz(a)anthracene-induced breast cancer in rats. *J Nutr* 118: 1577–1585
- 16. Wang Y *et al.* (1995) Decreased growth of established human prostate LNCaP tumors in nude mice fed a low-fat diet. *J Natl Cancer Inst* 87: 1456–1462
- 17. Aronson WJ *et al.* (1999) Decreased growth of human prostate LNCaP tumors in SCID mice fed a low-fat, soy protein diet with isoflavones. *Nutr Cancer* 35: 130–136
- 18. MacInnis RJ and English DR (2006) Body size and composition and prostate cancer risk: systematic review and meta-regression analysis. *Cancer Causes Control* 17: 989–1003
- 19. Gong Z *et al.* (2006) Obesity, diabetes, and risk of prostate cancer: results from the Prostate Cancer Prevention Trial. *Cancer Epidemiol Biomarkers Prev* 15: 1977–1983
- 20. N. Engl. J. Med., 2003:348:1625-38.
- 21. J. Clin. Oncol., 2004,22:439; J.Urol., 2005, 174:919
- 22. Katherine M. Flegal, PhD; Margaret D. Carroll, MSPH; Cynthia L. Ogden, PhD; Lester R. Curtin, PhD Prevalence and Trends in Obesity Among US Adults, 1999-2008. *JAMA*. 2010;303(3):235-241.
- Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. CA Cancer J Clin 1972;22 (4):232–240. [PubMed: 4625049]
- 24. Forti G, Salerno R, Moneti G, et al. Three-month treatment with a long-acting gonadotropinreleasing hormone agonist of patients with benign prostatic hyperplasia: Effects on tissue androgen concentration, 5 alpha-reductase activity and androgen receptor content. J Clin Endocrinol Metab 1989;68(2):461–468. [PubMed: 2465302]
- 25. Mohler JL, Gregory CW, Ford OH 3rd, et al. The androgen axis in recurrent prostate cancer. Clin Cancer Res 2004;10(2):440–448. [PubMed: 14760063]

- 26. Nishiyama T, Hashimoto Y, Takahashi K. The influence of androgen deprivation therapy on dihydrotestosterone levels in the prostatic tissue of patients with prostate cancer. Clin Cancer Res 2004;10(21):7121–7126. [PubMed: 15534082]
- 27 Geller J, Liu J, Albert J, et al. Relationship between human prostatic epithelial cell protein synthesis and tissue dihydrotestosterone level. Clin Endocrinol (Oxf) 1987;26(2):155–161. [PubMed: 3665114]
- Scher HI, Buchanan G, Gerald W, et al. Targeting the androgen receptor: Improving outcomes for castration-resistant prostate cancer. Endocr Relat Cancer 2004;11(3):459–476. [PubMed: 15369448]
- 29 Belanger B, Belanger A, Labrie F, et al. Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: Unique importance of extratesticular androgens in men. J Steroid Biochem 1989;32(5):695–698. [PubMed: 2525654]
- 30. Stanbrough M, Bubley GJ, Ross K, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 2006;66(5):2815–2825. [PubMed: 16510604].