Cell Proliferation and Cell Death (Apoptosis) in Adjacent Sections of White and African Americans Analyzed in Relation to the Gleason Histological Scores, Immunohistochemical and Immunofluorescence

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Abstract

Introduction: Our objective was to determine whether prostate cancer (PC) grew similarly or differently in White Americans (WA) and African Americans (AA) and race-specific treatment will improve survival of each race as indicated by analysis of data in relation to Gleason histological scores. We hypothesize that cell proliferation and/or apoptosis can occur differentially in the two races. Differential diagnosis can be followed by hormonal, focused beam radiation and/or chemotherapy treatments. Since more AA men die with PC than WA, we expect that Gleason l score-based diagnosis and treatments would improve survival of PC patients.

Materials and Methods: We studied 55 WA and 20 AA prostectomy specimens for localization of cell proliferation and cell death markers by immunohistochemical and immunofluorescence techniques. Briefly, formalin fixed, and paraffin-embedded and/or frozen tissues were used in localization of markers. Localization of black and white and coloured images were obtained directly from slides to a Zeiss microscope equipped with a digital Nikon camera.

Results: We found that cell proliferation increased in Gleason scores 5 and 7 of white patients and score 6 and 7 of African Americans. Our data indicates need for aggressive treatments in these scores. Our study is supported by three tables, statistics, graphs, histological, Immunohistochemical, and immunofluorescence figures.

Discussion: This is the first study to suggest that PC grows differently in WA and AA and race-specific diagnosis and treatment is expected to improve survival of PC patients using hormonal, radiation and/or chemotherapy treatments. The Author (AAS) had numerous discussions with Urologists, Pathologists, and scientists on the treatments of PC indicate. He has concluded that prostatectomy ought to be followed by focused beam radiation to prostatic fossa and adjacent areas to cure PC. Chemotherapy drugs circulate from head to toe in the body and often do not reach required concentration at cancer sites to kill malignant cells.

Keywords: Cell Proliferation; Cell Death (Apoptosis); White and African Americans; Differential Diagnosis Differential Treatment; Gleason Histological Scores; Targeted Hormonal; Radiation Treatment; and/or Chemotherapy; Improved Survival of Each Race

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Abbreviations

PC: Prostate Cancer; WA: White Americans; AA: African Americans; IRB: Institutional Review Board; ADT: Androgen Deprivation Treatment; BPH: Benign Prostatic Hyperplasia; MIB: Cell Proliferation Antibody.

Introduction

PC does not discriminate between rich and poor and races, yet more AA die of the cancer than WA patients. There are many reasons for the differences, including early and late diagnosis, and treatments. PC is among the diagnosed malignancy in the world [1]. Benign (normal) prostate develops as tubules which drain in prostatic ducts and finally in the ejaculatory duct. Initially, human prostate tumor is of pea size and avascular [2]. Prostate tumor has length, width, and height and it does not grow until vascularized by blood vessels and capillaries [1]. Benign prostate contains numerous acinar glands, and stroma with blood vessels and lymphatics [3,4]. Distribution of cancerous acini and stromal vascularity varies in cancer as shown by Gleason histological scores [4]. An acinar gland contains basal, stem, niche, and cuboidal/columnar (secretory) cells enclosing a lumen, whereas stroma contains connective tissue, blood vessels, lymphatics, fibroblasts, smooth muscles, collagen, nerves, and macrophages [3-5]. Homeostatic balance is maintained between cell proliferation and cell death in benign prostate and this balance is greatly altered in PC [6,7]. Cell proliferation is often involved in the growth of PC, but cancer growth can occur by the decreased cell death [8-11]. PC is often detected in men of 50 years or older, however it can be detected in younger men. The average age of patients with PC in the VA was 68.5 years.

Numerous studies on cell proliferation alone and cell death have been conducted on benign and PC [8-12]. Many separate studies on cell proliferation or cell death have not been conducted before but not in adjacent or serial sections and analyzed in relation to Gleason histological scores. Tumor growth usually occurs at the posterior portion of the gland, but it can grow on the underside as well. The relationship between cell proliferation and cell death is complex [8-12]. Complexity is further increased when PC is not studied in adjacent or serial sections. Complex tissue fixation and processing techniques require separate technique for parathion of samples [8-13]. These have resulted in incomplete and/or poor understanding of cell proliferation and cell death in PC. We hypothesize that PC can grow by increased cell proliferation, decreased cell death and/or their combinations. Complexity of PC is shown in Gleason score patterns [4]. This complexity is expected to show differences in the two races and treatments using hormonal, cytotoxic and/or radiation. Support for our hypothesis comes from the unregulated progression of PC as shown by the Gleason grades/scores [4].

An acinar gland contains basal, stem, niche, and cuboidal/columnar (secretory) cells [3,14]. Cancer stem cells are often associated with the basal cells and possess unique properties of self-renewal [3]. The exact numbers of stem cells and their genes in human prostate are unknown. Stem cells have been partially characterized by surface markers, namely, CD44, CD133, integrin α2 [15-17], Pluripotency factors like OCT4, NANOG, and SOX2 are present in stem cells. Several signalling pathways are also over-activated in stem cells, including Notch, PTEN/Akt/PI3K, RAS-RAF-MEK-ERK and HH [14-17]. Many genes have been implicated in PC, namely, BRCA1, BRCA2, ATM, PALB2, CHEK2, MLH1, MSH2, MSH6, and PMS2 [14]. PC stem cells are usually an obstacle in cancer therapy and are a major cause of drug resistance, recurrence, and metastasis [14-17]. Available treatments, targeting proliferating cancer cells are not effective in eliminating quiescent cells. Stem cells proliferate in prostate and its cancer [3]. Basal, niche, cuboidal/columnar cells usually do not proliferate and its cancer [3]. Columnar and cuboidal cells proliferate in castrated mice treated with testosterone [18]. Organogenesis establishes organ-specific stem cells and their genes during fetal life (such as in prostate, lungs, colon, brain [3]. The exact numbers of stem cells and genes are unknown. They become organ specific during organogenesis and therefore, gene therapy is not expected to be successful [3,14-17].

Materials and Methods

The former and current urology surgeons, Drs. Pratap K. Reddy and Eduardo T. Fernandes and their associates selected patients for radical prostatectomy at the Minneapolis Veterans Affairs Medical Center (VAMC). Prostate specimens were submitted to the Surgical Pathology Service and the extra specimens were provided for the research. Patients had not been treated with any hormone or chemotherapy prior to the surgery. All samples were collected after obtaining the consent of the patients and approval of the institutional review board (IRB) according to the guidelines in place at the VA and the University of Minnesota. For this study, we collected prostatectomy samples of 55 White Americans, and 20 African American patients. Our study involved laboratory coordination with surgery, collection of tissues, and processing specimens for distinctly two different techniques. Localizations of cell proliferation and cell death markers were conducted on adjacent sections using appropriately processed specimens. Each localization technique required differential processing of tissues requiring separate techniques. Localization of cell proliferation was conducted using MIB 1 antibody (ABCAM, Cambridge U.K). Cell death required processing by Tunnel assay (Roche Diagnostic

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Corporation, Indianapolis, IN, USA) [8-12]. Initially, we used fresh frozen, acetone or ethanol-fixed specimens which did not provide consistent results. Buffered formalin-fixed specimens required preparation of at least four adjacent sections. Briefly, sections were deparaffined in xylene and processed through graded ethanol to water for localization of cell proliferation antibodies using MIB1 antibody IgG (dilution 1 to 10) using immunohistochemical methods outlined by the company as reported before [3,9-11,13]. After localization, sections were processed prior to mounting using per mount.

We localized cell death using Tunnel localization techniques with protocols provided by Roche Diagnostic Corporation. We also used Apoptag techniques as detailed in the Manual (Invitrogen Company, Purchase, NY, USA). Briefly, formalin-fixed sections required antigen retrieval. Sections were treated with diluted acetic acid of pepsin before conducting localization of cell death marker. Localization was evaluated by light and fluorescence microscopy. Localization of polyclonal antibody IgG against actin (dilution 1: 30) (Dako Corp., Carpentaria, CA, USA) and the reaction product was developed using secondary antibody, as shown in the smooth muscles surrounding acini. Prostatic sections were graded using hematoxylin and eosin-stained sections by the former pathologists at the Minneapolis, Drs. Donald F. Gleason, and Stephen L. Ewing using Gleason histological scores [4,19]. Prostectomy patients had clinical stages B, C and D [20] (Figures 1A & 1B).
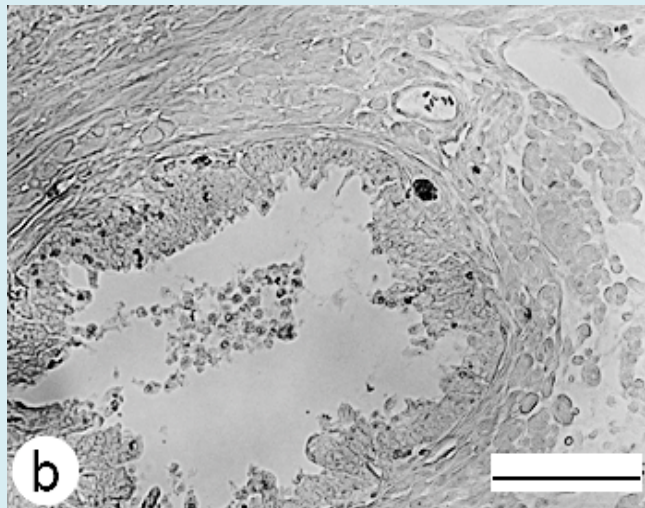
Figure 1(A): Prostate gland shows glandular cells, slooghed cells in the lumen, stroma vascularity and smoothe muscles.
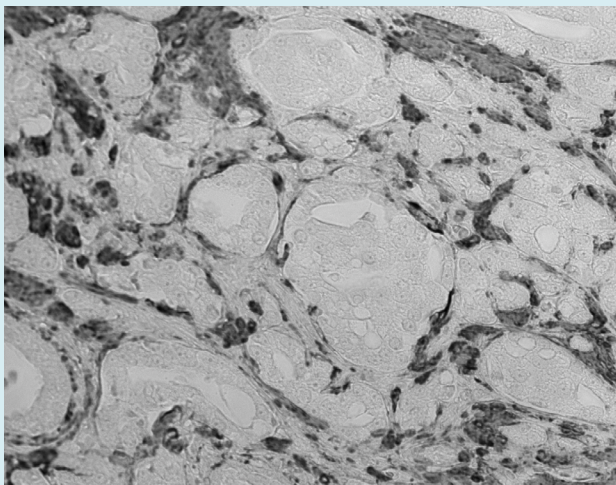

Figure 1(B): Micrograph shows localization of antibody IgG against actin in smooth muscles of the stroma surrounding cancerous prostate glands in cross sections.

Cell proliferation and cell death fluorescence markers have been assessed before [3,9-12,21,22]. Briefly, fluorescence isothiocyanate (FITC) was obtained (Jackson Research, West Grove, PA) and rhodamine (Cy3) from Oncogene Research (Cambridge, MA). Cell proliferation was localized with antibody (dilution 1:300) and FITC fluorescein produced (green) fluorescence. Cell death was localized with antibody (1:500) as shown by rhodamine Cy3 (red) fluorescence. Sections showing yellow florescence showed colocalization of green and red markers together. Some prostate gland cells did not localize markers indicating that cells were either dormant or inactive (Figures 1C & 1D). Fluorescence techniques required antigen retrieval in formalin-fixed and paraffin-embedded sections. Briefly, sections were treated with xylene followed by graded ethanol to water and phosphate buffer saline (PBS). Paraffin fixed sections required antigen retrieval and were processed via xylene, graded ethanol to water, and PBS before incubation with diluted pepsin for 30 minutes at room temperature.

Sections were washed several times in water and PBS before incubating with the diluted primary antibodies at room temperature and washed with PBS before incubating with the secondary antibodies. Reaction products were developed using peroxidase techniques detailed before [3,13,21-23]. Control studies were incubated with the normal serum of the animals used in preparation of the antibody. Images were acquired directly from the slides to a Zeiss microscope equipped with a Nikon digital camera and/or epifluorescence microscope to a computer. Images were photographed for the study and were adjusted using Photoshop for the uniformity of brightness or darkness as needed. AA samples were not available for immunofluorescence study. The laboratory study was completed before the author retired and the writing was completed after retirement.
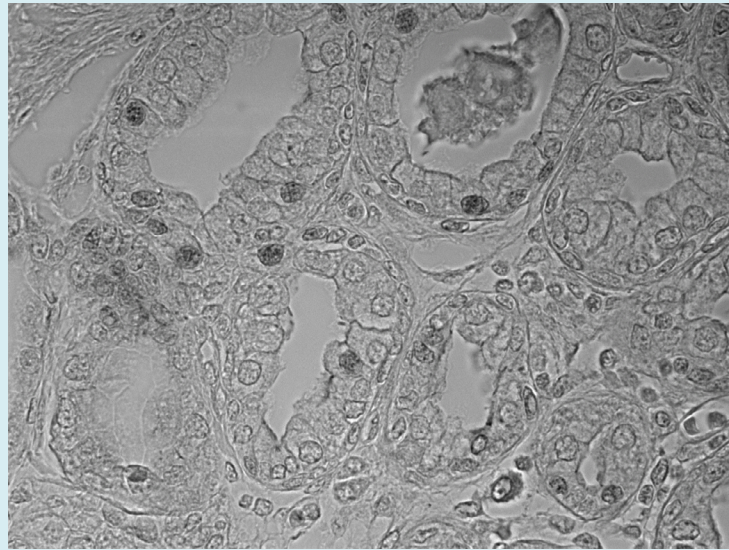
Figure 1(C): *Canceous acini* show a light cells (androgen-dependent) and dark cells (dark cells (androgen-independent) (see ref # 22). Some cancer cells are intrufing (nigrating) in stroma and forming flandular structure.
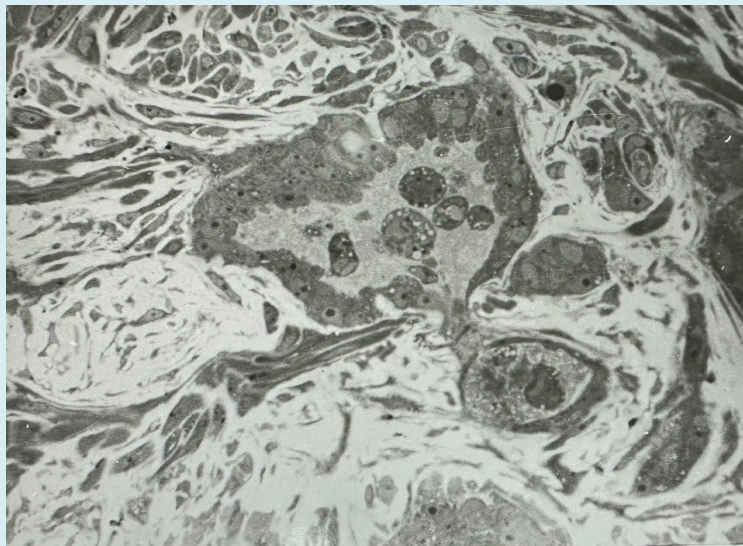

Figure 1(D): Cancerous cell is invading prosttic stroma and forming small glands. Lumen has many sloughed cells are present the lumen. Light and dark acinar cells are in the acinus. Gland is surrounded by smoothe muscles and vascular elements. Benign prostate in cross section illustrates normal cells and sloughed cells in the lumen. Stromal conectice tissue surrounds the gland.

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Results

We have presented data of WA and AA in three tables and statistics along with graphs. Age of patients ranged from 53.36 to 76.30 years, average age 66.79 years. Earlier study had shown that patients localizing <9ng/ml of PSA respond to treatment and survived longer than those with PSA more than 9ng/ml [24]. Antibody against actin was localized in smooth muscles of prostatic stroma (Figures 2A & 2B). Cell proliferation was localized by MIB 1 IgG antibody l in benign prostate, BPH and PC (Figures 2C & 2D). Localization was conducted on paraffin sections and data was presented, including statistical analysis, and graph figures (Tables 1 & 2 with Statistics, with and Graph Figures 1 & 2).
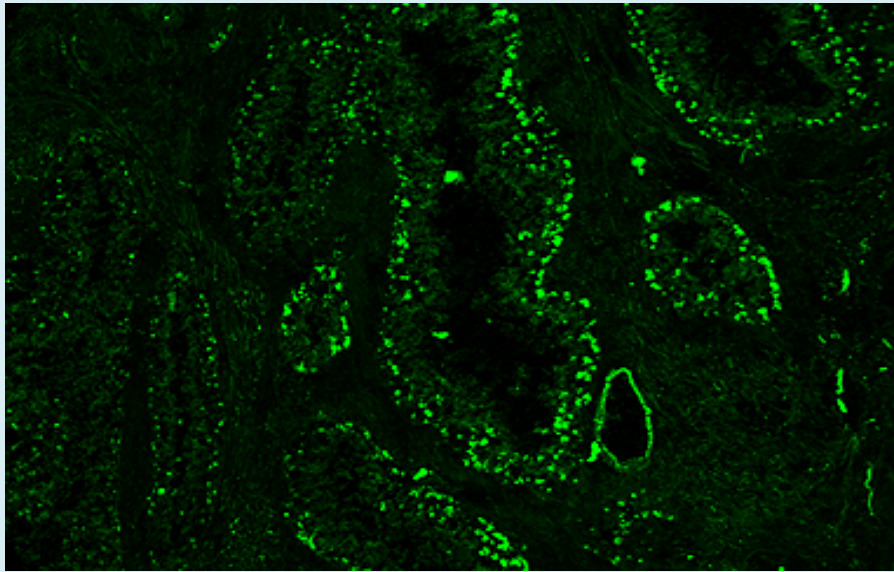

Figure 2(A): Immunofluorescence micrograph shows cell proliferation by green fluorescence of fluorescence isothiocyanate (FITC). Glands show lumina and stroma does not show Immunofluorescence.
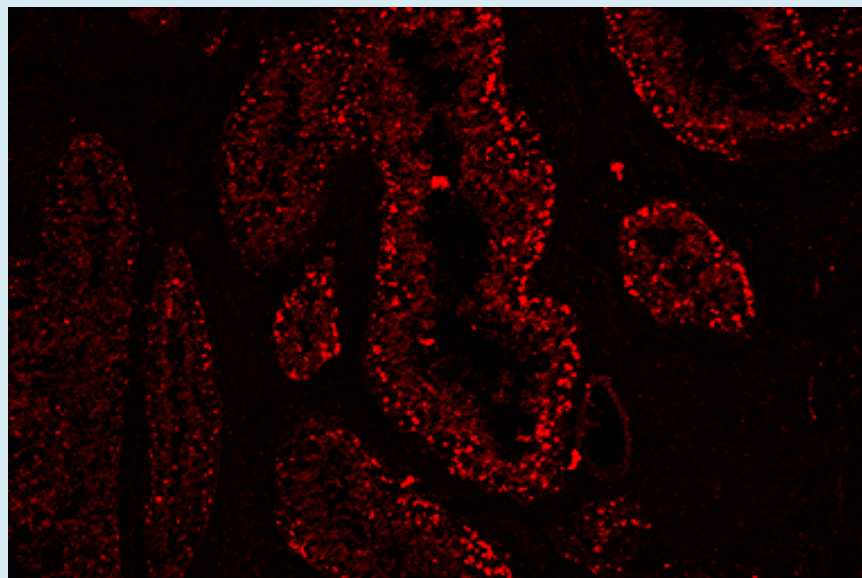
Figure 2(B): Immunofluorescence micrograph shows cell death by rhodamine (red) fluorescence. Stroma does not show Immunofluorescence.
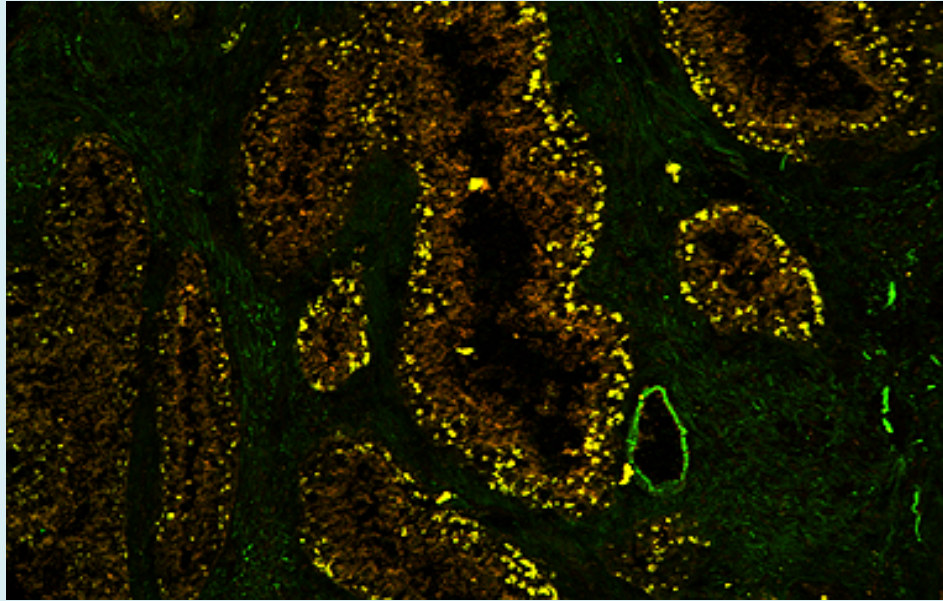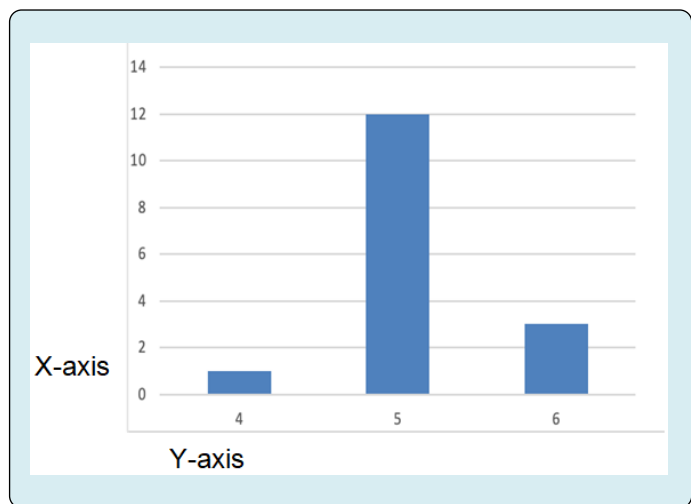
Figure 2(C): Yellow Fluorescence shows when cell proliferation and cell death localizations were combined. Micrograph also shows that many prostate cells did not localize indicating that they were either dormant or inactive. Stroma does not show Immunofluorescence.
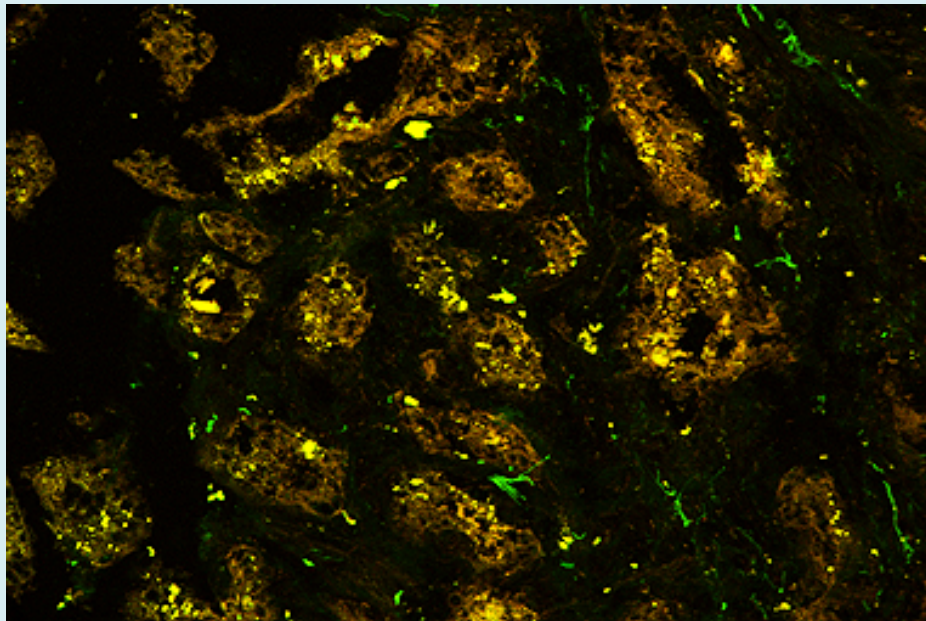
Figure 2(D): Another area of the prostate shows yellow fluorescence when cell proliferation and cell death localizations were combined. The micrograph also shows that many prostate cells did not localize indicating that they were either dormant or inactive. Stroma does not show Immunofluorescence.
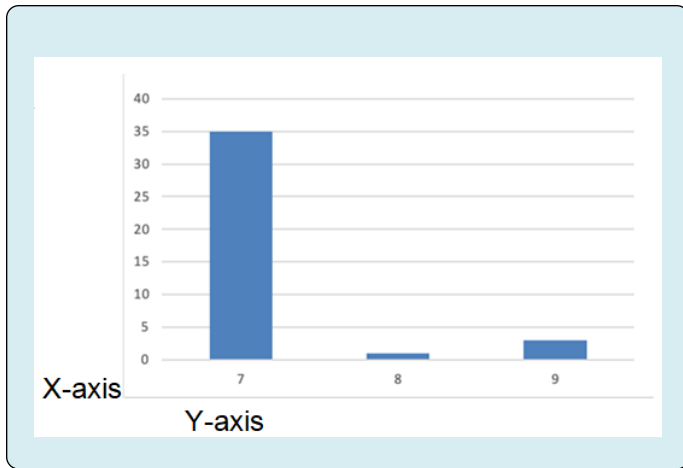
Patient #	Gleason	MIB	CD
346	5	0.156	0.819
523	5	0.126	0.234
421	5	0.142	0.202
427	5	0.068	0.088
454	4	0.209	0.125
469	5	0.081	0.044
473	5	0.041	0.004
479	5	0.116	0.038
504	5	0.198	0.104
373	5	0.156	0.059
477	5	0.244	0.08
423	5	0.342	0.163
403	5	0.29	0.096
415	6	0.259	0.021
393	6	0.355	0.072
526	6	0.491	0.148

Patient #	Gleason	MIB	CD
419	7	0.072	0.069
425	7	0.123	0.099
360	7	0.048	0.023
445	7	0.144	0.116
510	7	0.049	0.016
465	7	0.105	0.066
	7	0.074	0.034
312	7	0.155	0.111
443	7	0.288	0.222
321	7	0.469	0.4
370	7	0.168	0.06
351	7	0.194	0.071
453	7	0.185	0.055
376	7	0.267	0.107
535	7	0.451	0.228
500	7	0.285	0.053
430	7	0.323	0.091
330	7	0.439	0.046
503	7	0.797	0.183
439	8	0.118	0.073
410	9	0.593	0.153
537	7	0.13	1.516
521	7	0.146	1.319
534	7	0.162	1.213
498	7	0.106	1.138
381	7	0.185	1.08
387	7	0.505	1.35
491	7	0.437	1.265
379	7	0.292	0.992
392	7	0.317	0.898
475	7	0.191	0.664
413	7	0.436	0.803
483	7	0.16	0.513
412	7	0.041	0.231
467	7	0.154	0.3
466	7	0.153	0.249
518	7	0.275	0.308
489	9	0.093	1.181
344	9	0.079	0.17

	MIB	CD
# of Cases	16.000	16.000
Total Value	3.274	2.297
Range	0.041-0.491	0.004-0.819
Mean	0.205	0.144
Std Deviation	0.121	0.191
Increases	12	4
Decreases	4	12

Table 1, Graph Figure 1 and Statistics: Prostate Cell Proliferation & Cell Death in White Americans Gleason scores 4-6, scores 7 and 9.

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	MIB	CD
# of Cases	39	39
Total value	9.21	17.466
Range	0.041 - 0.797	0.016 - 1.516
Mean	0.236	0.448
Std Deviation	0.17	0.482
Increases	21	18
Decreases	18	21

Table 2, Graph Figure 2 and Statistics: Prostate Cell Proliferation & Cell Death in White Americans Gleason 7-9.

Patient #	Gleason	MIB	CD
86-41	5	0.268	0.187
85-13	5	0.149	0.082
91-65	6	0.12	0.073
84-43	6	0.098	0.074
97-02	7	0.462	0.425
83-36	7	0.128	0.053
96-52	7	0.15	0.057
81-10	7	0.209	0.055
91-55	7	0.214	0.031
98-08	8	0.282	0.105
92-35	9	0.891	0.553
X03-04	6	0.384	3.793
96-23	6	0.282	1.281
X X02-11	6	0.107	0.54
X 02-21	6	0.106	0.527
Feb-13	6	0.094	0.365
X00-71	6	0.177	0.351
97-66	7	0.193	1.947
01-Aug	7	0.198	0.952
X01-38	7	0.241	0.669

Y-axis
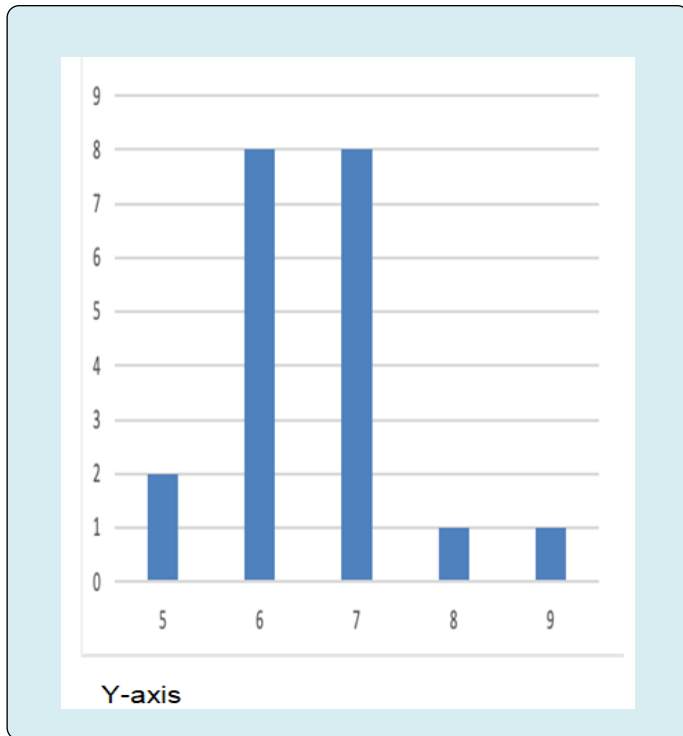
	MIB	CD
# of Cases	20	20
Total value	4.753	12.12
Range	0.094 - 0.891	0.031 - 3.793
Mean	0.238	0.606
Std Deviation	0.182	0.895
Count of Increases	11	9
Count of Decreases	9	11

Table 3, Graph Figure 3 and Statistics: Prostate Cell Proliferation & Cell Death in African Americans.

Morphology of prostatic acini in cross sections is shown by histology, histochemistry, immunohistochemistry, and immunofluorescence by light microscopic study [3,13,21-23,25-27] (Table 3). The prostate glands are separated from prostatic stroma by the acinar basement membrane (Graph Figure 1). Antibody IgG against actin localizes in smooth muscles in the stroma separating cancerous acini (Graph Figure 1). Many acini show light and dark cells and acinar lumen (Graph Figure 2). Previous study had shown that light cells were androgen-dependent (or sensitive) and dark cells were androgen- insensitive (androgen -independent) [28]. Micrograph shows cancer cells invading prostatic stroma and the acinar lumen shows sloughed prostatic cells (Graph Figure 3). Benign prostate has intact basement membrane and stroma. Green fluorescence of FITC shows cell proliferation, whereas rhodamine (red) fluorescence shows cell death. When cell proliferation and cell death localizations are combined yellow fluorescence is observed.

Discussion

The relationship between cell proliferation and cell death is poorly understood when studied separately. We did not get consistent results on cell proliferation and cell death for nearly 10 years. Others have studied cell proliferation and cell death separately [3,18,21-23,25,29,30]. Adjacent and/or serial sections are required for consistent localization results of cell proliferation and cell death markers. We have shown that PC grows by cell proliferation in WA with Glasson scores 5 and 7 whereas, it grows AA with scores 6 and 7 tumors. Differences in the two races require differential diagnosis and treatment [31-33]. Specific reasons for differences are unknown, but could be related to food habits, herbicides and pesticides in foods and water, education, poverty, etc. Utilization of Gleason score-based diagnosis and treatment is expected to improve survival in each race. Current study in WA, AA races differs from our previous study of cathepsin B (a proteolytic enzyme) in Japanese Americans, and Japanese in Japan [23,26]. Cathepsin B was similar in the above races. Cell proliferation and cell death study support the need for differential diagnosis and treatment in each race. We also suggest that aggressiveness of cancers ought to be determined prior to using hormonal, cytotoxic and/or radiation treatments.

We studied the ultrastructure of acinar secretory cells in benign prostate and benign prostatic hyperplasia (BPH) [28]. Study of untreated and DES-treated cases showed two types of PC, namely, androgen-dependent (sensitive) and androgen-independent (insensitive) [25]. Androgen deprivation treatment (ADT) treats only androgen-dependent (androgen-sensitive) cancer and not androgen independent cancer which is dependent on estrogen [25]. Androgen-independent (insensitive) PC can be treated by antiestrogen drug such as Tamoxifen. Subsequent study showed androgen-dependent PC has androgen-specific stem cells, and estrogen dependent PC has estrogen-specific-stem cells and their receptors [21]. As a reproductive biologist, the Author (AAS) studied pluripotential cells in blastocysts and found that these cells become organ-specific during organogenesis (3). For example, pluripotential cells in prostate, including stem cells and genes, become organ-specific including during organogenesis [3,6,8,9,15-17,34-36]. Such specificity precludes application of gene therapy via unrelated organs. This may have been the reasons for the failure of gene therapy so far. Gleason study prostate tumor led to five patterns and the Gleason grading system [4,19]. Gleason grading system is being used worldwide in diagnosis of PC.

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The above discussion indicate that other solid organs cancers (such as, lungs, liver, colon, breast, pancreas, brain) can be dissected using a variety of techniques, namely, markers, phase contrast, light, and electron microscopy, etc. in the hope of finding variants in each solid organ cancer. Each variant could be readily targeted resulting in improved survival. Since President Richard Nixon declared war on cancer in 1971, numerous cancer patients have died throughout the world despite over several trillion dollars of expenses. Additional efforts are in dissecting solid organ cancers can result in improved diagnosis and treatments resulting improved survival of some PC patients in the two races.

Conclusion

PC grows due to the increased cell proliferation in scores 5 and 7in WA and scores 6 and 7 in AA when data is analyzed in relation to the Gleason scores. Our conclusion is supported by data in tables, statistical analysis, histological, Immunohistochemical and immunofluorescence figures. The differential growth of PC in the two races requires race-specific hormonal, radiation and/or chemo therapy treatments. The sequence and dosage have been determined in clinical trials.

Postscript comments: Black men are diagnosed with PC more than WA and die at a higher rate than WA. The reasons are complex and not fully understood. Some of the reasons may be associated with foods contaminated by herbicides and pesticides in food, and water. Disparity health care for AA men, women and children in the USA and the world, including the VA Medical System may contribute to the problem. Active efforts and surveillance are expected to reduce mortality in AA and other low income patients. There is a general mistrust in men and women of AA origin than in the VA Healthcare system. Efforts of surgeons and their associates did not result in obtaining consents of many AA patients. Inclusion of veterans' family in the VA Health Care system has the possibility of improving the situation.

Scientist (AAS) published cell biology of benign (normal) prostate and benign prostatic hyperplasia (BPH) in 1973. He has had numerous discussions with Urologists, Pathologist, and prostate scientists including Dr. Donald F. Gleason on the treatments of prostate cancer. AAS has concluded that the best treatment for PC is prostatectomy followed by focused beam radiation to prostatic fossa and surrounding area between 4 and 8 months after sugary.

Acknowledgements

Conflicts of Interest

The authors have no conflict of interest.

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