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Prostate Cancer
Old Problems and New Approaches

Part I. Epidemiology, Incidence and Genetic Alterations

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Rates of prostate cancer (PCa) have increased so dramatically over the last decade that the age adjusted incidence rate for PCa is now greater than that any other cancer among men in the United States. This review, published as a three part series, provides a state-of-art assessment of the PCa problem in its divergent aspects. *Part 1* covers epidemiology, incidence and progression. Several epidemiological studies have demostrated that first degree male relatives of men with PCa are at increased risk of developing the disease. Familial and genetic factors as well as medical, anthropometric, dietary, hormonal and occupational factors involved in PCa are discussed. Postmortem examination of the prostate in men without evidence of PCa documented a high frequency of adenocarcinoma. Latent disease occurred as early as the second decade of life. Although there is no significant difference in incidence between Caucasian and African–American males, high grade prostatic intraepithelial neoplasia (HGPIN) is higher in the latter group. While dietary fat, androgens and certain environmental factors may be determinants for PCa, the exact mechanism of tumorigenesis is still relatively unknown. The current thinking of the role of genomic instability, chromosomal alterations, tumor suppressor genes and the androgen receptor are explored. (Pathology Oncology Research Vol 2, No1–2, 98–109, 1996)

Key words: prostate cancer, epidemiology, incidence, genetics, progression

Introduction

Prostate Cancer (PCa) is the most common newly diagnosed cancer among men in the U.S. today. With the advent of the Prostatic Specific Antigen (PSA) test the number of newly diagnosed cases has increased tremendously. With this increase, two important clinical issues have surfaced. The first is whether or not therapy is necessary in a given patient, and the second is, if therapy is indicated, which treatment is most appropriate. Several groups have recommended an approach of "watchful waiting" in selected patients. Autopsy studies conducted at Wayne State University have found that many PCa start when a man may be in his 30's or 40's and thus may take twenty or more years to become clinically important. In fact, many of these small cancers do not progress at all. Therefore, reliable methods are needed to differentiate cancers that will remain latent from those that will ultimately grow and metastasize. Unfortunately biological markers are not yet available to distinguish insignificant cancers from those with potential to progress. In addition, for patients with localized disease, considerable debate exits on the pros and cons of surgery versus radiation therapy although considerable advances have been forthcoming in the use of the latter treatment for localized and systemic disease as discussed below.

The ability to predict the ultimate aggressiveness of PCa could significantly impact on therapeutic decision making. Unfortunately, so far there is no single molecular marker which has been proven to predict the clinical

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aggressiveness of PCa. Clearly novel prognostic markers are required.

Even though advances have been made in the chemotherapy of PCa (see below), new, innovative therapies need to be developed for the treatment of PCa. In this regard novel therapy such as induction of apoptosis offer new potential modalities. In patients with metastatic disease, androgen ablation prolongs the disease free interval but does not significantly enhance survival. Therefore, understanding the mechanisms of key steps in the metastatic spread of PCa may impact on therapy. Since the growth of human PCa epithelial cells is controlled by an intricate network of growth factors and their receptors, understanding the operative cellular signaling mechanisms may identify new targets designed to interfere with PCa cell proliferation. In the second part we begin with a discussion of the epidemiology and incidence of PCa followed by an update on our understanding of the genetic basis for PCa progression. The status of diagnostic and prognostic markers for PCa are discussed as well as the currently accepted pathological staging system. Following a discussion of the pathology of PCa, we address two important aspects of the biology of this disease, apoptosis and metastasis. In Hu Hird part, attempts at the development of prevention strategies are discussed as well as the latest advances in the treatment of PCa by surgery, radiation therapy, androgen ablation and chemotherapy. Clearly, there are vast areas which remain unexplored and thus there is a tremendous opportunity for a wide diversity of new research and therapeutic initiatives.

1. Epidemiology

1.1. Descriptive Epidemiology

Rates of PCa have increased so dramatically over the last decade that the age adjusted incidence rate of PCa is now greater than that for any other cancer among men in the United States.[1] The American Cancer Society has estimated that 317,100 new cases of PCa will be diagnosed in the US in 1996.[2] Although PCa rates have risen steadily since 1973, there has been a dramatic acceleration in the late 1980's which has been associated with the introduction and use of PSA for screening and early detection.[3] There is now some evidence that the rates may be levelling off and even decreasing in some areas.[4] After lung cancer, PCa is the leading cause of deaths due to cancer among men in the US, with 41,400 PCa deaths expected in 1996.[2] In spite of this substantial impact on our society, PCa remains a relatively understudied disease with an essentially unknown etiology.

Although PCa can occur in younger men, it is essentially a cancer of elderly men. Among men greater than or equal to 85 years of age in the Detroit metropolitan area in 1992, the age-specific incidence rate was 2081.7 and 1996.3 per 100,000 among African–American and Caucasian men, respectively.[5] The highest rates of PCa in the world occur among African–American men in the US.[6] African- Americans have higher rates than Caucasians at all age levels in the US, and adjusting for socio-economic status does not appear to account for this difference to any appreciable extent.[7] There are no clear reasons why PCa rates are so much greater among African- Americans than Caucasians in the US. The reported rates in Africans are substantially lower than those in African–Americans,[6] suggesting that environmental factors have an influence on PCa.

1.2. Familial and genetic factors

Several epidemiological studies have demonstrated an increased risk of PCa in the first degree male relatives of men with PCa.[8,9] A study in Utah reported a strong correlation in serum testosterone levels between men with PCa and both their brothers and sons.[10]

Over two decades ago, a case-control study suggested that there was a significant excess of PCa in male relatives of females with breast cancer.[11] These results went essentially unnoticed until two recent studies in the US and Iceland provided additional evidence that the risk of PCa may be increased in men who have female relatives with breast cancer.[12,13] The cohort study in Iceland reported a significantly increased risk of PCa in male relatives of women with breast cancer. The US study reported a four fold increased risk for breast cancer in female relatives of male breast cancer patients with a family history of PCa.

A study of seven Icelandic breast cancer families examined by linkage analysis (to 17q) found that PCa was the most frequent cancer, after breast cancer, found in these families.[14] Although no formal statistical testing was done, a total of 13 cases of PCa was reported and PCa appeared to be especially prevalent in males presumed to carry the trait. The authors suggest that breast cancer genes may predispose to PCa in male carriers. These data support the hypothesis that PCa aggregates in families and that PCa and breast cancer may also aggregate. Of course, these studies do not fully address the question of genetic factors in PCa, since family members may share common environmental exposures (such as diet) which have been linked to PCa. A few studies have examined directly the role of molecular genetic factors which may contribute to the development of PCa and there has been some suggestion that p53 gene alterations may play some role (see below).[15] However, this area needs much additional work before any conclusions may be drawn.

1.3. Medical and anthropometric factors

Benign prostate hypertrophy (BPH) is common in elderly men. BPH usually occurs in the interior portion of the prostate gland, as opposed to malignant neoplasms which

occur in the outer parts of the gland. Even if BPH does not directly contribute to the development of PCa, however, BPH and PCa may share one or more etiologic factor(s). Several studies have evaluated the potential relation between BPH and the subsequent development of PCa, with equivocal results.[16,17] The exceptionally high prevalence of BPH in elderly men may prevent epidemiological studies from contributing substantially to the issue of whether or not BPH increases the risk of PCa.

A multitude of studies have considered the role of vasectomy in the development of PCa.[18-21] Although some of the earlier reports were positive, most of the more recent studies have not found an association. Several reviews have suggested that the evidence for an effect is weak and further studies are needed. A number of studies have considered whether obese men are at increased risk of PCa.[22,23] While the evidence is essentially equivocal, body mass index reflects both lean and fat tissues. A cohort study based on anthropomorphic measurements found that muscle area of the upper arm was significantly associated with PCa while fat area was not.[24] This may be an indication that increased muscle mass is related to higher androgen levels in men at risk of PCa. Increasing one's level of physical activity may also increase muscle mass, but there is minimal evidence that physical activity is related to PCa.

1.4. Diet

The possibility that diet may play a role in the etiology of PCa is supported by the large differences in PCa rates between nations such as Japan and the US, and the observation that PCa rates increase in Japanese men who migrate to Hawaii.[25] In spite of this, the exact role of dietary factors remains unknown, due, at least in part, to the difficulties associated with accurate measurement of dietary factors. A multitude of epidemiological studies have examined the relation between dietary fat and PCa. A recent review of 17 separate studies[26] concluded that most studies have shown at least some positive association between various measures of dietary fat and PCa. More recent studies[21,27] have confirmed this observation. Exactly how dietary fat may be related to PCa, which specific component(s) of dietary fat may be important, and etiologically relevant exposure periods are not yet clear. The only other dietary components which have received extensive scrutiny are micronutrients and vitamins, especially vitamin A. Studies of vitamin A have been equivocal,[28] as have been studies of serum vitamin A levels.[29] It has been suggested that vitamin A from plants reduces risk, while vitamin A from animals increases risk.[30] Of course, these sources may also reflect dietary fat intake.

A hypothesis was recently advanced that vitamin D deficiency might be responsible, at least in part, for the observed association with increasing age and the increased rates in African–Americans.[31] Both of these factors are associated with decreased synthesis of vitamin D and the hypothesis is also consistent with the antitumor properties of vitamin D. In addition, PCa mortality rates are inversely correlated with ultraviolet radiation, which is a major factor in the synthesis of vitamin D. This idea has received some preliminary support[32] and several nested case-control studies based on stored sera have shown that PCa risk decreased with higher levels of vitamin D metabolites in the serum.[33] The most recent nested case-control study found no association between vitamin D metabolites and PCa.[34] A recent editorial has pointed out that vitamin D metabolites inhibit other human cancers such as breast cancer, colon cancer, and leukemia, and has suggested that further investigation of this hypothesis is warranted.[35]

1.5. Hormones

It seems reasonable that sex hormones should have some role in the development of PCa. Androgens are involved in the normal growth and activities of the prostate gland. PCa has never been known to occur in eunuchs and castration has been used as a treatment for PCa (see below). In spite of this, epidemiological studies of hormones (both male and female) have been inconsistent.[20,28,36]

Several facts may account for this inconsistency. First, testosterone levels in the blood follow a circadian rhythm, with a peak level in the morning and the lowest level in the evening. This might cause difficulties if blood samples are collected on different subjects at different times of the day. Second, the metabolic clearance rate of testosterone may be greater in men with PCa compared to disease-free men.[37] If so, then case-control differences in testosterone levels may not be detectable even though testosterone production is greater in these cases. Third, serum testosterone levels may be affected by stress,[38] which suggests that serum measurements made around the time of diagnosis or treatment of PCa may not be ideal. Measurement of hormones in prostate tissue may be a more appropriate method. However, it is usually quite difficult (if not impossible) to obtain prostate tissue from normal subjects, rendering this method impossible in the vast majority of epidemiological studies.

1.6. Occupation

Although a variety of specific occupations and industries have been associated with PCa in case reports, the few epidemiological studies of PCa and occupation have concentrated on exposure to cadmium, employment in the rubber/tire manufacturing industry, and farming. Cadmium has received the most scrutiny, with some,[39] but not all,[40] studies suggesting that occupational exposure increases the risk of PCa. One possibility is that cadmium increases the risk of PCa by being a zinc antagonist in the prostate gland.[41] While the prostate gland contains very

high levels of zinc, it remains unclear whether or not zinc levels are associated with PCa.

Several studies have suggested that workers in the rubber and tire manufacturing industry are at increased risk of PCa,[42] although not all studies have been positive.[43] It has been suggested,[44] but not confirmed,[45] that rubber workers involved in batch preparation might be at the highest risk. A review panel from the International Agency for Research on Cancer reported that the data are too limited at present to infer causality.[46]

Farmers have generally been thought to be at increased risk for several cancers, including PCa. A review of 24 studies by Blair and Zahm[47] found that 17 have reported an increased risk of PCa among farmers. Previous studies have typically evaluated all farmers combined without consideration of details on specific types of farm exposures. Two recent studies have suggested that farmers with exposure to herbicides[48] and pesticides[49] may be especially at increased risk for PCa.

In the most intensive case-control study yet undertaken of occupational risk factors for PCa, Aronson *et al*[50] evaluated the possible associations of about 450 occupational substances, categories, and industries with PCa. A number of jobs, industries, and substances were found to be associated with PCa. The substances included: aluminum alloy dust, nickel compounds, liquid fuel combustion products, lubricating oils and greases, alkanes (C18+), PAHs, pesticides, and ultraviolet radiation; the occupations included: metal product fabricators, aircraft fabricators, structural metal erectors, and electrical power workers; and the industries included: agriculture, railway transport, and water transport.

While these studies provided some important leads, to date there are no well established risk factors (other than cadmium) for PCa. Nomura and Kolonel[26] have suggested that further occupational studies of PCa are necessary to continue to identify high-risk groups and to further our understanding of the mechanisms involved in the development of PCa.

As the population continues to age, PCa is likely to increase even more in prominence. In spite of the importance of PCa, relatively little is known about its etiology. Current leads involve the potential roles of dietary factors, lifestyle, occupation, family history of cancer, and hormone metabolism in the etiology of PCa, and clearly additional studies in each of these areas are warranted.

Even though in the last several years, we have witnessed a dramatic increase in the number of newly diagnosed cases of PCa the actual incidence of the disease, as discussed below, may be significantly higher than anticipated.

2. Incidence of prostatic carcinoma and premalignant lesions of the prostate

Despite the impressive increase in the incidence of clinically diagnosed PCa, this form of the disease is remarkably less common when compared to the high prevalence of latent cancer which is defined strictly as PCa discovered in *post mortem* examination. The discrepancy between the occurrence of these two forms of the disease is one of several characteristics which distinguish PCa from other human malignancies. Another peculiar aspect of this disease is the wide variation in the incidence and mortality rates among different ethnic, geographic and racial groups as discussed above. Both the incidence of the clinically detected form of PCa and the prevalence of the latent form increase with advancing age in men of different races and ethnic origins. Key questions regarding the natural history of latent and clinically detected cancers, the definition and role of precursor lesions and the ability to predict progression in this tumor system remain largely unanswered.

2.1. Latent Carcinoma

Post mortem examination of the prostate in men without evidence of PCa has documented a high frequency of adenocarcinoma of the prostate. Several classic studies, some dating to the earlier part of the century, repeatedly have documented the high prevalence of latent PCa primarily in men over age 50. In these studies the frequencies by which latent carcinoma was documented varied according to tissue sampling and examination techniques. Understandably, studies which examined the entire glandular tissue using thin step sections documented higher frequencies than autopsies which utilized partial or random sampling of the prostate. Compiling figures from these series demonstrates a high prevalence of latent disease, with the prostates of approximately 30% of men over age 50 harboring foci of PCa. At Wayne State University, a contemporary autopsy study, was conducted in collaboration with the Wayne County Medical Examiner's Office. This endeavour was a comprehensive morphologic study designed to provide information regarding important aspects of the latent disease which were not addressed in previous studies. For example, comparison was made of the prevalence of latent cancer between Caucasian and African–American males. With the exception of one report,[51] this aspect is lacking in most other studies. In addition, the prevalence of latent disease was documented at an age younger (i.e. 20 yr.) than reported previously. Finally, the documentation and comparison of the presence and the extent of precursor lesions of PCa was observed at a young age. The frequency of detecting small microscopic foci of adenocarcinoma in individuals aged 30 to 50 was between 25% and 32%.[52] The majority of these cancers were microscopic and of the well to moderately differentiated type *(Fig. 1)*. In clinically detected PCa African–Americans have twice the incidence rate compared to Caucasians. However, the findings of the autopsy series indicated no statistical difference in the prevalence of the latent form of the disease between the two races.[52,53] Further, the type of cancers detected [i.e. in histological

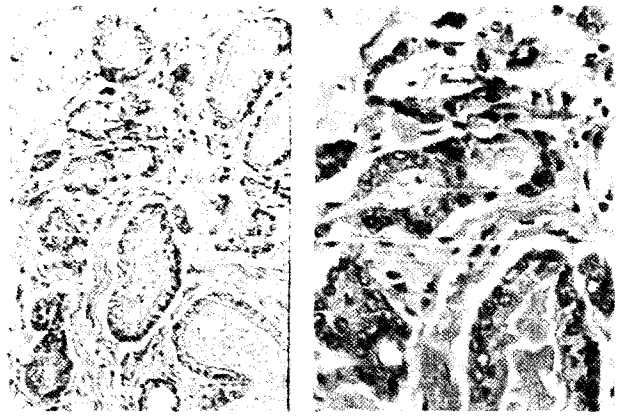

Figure 1. Typical microscopic focus of latent prostatic adenocarcinoma discovered during post mortem examination. Few infiltrative acini with single cell lining (lacking basal cell layer) can be recognised. The epithelial cells are enlarged with high nuclear/cytoplasmic ratio with many containing prominent nucleoli.

differentiation (Gleason grade), volume, number of cancer foci (multifocality), or the anatomic distribution of cancers within the gland] in the two racial groups were not different. This was particularly true for cancer detected in individuals under age 50.

The high prevalence of latent PCa compared to the lower incidence of clinical disease is indeed one of the conundrums of this malignancy. Two possibilities present themselves which may not be mutually exclusive. It is possible that the vast majority of the epithelial foci which are diagnosed as latent adenocarcinoma will not progress into clinically significant cancer during the expected lifetime of the individual. However, it is possible that a subset of these latent cancers will progress to become clinical disease. In contrast, it may be argued that latent adenocarcinoma and the clinically significant forms of PCa are two separate entities which may bear no direct relationship to each other. The latent disease may be a slow progressing form which is not life threatening during the normal life span while the clinically significant form progresses in a life threatening fashion. The question can then be asked what are the precursors of clinically significant PCa. Are the premalignant lesions to be found in the latent foci?

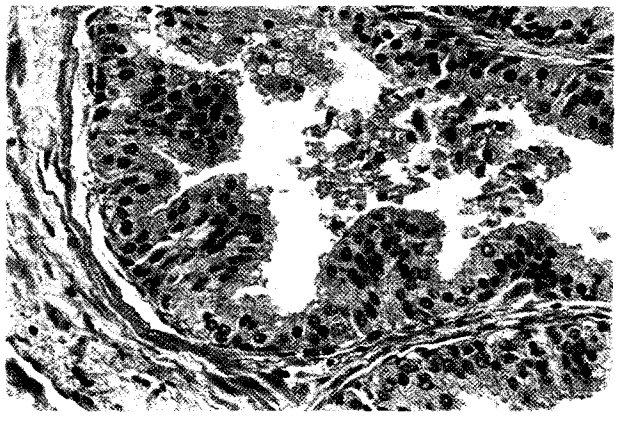

Figure 2. Low grade prostatic intraepithelial neoplasia: stratification and mild architectural complexity of the epithelium lining the duct. There is nuclear pleomorphism and only occasional prominent nucleolus.

2.2. Premalignant lesions of prostate cancer

Historically premalignant lesions of the prostate have been poorly defined. The literature addressing this concept suffers from confusing terminology superimposed on morphologically ill-defined entities. In recent years it has become increasingly evident that atypical or "dysplastic" epithelial lesions of the prostate can be grouped into two major categories.

The first is represented by a process of "architecturally" abnormal proliferation which includes the formation of new units possessing small ducts and acini and which occur in both the peripheral and transition zones of the gland. Lesions in this category have been named adenosis, atypical small acinar proliferation, atypical hyperplasia, and atypical adenomatous hyperplasia among others.[54] The second category consists of lesions which do not form new units, but rather involve or affect the lining of preexisting ducts and demonstrate a host of architectural and more importantly, cytological changes that signify malignant transformation. While an array of terms similar to those mentioned above have been used to describe the latter lesion, a recent workshop on prostatic dysplasia, adapted the term prostatic intraepithelial neoplasia (PIN) for a specific lesion in the spectrum of atypical epithelial proliferations described above *(Fig.2)*. Since then, a number of studies have investigated the epidemiological, morphologic, genomic, and clinical aspect of this lesion with the aim to clarify the role of PIN and a more dysplastic form termed high grade PIN (HGPIN) in prostatic carcinogenesis.[55,56] A general agreement regarding the clinical significance of identification of HGPIN in prostate tissue samples (usually needle biopsies) has been reached i.e. that HGPIN is frequently associated with adenocarcinoma *(Fig.3,4)*. In fact several studies indicate that between 30-70% of patients with HGPIN in biopsies will harbour a concurrent or subsequently discovered adenocarcinoma.[57,58]

2.3. Prevalence of latent PCa and PIN in Caucasian and African-American males

Foci of PCa and PIN increase in frequency with increasing age in both groups. Six percent of African-American males in the third decade harboured foci of adenocarcinoma which increased to 31, 39 , 48, 78 and a 100% in the 4th, 5th, 6th, 7th, and 8th decades respectively. The corresponding figures

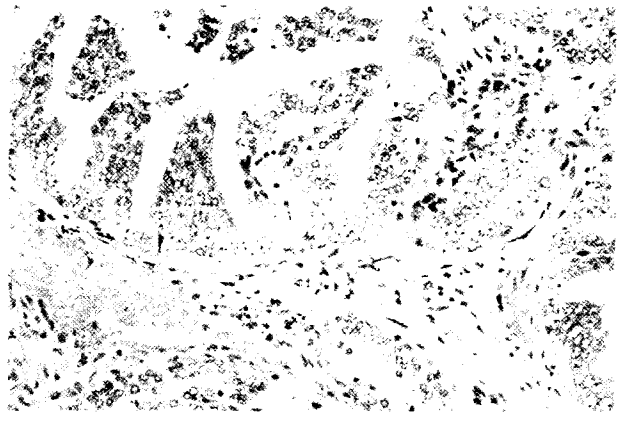

Figure 3. High grade prostatic intraepithelial neoplasia: The architectural complexity may vary the cytologic abnormalities however are pronounced; the cells are uniformly enlarged with most containing prominent nucleoli. The process is confined within the boundaries of the involved ducts.

for Caucasian males beginning with the third decade were 10, 29, 38, 31, 63 and 81% respectively *(Table 1)*.

High grade PIN also was detected with increased frequency with advancing age in both races. Prostate ducts showing cytological and architectural changes meeting the criteria for HGPIN were identified in 2, 18, 31, 69, 79, and 86% of African–American males in the 2nd through 7th decades respectively. The numbers for Caucasian males were 10, 14, 21, 38, 50, and 63% respectively. A similar prevalence of latent PCa was observed in both racial groups particularly in those under fifty years of age.

***Table 1.* The frequency of latent PCa and HGPIN in two races by decades**

Age		*PCa*	*HGPIN*
20–29	AA	4/62 (6%)	1/62 (2%)
	C	2/20 (10%)	2/20 (10%)
30–39	AA	16/51 (31%)	9/51 (18%)
	C	10/35 (29%)	5/35 (14%)
40–49	AA	20/51 (39%)	16/51 (31%)
	C	15/39 (38%)	8/39 (21%)
50–59	AA	14/29 (48%)	20/29 (69%)
	C	8/26 (31%)	10/26 (38%)
60–69	AA	14/18 (78%)	14/18 (78%)
	C	10/16 (63%)	8/16 (50%)
>=70	AA	7/7 (100%)	6/7 (86%)
	C	13/16 (81%)	10/16 (63%)

A – African–American; C – Caucasian; Pca – prostate cancer; HGPIN – high grade prostatic intraepithelial neoplasia

2.4. Extent of HGPIN and it's anatomic relationship to PCa

The distribution of HGPIN and its relationship to latent PCa is complex. In a significant number of cases the two lesions were identified in the same gland. Seventy three of the 372 prostate glands (20%) examined harboured both HGPIN and PCa. However, considering that 29% of the 372 glands contained areas with HGPIN alone while 36% of the same 372 glands contained foci of PCa alone, it became clear that the two lesions can occur in isolation of each other and that the patterns of association between the two warrant further analysis. Our results demonstrate that if HGPIN existed within a prostate, the probability of this gland harbouring a concurrent PCa is 67% compared to a 23% probability of PCa occurring in prostates which do not contain HGPIN. Further, the association between the two lesions was age dependent in both races. Two thirds of histological PCa in individuals under fifty years of age occurred in prostates not harbouring HGPIN *(Table 2)*.

***Table 2.* Default Paragraph Fontccurrence of HGPIN and PCa in the same prostate**

Age		*PIN with PCa*	*Isolated PCa*	*Isolated PIN*
20-29	AA	0	4/4 (100%)	1/1 (100%)
	C	0	2/2 (100%)	2/2 (100%)
30-39	AA	7/16 (44%)	9/16 (56%)	2/9 (22%)
	C	3/10 (30%)	7/10 (70%)	2/5 (40%)
40-49	AA	9/20 (45%)	11/20 (55%)	7/16 (44%)
	C	4/15 (27%)	11/15 (73%)	4/8 (50%)
50-59	AA	11/14 (79%)	3/14 (21%)	9/16 (44%)
	C	6/8 (75%)	2/8 (25%)	4/10 (40%)

The disparity in the occurrence of the two lesions appears to diminish with advancing age. Only 24% of the PCa encountered in the 6th decade occurred in glands without HGPIN. In addition when HGPIN and PCa were found in the same gland, the frequency of close anatomic proximity to each other increased with age and was to an extent race dependent. For example, a higher proportion of HGPIN associated cancers were evident in males over age fifty in both races, and African–Americans were more likely to

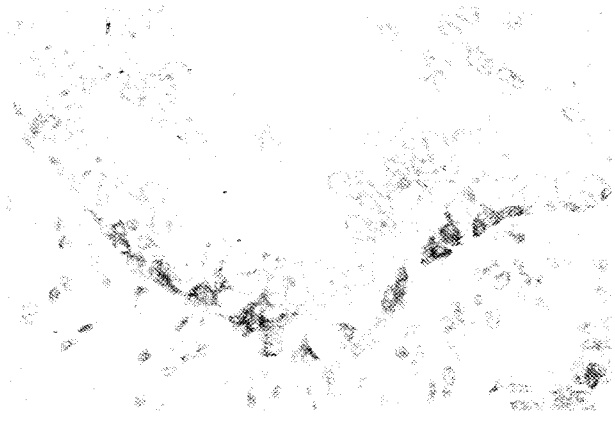

Figure 4. Immunohistochemical staining for high molecular weight cytokeratin showing partial loss of the basal cell layer around ducts with high grade prostatic intraepithelial neoplasia.

harbour these HGPIN/PCa lesions. Of particular interest, was the distribution and the "extent" of HGPIN. When HGPIN was identified in a gland either with or without concurrent carcinoma, it was classified as focal, multifocal or extensive. The appearance of extensive HGPIN occurred at a younger age in African–American compared to Caucasian males. For example, in one study the percentage of African–Americans with extensive HGPIN was 11, 25 and 45% in the 3rd, 4th and 5th decades respectively. In contrast, the corresponding numbers for Caucasian males in the same age groups were 0, 13 and 30%.

2.5. Differentiation (histological tumor grade)

The mean Gleason's score (i.e. 5) was similar for all the cancer foci encountered in individuals of both races under fifty years of age. Similar results were observed if one examined the mean Gleason score of the largest foci of PCa for individuals in the 6th decade. The mean Gleason score for the all PCa foci in African–American males was 5.59, while that of Caucasian males was 5.45. When the study was extended to individuals in the 7th decade the difference increased between the two races. The mean Gleason score of African–Americans was 6.01 compared to a mean of 5.12 for comparable Caucasian males.

2.6. Tumor volume

The majority of latent PCa foci were remarkably small with means of 0.005, 0.022, 0.069, and 0.077 cc for individuals in the 3rd, 4th, 5th, and 6th decades respectively, and was similar for both racial groups under 50 years of age. Modest differences began to appear in the 6th decade where the mean volume of the largest tumor foci in African–American males was 0.098 cc compared to 0.0527 cc for their Caucasian counterparts. In this age group, the mean volume of all latent PCa foci in African–Americans was 0.249 cc compared to a mean of 0.0897 cc in Caucasian males.

2.7. Anatomic Distribution

The majority (80%) of latent PCa foci in African–Americans and Caucasians were found in the peripheral zone. In keeping with previous observations, PCa of the peripheral zone were generally larger and less differentiated than PCa of the transitional zone.

3. Genetic alterations during prostate cancer progression

Dietary fat, androgens, and unknown genetic and environmental factors may be risk determinants for PCa. However, the exact molecular mechanism of prostatic tumorigenesis is still largely unknown. This section, will summarise some recent data on genetic alterations associated with PCa progression.

3.1. Genomic instability

Microsatellites are highly polymorphic, short tandem-repeat sequences dispersed throughout the genome. Microsatellite instability has been shown to be involved in human cancer and other diseases. This type of genomic alterations may be the direct result of DNA replication and/or repair errors. To examine possible microsatellite instability during the development of PCa, 57 patients with prostatic adenocarcinoma were screened at 18 microsatellite marker loci on 12 chromosomes (3p, Sq, 6p, 7p, 8p, 10q, 11p, 13q, 16q, 17p, 18q, and Xq). Overall, in 37 of 57 patients (65%), microsatellite instability was found in at least one of the loci analysed. A significantly greater number of cases were found to be positive for this phenotype among the poorly differentiated than the moderately- and well-differentiated prostatic adenocarcinomas. This data suggests that genetic instability may play an important role in the development and progression of human PCa.[59] Subsequently, several other groups also reported microsatellite instability in PCa, with varied frequencies.[60,61] The general consensus is that the genomic instability, reflected by microsatellite sequence expansion or contraction, may play a role in the development of a subset of PCa cells. This is no surprise since tumors progress to more aberrant phenotypes with time.

Conventional cytogenetics has yielded useful information on chromosomal alterations in PCa. However, some artefacts may be introduced by the cell culture procedure during this type of analysis. Recently, comparative genomic hybridization (CGH) has been introduced into the field of PCa. This technique allows one to directly determine the overall chromosomal alterations in tumor samples. Cher *et al*[62] have shown that the loss of 8p, 13q, 16p, 16q, 17p, 17q, 20q, Y and gain of 8q are frequently associated with primary prostate tumors. Visakorpi *et al*[63] have recently reported similar results. Interestingly, the latter group has observed significantly more gain of 8q, X, 7 and more loss of 8p in local recurrences that developed during endocrine therapy. Based upon the previous cytogenetic analysis and recent data from CGH, alterations on chromosome 8p, 10q, 11p, 16q and 17q appear to be most prevalent in PCa. Therefore, genetic changes on each of these chromosomes will be discussed briefly in the next section.

3.2. Chromosomal alterations

Allelic loss at chromosome 8p is one of the most frequent genetic changes associated with PCa.[64] Introducing chromosome 8 into rat prostate tumor cells suppresses their metastasis.[65] These results strongly indicate that

human chromosome 8 harbours (s) prostate tumor suppressor gene(s). Subsequently, regions of highly frequent deletion have been mapped to 8p22 and 8p12-21 loci.[66-68] Recently, Macoska *et al*[69] have reported evidence of three tumor suppressor gene loci in the proximity of 8p22, 8p21 and 8p12. A detailed map of chromosome band 8p22 has been constructed,[70] and should be useful for positional cloning of PCa cancer specific suppressor genes.

Chromosome 10q has been shown to be involved in PCa, however less information is available compared to the chromosome 8p. Interestingly, the MXI1 gene maps to chromosome 10q24-25, a region that is deleted in some cases of PCa. MXI1 negatively regulates myc oncoprotein activity and thus potentially serves a tumor suppressor function. It has been reported that MXI1 displays allelic loss and mutation in prostate tumor cells.[71] However, it is still unclear whether the MXI1 gene constitutes a candidate prostate tumor suppressor, as contradictory results also have been observed.[72]

Metastasis is the main cause of death among patients with cancer, including PCa. Previously, it has been shown that the metastatic ability of rat prostate tumor cells can be suppressed when they are fused to nonmetastatic tumor cells.[73] The putative metastasis suppressor gene was then mapped to human chromosome 11p11.2-13.[74] Recently, a metastasis suppressor gene, named KAI1, has been cloned.[75] The KAI1 gene encodes a protein of 267 amino acids with resemblance to membrane receptors. Genetic and biochemical study of the KAI1 gene may provide important information for the management of PCa.

In addition to human chromosome 8p, 10q and 11p, deletions of chromosome 16 in PCa have been mapped to band 16q24.[76] Frequent genetic alterations on chromosome 17 in PCa also have been observed.[77] A region on the long arm of chromosome 17 distal to the BRCA1 gene may contain prostate-specific suppressor genes, but the BRCA1 itself plays only a minor role in PCa development.[78] Introducing chromosome 17q12-22 into the PCa cell line PPC-1 suppresses the malignant phenotype of these tumor cells.[79]

3.3. Alterations of the known tumor suppressor genes

Inactivation of the p53 tumor suppressor gene has been the subject of intensive investigation in human PCa. The consensus is that p53 is mutated in many prostate tumors, and the mutation frequency increases with the progression of PCa.[80] It is interesting that a majority of mutations are A to G or T to C transitions.[15] However, the frequency of p53 abnormality may be underestimated in human PCa. In principle, p53 can be inactivated by mutation, loss of expression and association with viral or cellular proteins such as SV40T and mdm2. Chen *et al* have observed a high frequency of LOH (42%) and decrease in p53 expression (36%) in PCa tissue samples. In 18 cases which expressed similar amounts of p53 mRNA compared to their matched normal prostate tissues, 7 (39%) had mdm2 overexpression. These results suggest that p53 abnormalities (allelic deletion, low expression, mutation and mdm2 overexpression) occur at a high rate (71%) during PCa development.[81]

Bookstein *et al* have shown that the Rb promoter sequence is mutated in some prostate tumors, resulting in a loss of Rb expression,[82] and that Rb protein is inactivated in human PCa DU 145 cells due to exon 21 deletion.[83] Recently, it has been reported that the frequency of Rb mutation and loss of heterozygosity is approximately 20% in human PCa.[80,84] These results indicate that inactivation of the retinoblastoma gene may be an important event in prostate carcinogenesis.

p21WAF1/CIP1 is a mediator of p53 tumor suppressor function and a cyclin-dependent kinase inhibitor. The p21WAF1/CIP1 mutation has been analysed in 18 matched normal and tumor prostate tissues by nested reverse transcription-polymerase chain reaction/single strand conformation polymorphism and DNA sequencing analysis. Four sequence alterations were identified including a C to A transversion, a G to A transition and two A insertions.[85] The base substitutions may represent point mutation or polymorphism. However, the frameshift mutations result in a truncated p21WAF1/CIP1 protein of 34 amino acids that lacks the cyclin-dependent kinase and proliferating cell nuclear antigen binding domains.

E-cadherin is a transmembrane glycoprotein involved in calcium-dependent cell-cell adhesion. This adhesion is mediated by catenins which bridge the cadherin molecule to the microfilament cytoskeleton. A reduction or absence of E-cadherin expression has been reported in high grade PCa tissues,[86] and a reduction of E-cadherin expression or deletion of the α-catenin gene in human PCa cell lines.[87] Re-expression of α-catenin restores the function of E-cadherin and correlates with the suppression of tumorigenicity of PC3 PCa cells.[88] The down-regulation of E-cadherin expression may result from hypermethylation of the E-cadherin gene.[89] The level of E-cadherin and catenin expression may be useful prognostic markers, since decreased E-cadherin expression is associated with poor outcome of PCa patients.[90]

The putative tumor suppressor genes APC and MCC have been mapped to human chromosome 5q21 and cloned. The mRNA expression of the APC and MCC genes and LOH at the APC and MCC loci have been determined in PCa tissues from 28 patients and 5 human prostatic adenocarcinoma cell lines. Of the informative cases, the frequency of LOH at the APC and MCC loci was 63% (10/16) and 54% (7/13), respectively. Overall, 65% (15/23) of the informative cases showed LOH at the APC and/or MCC gene. All PCa cell lines showed homozygosity at all APC and MCC polymorphic sites studied. Approximately half (57%) of the tumor tissues examined

showed a decreased expression of APC and MCC mRNA. These data suggest that the APC and MCC genes may be involved in the formation of human PCa.[91] Although direct evidence of prostate tumor suppression by APC and MCC is still lacking, introduction of chromosome 5 suppresses tumorigenicity of PC3 PCa cells.[88]

The tumor suppressor gene DCC has been shown to be frequently lost or expressed at low levels in colorectal, gastric, pancreatic and esophageal carcinomas. The DCC gene and its mRNA expression in human and rat PCa cells as well as in PCa tissues were examined by RT-PCR and PCR-LOH. The DCC gene was present and expressed in normal prostatic cells. However, its expression was decreased or undetectable in all PCa cell lines either from human (4 cell lines) or rat (5 cell lines). In patients, 12 out of 14 cases (86%) showed reduced DCC expression and 5 out of 11 informative cases (45%) showed loss of heterozygosity at the DCC locus. These results demonstrate that loss of DCC expression and loss of heterozygosity at the DCC locus are a frequent feature of PCa. Previously, Carter *et al*[92] reported that the frequency of LOH at the DCC locus in 28 PCa patients was approximately 17%. The difference in frequency between Carter *et al* (17%) and Gao *et al* (45%)[93] could be due to the methods used to detect LOH. Carter *et al* determined the M2 and M3 site LOH by Southern blot analysis, whereas Gao *et al* used three sites for LOH detection which increases assay sensitivity. In fact, M2 plus M3 site LOHs also were found in 18% of the informative cases in the latter study.[93] While these data suggest a correlation between loss of DCC expression or LOH of the DCC locus and PCa, the function of DCC in prostate carcinogenesis is still unknown.

3.4. Mutation of the androgen receptor

Androgens are required for prostate differentiation and growth, and also play an important role in prostate tumor progression. The androgen receptor (AR) gene contains a polymorphic CAG microsatellite that codes for a variable length of glutamine repeats. A somatic contraction of the CAG repeats from 24 to 18 has been found in a PCa patient who manifests a paradoxical agonistic response to hormonal therapy with the antiandrogen flutamide.[94] In addition, a codon 877 mutation in the AR gene which alters ligand-binding specificity has been observed in a PCa cell line[95] and patients.[96] The prevalence of androgen gene mutations has recently been reported in a Japanese man with latent PCa.[97] Mutations in the AR gene also have been identified in metastatic androgen-independent PCa.[98] The latter two studies are instrumental to our understanding the role of AR in human PCa progression. Based upon their results, it appears that the prevalent inactivating mutation of AR in latent PCa may account for the low incidence rate of clinical PCa among Japanese men. Further, a high level of AR expression and AR mutations which confer to the receptor affinity towards a wider spectrum of steroids may explain, at least partially, why most patients relapse as a result of the outgrowth of androgen-independent tumor cells after the initial success of androgen ablation treatment.

In summary, it is clear that PCa development is a multistep process. Steady progress has been made in identifying genetic alterations in human PCa. However, at the present, few of the genomic loci and genes identified have significant diagnostic and/or prognostic values. Thus there is an urgent need for a clear understanding of the molecular progression of PCa, and the development of prognostic markers.

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