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APPLICATION OF ADHESION MOLECULES (BETA-CATENIN) AS A PROGNOSTIC MARKER IN PROSTATE CANCER

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REGUIRMENT FOR MASTER DEGREE IN PATHOLOGY

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صدق الله العظيم

سورة العلق الأية 1

Dedication

This dissertation is dedicated to my husband, and my children, Moad and Malek. I give my deepest expression of love and appreciation for the encouragement that you gave and the sacrifices you made during this graduate program.

CERTIFICATE

We the under signed, certify that on March the 25th, 2013
WARDA. M. MUSBAH. SAID was examined for her thesis entitled:

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The thesis has been accepted for the partial fulfillment of the requirements for the Master degree in pathology.

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LIST OF ABBREVIATIONS

AAH	Atypical adenomatous hyperplasia
AI	androgen-independent
AIPC	androgen-independent prostate cancer
AMACR	Alpha-methylacyl CoA racemase
APC	adenomatous polyposis coli
AR	Androgen receptor
BCR	Benghazi Cancer Registry
BCR	biochemical recurrence
BMI	Body mass index
BPH	Benign prostatic hyperplasia
CAMs	calcium-dependent cell adhesion molecules
CAP	College of American Pathologists
CI	confidence interval
CK	cytokeratin
CKI	casein kinase I
CRPCs	castration-resistant prostate cancers
CXCR4	CXC chemokine receptor 4
DHT	5α –dihydrotestosterone
DRE	Digital rectal examination
DTCs	disseminated tumor cells
EGFR	Epidermal growth factor receptor
EPE	Extraprostatic Extension
ERG	v-ets erythroblastosis virus E26 homolog (avian)
ERSPC	European Randomized Study for Screening for Prostate Cancer
EZH2	Enhancer of zeste homolog 2
FDRs	first-degree relatives
GSK3β	glycogen synthase kinase 3β
GSTP1	G lutathione-S-transferase P1
HA	hyaluronan
HGPIN	High-grade prostatic intraepithelial neoplasia
HMCK	High molecular- weight cytokeratin
HPC	Hereditary prostate carcinoma
HPC1	hereditary prostate carcinoma 1
HSPs	Heat shock proteins
IGF-1	Insulin-like growth factor-1
MVD	Microvessel density
NCCN	National Comprehensive Cancer Network

PCa	Prostate cancer
PCAP	prostate cancer predisposing
PCTA-1	prostate carcinoma tumor antigen-1
PIN	prostatic intraepithelial neoplasia
PLCO	Prostate, Lung, Colorectal, and Ovarian Cancer Screening trial
PSA	prostate-specific antigen
PTEN	phosphatase tensin homolog
RR	Relative risk
SDF-1	stromal cell–derived factor-1
STIs	sexually transmissible infections
TCF/Lef	T-cell factor/lymphoid enhancer factor
TGF	transforming growth factor
TGF-βR	transforming growth factor β receptor
TMPRSS2	transmembrane protease, serine 2
TRUS	Transrectal ultrasound
TSG	Tumor suppressor gene
VACURG	Veterans Administration Cooperative Urological Research Group
WHO	World Health Organization
XMRV	Xenotropic murine leukemia virus

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CHAPTER I

1.1. ABSTRACT

Background and Aims: β-Catenin is a critical end component of the wnt signaling pathway that regulates cell growth, apoptosis, and migratory behavior in response to intercellular adhesion molecules**.** The aim of this study was to evaluate abnormalities of β-catenin protein expression, subcellular localization and determine its relation to different clinicopathological features and disease free survival.

Patients and Methods: Forty prostate cancer specimens, obtained from patients with different stages of prostate cancer (83% stage IV) who underwent a radical prostatectomy or TURP between 2006 and 2011, β-Catenin was determined by immunohistochemistry. The membranous expression was semiquantitatively evaluated in four scores (0, 1+, 2+, 3+). Clinical records of these patients were studied for follow-up data .

Results: β-catenin immunostaning results show overexpression of β-catenin in PCa Libyan patients. There was no statically significant difference in β-catenin immunoexpression as regards histopathological type, perineural invasion, tumor stage, biological recurrence. However, β-catenin overexpression showed significant correlation with old age ($p<0.024$), Gleason score ($p<0.014$).

Conclusions: We concluded that changes in expression and cell distribution of β-catenin correlated with the progression degree of prostate adenocarcinoma, suggesting a role of this molecule as marker of progression and prognosis.

Key Words: Prostate cancer, β-catenin expression, Immunohistochemistry, Gleason score, Prognosis.

1.2. Introduction

Prostate cancer (PCa) is the second most common cause of cancer and the sixth leading cause of cancer death among men worldwide with an estimated 899 000 new cases and 258 000 new deaths in 2008. The worldwide PCa burden is expected to grow to 1.7 million new cases and 499 000 new deaths by 2030 simply due to the growth and aging of the global population (Center et al, 2012). Recent statistics reveal that PCa continues to remain the most commonly diagnosed lethal malignancy in men in the United States with 1 out of 6 men developing PCa and 1 out of 35 dying from it (Jemal et al, 2009).

The biologic heterogeneity in PCa has been brought into sharp focus as a result of widespread adoption of prostate specific antigen (PSA) screening in many countries, with a resulting marked migration towards the diagnosis of lower-risk prostate cancer (Shao et al, 2009). There are now more than 240,000 men in the United States diagnosed with prostate cancer each year (Siegel et al, 2011), and 90 percent of prostate cancers are clinically localized and occult disease at time of diagnosis (Shao et al, 2009). Data from the randomized trials of PSA screening (Andriole et al, 2009; Schröder et al, 2009; Stephenson et al, 2009) highlight the considerable over diagnosis and overtreatment of men with screen-detected PCa with very low prostate-cancer specific mortality rates in modern series (Hugosson et al, 2010).

The well-established risk factors for PCa are older age, black race /ethnicity, and a family history of the disease (Platz et al, 2006). The wide variation in international PCa incidence rates and trends is in part due to the substantial differences worldwide in the diagnosis of latent cancers through PSA testing of asymptomatic individuals as well as during prostate surgery. PCa mortality rates and trends are less affected by diagnostic practices but reflect differences in PCa treatment worldwide as well as underlying risk (Center et al, 2012).

In patients with localized PCa, the 5-year survival approximates 100%; however, in patients in whom distant metastases have occurred, the 5-year survival drops to 31% (Jemal et al, 2010). Like most other solid malignancies, PCa can metastasize to distant organs such as the liver, lungs and brain, but it has an unusually high propensity for

metastasizing to the bone. In one autopsy study, ~80% of the men who had died from PCa possessed bone metastases (Bubendorf et al, 2000).

To assist with patient counseling and guide treatment selection, the National Comprehensive Cancer Network (NCCN) has recommended risk stratification of patients with newly-diagnosed PCa according to PSA level, biopsy Gleason score, and clinical stage. Despite the fact that the widespread use of PSA testing has altered the clinical and demographic characteristics of men with newly-diagnosed, PCa men with what is characterized as high-risk disease continue to be encountered. Indeed, the management of these patients represents one of the most significant current challenges in PCa treatment, as the optimal therapeutic strategy remains to be established (Boorjian et al, 2011).

Several genes and signaling pathways have been implicated in PCa initiation and progression, such as p53, C-MYC, Nkx3.1, PTEN, androgen receptor (AR), and Wnt/β-Catenin (Kasper, 2005). Wnt/β-Catenin signaling has been implicated in both normal prostate development and in PCa progression (Yu et al, 2009).

The cytoplasmic protein β-Catenin (encoded by the **CTNNB1** gene) is crucial in many steps of embryogenisis and is involved in a number of cancers. β-Catenin forms part of the adherens junction with E-Cadherin and is also a component of canonical Wnt signalling. In the absence of Wnt ligand, a destruction complex of Axin, APC, GSK3β and CK1α promotes the phosphorylation and subsequent degradation of β-Catenin via the ubiquitin pathway. When Wnt ligand binds to the Frizzled/LRP receptor complex, the destruction complex is destabilized and GSK3β is unable to phosphorylate β-Catenin. This leads to an accumulation of β-Catenin that translocates to the nucleus and interacts with the transcription factors TCF/LEF to activate target genes (Francis et al, 2013).

Currently, the function of β-Catenin in human PCa is unclear (Kypta & Waxman, 2012). **CTNNB1** mutations in PCa occur rarely, in only 5% of cases (Voeller et al, 1998). It has been observed that β-Catenin expression and localization change during human PCa progression, however, results are inconsistent. Several studies have seen an increase in β-Catenin expression and nuclear localization in late stage cancer samples, while others have reported a loss in nuclear expression in advanced tumours (Morita et

al, 1999; de la Taille et al, 2003; Chen et al, 2004; Whitaker et al, 2008; Szász et al, 2010). In addition to its role in Wnt signalling, β-Catenin can act as a co-factor with AR, suggesting it has a role in castration-resistant disease. In cell PCa lines, β-Catenin enhances androgen-stimulated AR transcriptional activation and increases sensitivity to low levels of androgens and to non-androgen ligands (Truica et al, 2000; Song et al, 2003; Verras et al, 2004). Nuclear localization of β-Catenin may also result in increased complexes between AR and β-Catenin in PCa cells, changing target gene activation (Pawlowski et al, 2002).

In this review, we present an overview of evidence on prognostic features in PCa. We discuss clinical and pathological features, as well as molecular markers in tumors and circulation and by immunohistochemical we also evaluated β-catenin expression in a series of Libyan PCa, and its relationships with several clinicopathological features, disease recurrence and outcome.

1.3. Aims of study

1- Histopathological study of PCa in Libyan patients by H&E stain to detect differentiation grades by Gleason score.

2- To evaluate the expression β-catenin in Libyan patients with PCa.

3- To observe the relationship between traditional prognostic parameters such as histological type, Gleason score, stage, perineural invasion, and lymphovascular invasion in correlation with β-catenin expression of tumor cells.

CHAPTER II
REVIEW OF LITERATUR

2. REVIEW OF LITERATURE

The prostate gland is a functional conduit that allows urine to pass from the urinary bladder to the urethra and adds nutritional secretions to the sperm to form semen during ejaculation. The function of the many secreted products of the prostate, including PSA is incompletely understood (Mills et al, 2004).

2.1.Embryology of prostate

The prostate appears in early embryonic development as a condensation of mesenchyme along the course of the pelvic urethra. By 9 weeks of embryonic life, a number of features that are characteristic of adult contour and location are evident. The mesenchymal condensation is most dense along the posterior (rectal) aspect of the urethra and distal (apical) to its midpoint. This is the only region where highly condensed mesenchyme is in immediate contact with urethral lining epithelium, and only here is the urethra lined by a tall columnar epithelium. Between its midpoint and the bladder neck, the proximal urethral segment shows a sharp anterior angulation. However, the strip of highly condensed mesenchyme continues directly proximally to a dome-shaped base, leaving a gap between condensed prostatic mesenchyme and proximal urethra. The ejaculatory ducts penetrate the mesenchyme toward the future verumontanum, which is located at the urethral midpoint. This is wolffian duct tissue, but its stroma is indistinguishable from the remaining prostatic mesenchyme, which is mainly derived from the urogenital sinus. However, that portion of the mesenchyme that surrounds the ejaculatory ducts and expands proximally to occupy nearly the entire prostate base is distinguishable in the adult as the central zone, which is probably also derived from the wolffian duct, as are the seminal vesicles. In this concept, the prostate is of dual embryonic derivation. At about 10 weeks, epithelial buds begin to branch, mainly posteriorly and laterally from the posterior and lateral walls of the distal urethral segment into the condensed mesenchyme. Recent computer reconstructions of serially sectioned specimens have shown that the branching pattern that is established initially is identical to that described for the adult later (Mills, 2007).

Postnatally the prostate grows at a slow rate, reaching less than 2 cm in diameter by the time of puberty. During this period, the ducts and acini are lined by epithelium, which

undergoes little change from the neonatal period. Gland spaces are lined by cells that are crowded with multilayered dark nuclei. There is a superficial resemblance to adult postinflammatory atrophy, but the histologic features are quite different (Mills, 2007).

2.2. Anatomic consideration

The normal prostate weighs 20g by early adulthood and is best thought of as having an inverted pyramid shape, with anterior, posterior and lateral surfaces, a narrow apex anteroinferiorly and a board base superiorly which lies against the bladder neck. It is related anteriorly to the symphysis pubis, laterally to the anterior fibers of the levator ani muscle and posteriorly to seminal vesicles and rectum, separated from the latter by Denonvilliers fascia (Figure 2.1) (Derek & Cameron, 2004).

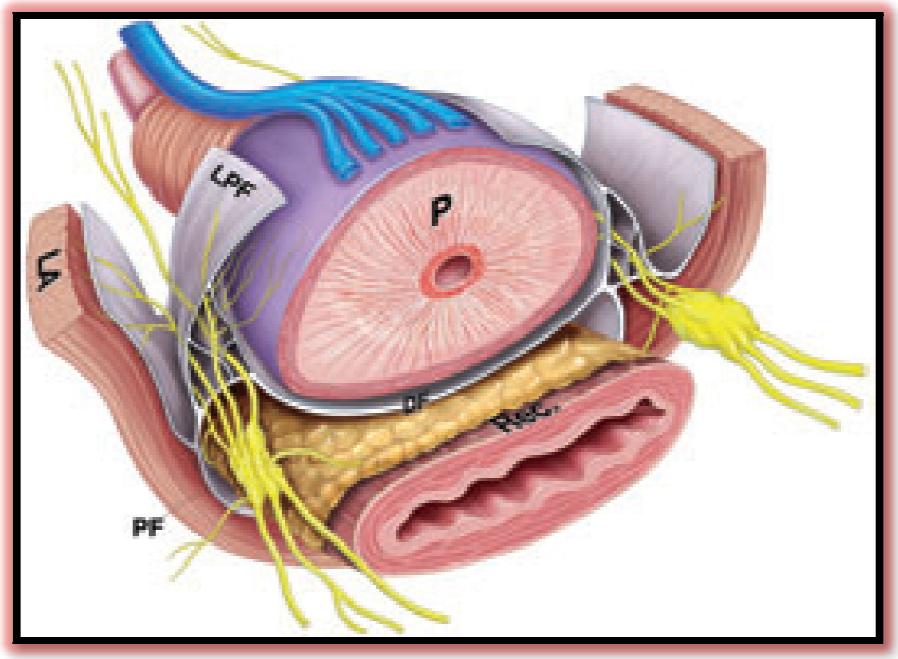

Figure 2.1. Graphical illustration of the peri-prostatic anatomy. Denonviller's Fascia (DF), Levator ani (LA), lateral prostatic fascia (LPF), pararectal fat (PF), and prostate (P), rectum (Rec) (Costello et al, 2011).

The prostae is surrounded by an ill defined fibrous capsule which blends with the pelvic fascia. Numerous neurovascular bundles are found within this connective tissue. At the apex, skeletal muscle fibres of the urethral sphincter are admixed with occasional benign prostatic glands and at the base, fibres from the bladder detrusor muscle blend imperceptibly with the prostate capsule. At these points the boundaries of the organ are particularly obscure, rendering difficult in resection specimens the interpretation of capsular penetration by carcinoma and capsular incision during surgery. Adipose tissue is occasionally found just inside the prostatic capsule. The prostate is composed of

branching tubuloalveolar glands lined by cuboidal or columnar epithelium and invested and surrounded by fibromuscular stroma which is continuous with the prostatic capsule. The urethra transverses the full diameter of the prostate in a curved fashion, entering at the centre of the prostate base and exiting just anterior to the apex. Prostatic ducts empty into the prostatic urethra. The ejaculatory ducts, formed at the juncture of the vasa deferentia and seminal vesicle, also secrete into the prostatic urethra (Derek & Cameron, 2004).

The prostate gland consists of concentric inner and outer zones, where clinically detectable carcinomas predominantly affect the outer region of the gland, and benign prostatic hyperplasia primarily involves the central inner aspect of the gland. For practical purposes, McNeal's model is often simplified such that the central inner periurethral aspect of the prostate is termed the “transition zone,” and the outer peripheral aspect is referred to as the “peripheral zone” and includes the “central zone, ”which is located toward the base of the prostate (Figure 2.2) (Mills et al, 2004).

The peripheral zone contains approximately 70% of the volume of the prostate and is the most common site of the prostatic intraepithelial neoplasia (PIN) and carcinoma. The central zone is a cone-shaped area that includes the entire base of the prostate and encompasses the ejaculatory ducts, it comprises approximately 25% of the volume of the prostate. The existence of the central zone has been questioned, and most authors now combine it with the peripheral zone (awkwardly referred to together as the nontransition zone). Digital rectal examination often includes a description of the left and right lobes based on palpation of the median furrow in the midline that divided the nontransition zone into left and right halves. The transition zone contains the smallest volume of the normal prostate (≈5%) but usually enlarges together with the anterior fibromuscular stroma to massive size besause of benign prostatic hyperplasia (BPH) and dwarfs the remainder of the Prostate (Weidner et al, 2009).

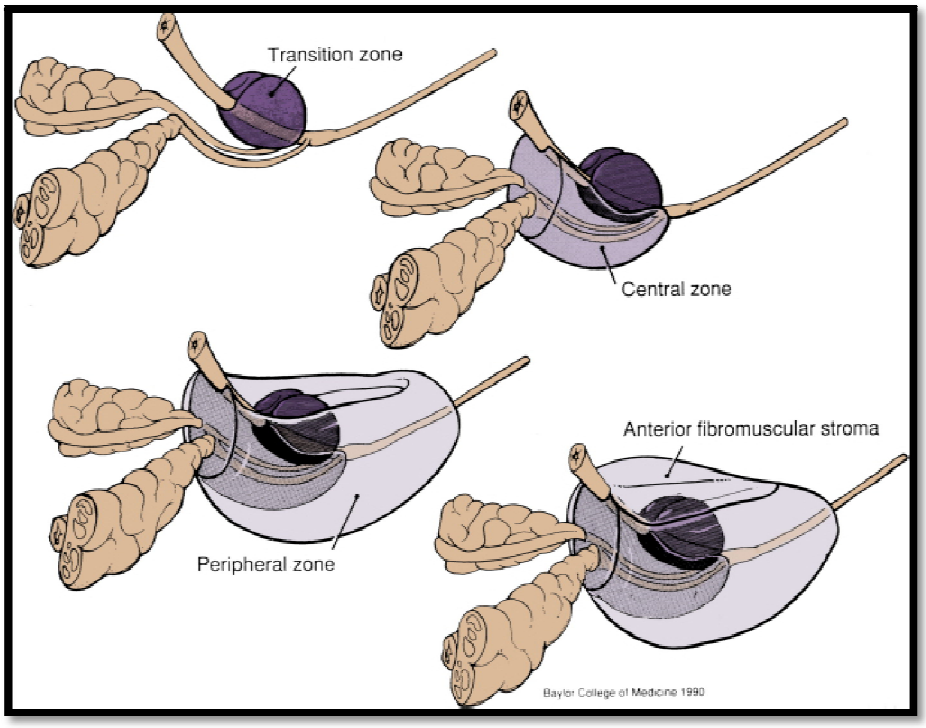

Figure 2.2. Zonal anatomy of the prostate as described by J.E. McNeal.The transition zone surrounds the urethra proximal to the ejaculatory ducts. The central zone surrounds the ejaculatory ducts and projects under the bladder base. The peripheral zone constitutes the bulk of the apical, posterior, and lateral aspects of the prostate (Wein et al, 2012).

Blood supply of the prostate

The main arterial supply is from the prostatic branch of the inferior vesical artery, with some small branches from the middle rectal and internal pudendal vessels. The veins run into a plexus between the true and false capsules and joins the vesicoprostatic plexus situated at groove between bladder and prostate. This plexus receives the deep dorsal vein of the penis, and drains backwards into the internal iliac veins (Sinnatamby, 2006).

Lymph drainge

The lymphatic of the prostate pass across the pelvic floor mainly to internal iliac nodes, a few may reach external iliac node (Sinnatamby, 2006).

Nerve supply

The acini receive parasympathetic (cholinergic) innervation from the pelvic splanchnic nerves via the inferior hypogastric plexus. The muscle fibres of the stroma, which contract to empty the glands during ejaculation, are under smpathetic (adrenergic) control from the inferior hypogastric plexus (Sinnatamby, 2006).

2.3. Histology of Prostate

The prostate gland, a conglomeration of 30 to 50 individual compound tubuloalveolar glands, is arranged in three discrete, concentric layers: mucosal, submucosal, main. Each tubuloalveolar gland has its own duct that delivers the secretory product into prostatic urethra. The mucosal glands are closest to the urethra and thus are the shortest of the glands. The submucosal glands are peripheral to the mucosal glands and are cosequently larger than the mucosal glands. The largest and most numerous of the glands are the peripheral-most main glands, which compose the bulk of the prostate (Gartner & Hiatt, 2008).

The prostate epithelium in the human is composed of two major cellular compartmen: Epithelial cells and stromal cells. The prostate epithelial compartment consists of basal epithelial cells, intermediate cell, neuroendocrine cells, and luminal secretory epithelial cells (De Marzo et al, 1998). The stromal compartment architecturally serves as structural support and consist predominantly of connective tissue, smooth muscle cells, and fibroblasts (Peehl, 2005). In most glands with renewing cell populations there is a steady state flow of cells from mostly quiescent stem cells to a more rapidly dividing pool of transient proliferating cells.This proliferating population finally reach terminal differentiation, characterized by metabolically active secretory epithelium. In prostate, cell lineage has not been rigorously determined but has been inferred from a variety of sources A hypothetical differentiation scheme for prostate epithelium is presented in (Figure 2.3). As in most multilayered epithelia, stem cells reside in the basal compartment and appear to give rise to all of the other epithelial cell types, as well as neuroendocrine cells. These include fully differentiated secretory cells that line glandular lumina (luminal cells), neuroendocrine cells that secrete bioactive peptides, and intermediate cells that show phenotypic features that are intermediate between basal cells and luminal cells (Wein et al, 2012).

Basal Cells

The basal cells possess the highest proliferative activity of the prostatic epithelium, albeit low, and are thought to contain a subset of stem cells that repopulate the secretory cell layer. Basal cells retain the ability to undergo metaplasia, including

squamous differentiation in setting of infarction and myoepithelial differentiation in sclerosing adenosis. Antibodies against high–molecular weight cytokeratin(34βE12) and P63 are frequently used basal cell markers, a property that is exploited immunohistochemically to aid in separating benign acinar processes such as atrophy (that retains a basal cell layer) from adenocarcinoma(that lacks basal cell layer) (Weindner et al, 2009).

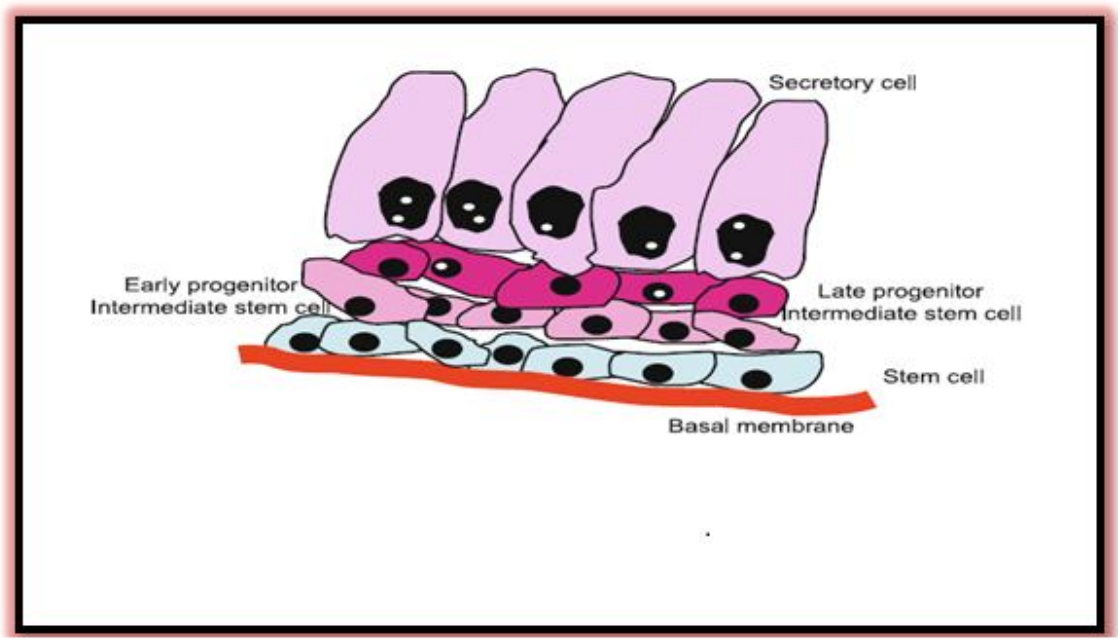

Figure 2.3. Hypothetical cell differentiation in adult prostate. Basal stem cells populate the basal cell compartment and, eventually, intermediate cells. Intermediate cells proliferate and differentiate into quiescent luminal cells. Neuroendocrine cells are also believed to derive from epithelial stem cells (Algaba et al, 2007).

Luminal Epithelial Cell

The luminal epithelial cell is the "workhorse" of the prostate gland, responsible for epithelial barrier integrity and production of prostatic secretion. Luminal cells constitute most of the prostate epithelium. These tall (10 to 20μm) columnar secretory epithelial cells are terminally differentiated and have a low proliferative index (De Marzo et al, 1998); they are easily distinguished by their morphologic features and abundant secretory granules and enzymes. Secretory cells produce a variety of protein that characterize prostatic differentiation, including PSA, acid phosphatase, AR, leucine aminopeptidase, and15–lipoxygenase–2 (Shappell et al, 1999; Bhatia et al, 2003).

They are also rich in keratin filaments (subtypes 8 and 18) (Van Leenders & Schallken, 2003). These tall, columnar secretory cells appear in rows like a picket fence with each cell connected to the next by cell adhesion molecules; the apical aspect of these cells projects into the lumen, with the base attached to a basement membrane through integrin receptor. The nucleus is at the base just below a clear zone (2 to 8μm) of abundant Golgi apparatus, and the upper cellular periphery is rich in secretory granules and enzymes. The apical plasma membrane facing the lumen possesses microvilli, and secretions move into the open collecting spaces of the acinus. These epithelial cells ring the periphery of the acinus and produce secretions into the acini that drain into ducts connected to the urethra (Figure 2.4) (Wein et al, 2012).

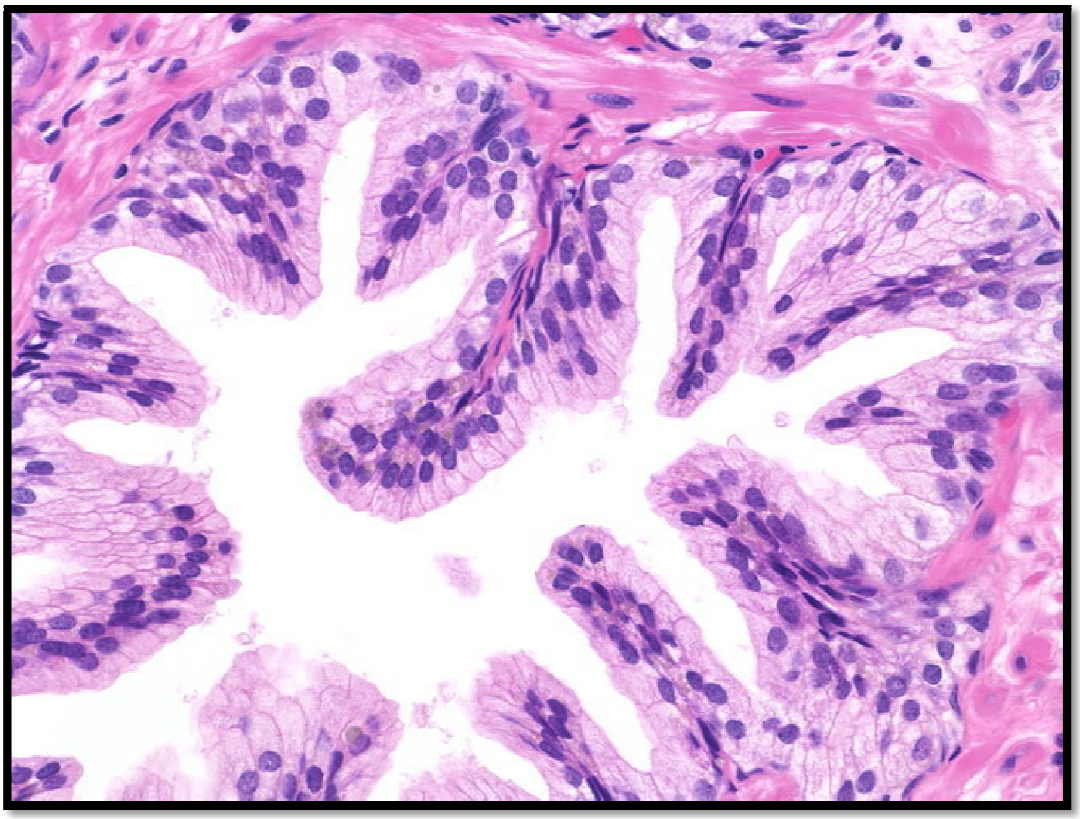

Figure 2.4. In this benign gland, the luminal contour shows tufts and papillary infoldings. The tall secretory epithelial cells have pale clear cytoplasm and uniform round or oval nuclei. Prominent nucleoli are not seen. Many basal cells can be identified. http://www.pathologyoutlines. com

2.4.Epidemiology

2.4.1. Incidence

PCa is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males, accounting for 14% (903500) of the total new cancer cases and 6% (258 400) of the total cancer deaths in males in 2008 (Jemal et al, 2011). The American Cancer Society estimated that, in the USA, (241 740) males would be diagnosed with PCa in 2012, an (28 170) would die of their disease (American Cancer Society, 2012).

The rate of microscopic (i.e. ., latent) carcinomas found at autopsy, increases with age (Sakr et al, 1994). After the introduction of PSA to PCa diagnosis (Wang et al, 1979), and initiation of PSA-based cancer screening in some countries (Catalona et al, 1991), the incidence of the disease has dramatically increased. Moreover, the common practice of biopsying cancer-suspected prostates has led to finding an increased number of carcinomas. The number of biopsy cores taken has gradually increased, being 10-12 nowadays, which has additionally enhanced the detection of cancer. At the same time, the disease-specific mortality rate has not increased, which has improved the disease-specific survival at statistical point of view.

The incidence of PCa varies considerably between countries and ethnic populations (Figure.2.5). Availability of and differences in health care services partly explain the variation in cancer registration (Parkin et al, 2005; Sim & Cheng, 2005). The incidence is being very high in the USA, Australia, and Scandinavian countries (probably due to screening). Incidence rates in Europe are variable with a higher incidence in the countries of Northern and Western Europe and lower in the countries of Eastern and Southern Europe. PCa are relatively rare in Asian populations (Fletcher, 2007). These international differences are clearly reflected within the united states, where the Black population has the highest incidence and mortality rates, some 70% higher than Whites, who in turn have rates considerably higher than populations of Asian origin (Chinese, Japanese and Korean males). Similarly in Sảo paulo, Brazil, the risk of PCa in Black males was 1.8 (95% cl 1.4− 2.3) times that of White men (Eble et al, 2004).

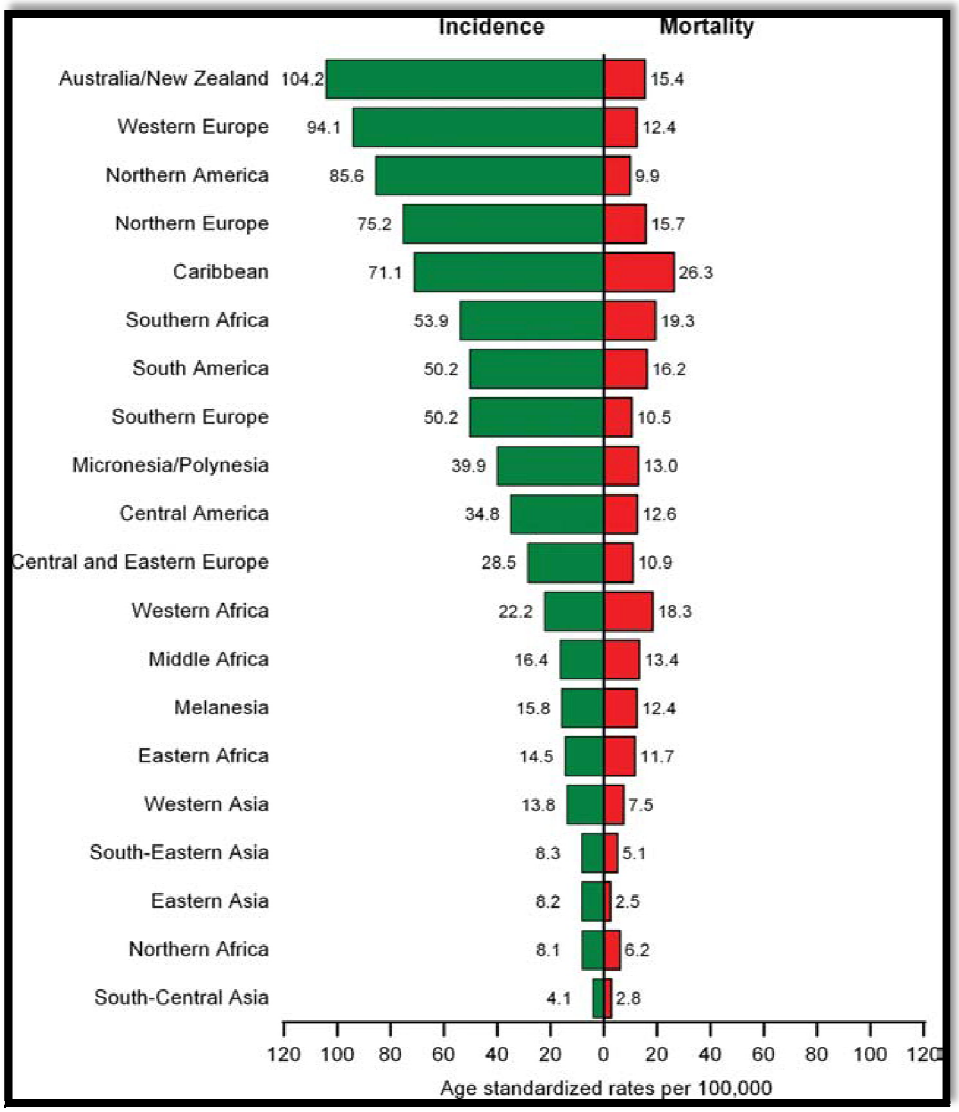

Figure 2.5. Age-Standardized Prostate Cancer Incidence and Mortaility Rates by World Area. Source: GLOBOCAN 2008 (Jemal et al, 2011).

The lowest cancer incidence rates (1.8/100 000) are among Chinese men (Parkin et al, 2005; Sim & Cheng, 2005) and the highest (272.1/100 000) among African-American men in the USA (Gloeckler Ries et al, 2003). Finnish men have the highest incidence in Europe with 95.3 new cases per 100 000 men (Finnish Cancer Registry, 2003). In many African countries the incidence is much higher than in Asia but does not come even close to the incidence rates of Northern Europe or North America (Parkin et al, 2005). The underling causes of this variation are mostly unknown but there is growing evidence for enviromental, dietary and genetic factors explaining the Differences (Shimizu et al, 1991). The importance of dietary, socioeconomic and enviromental factors is illustrated by the increasing risk of PCa in Asian immigrants in the USA

(Whittemore et al, 1995). Genetical alterations probably also contribute to the huge geographical variation of PCa incidence. In conclusion, differences in risk by race may be due to one or more factors: genetics, exposure to carcinogens and life style factors such as diet and decision–making (e.g. detection of cancer).

A total number of 1097 new cases were registers by Benghazi Cancer Registry (BCR) during 2004. Excluding non – melanoma skin cancers, 564(52.4%) were male and 513(47.6%) were females. PCa was the fourth most cancer of men after Lung (19%), Colon and Rectum (11%), and Bladder (9%). Between January and December 2004, there were 42 new prostate cancers, accounting for 7% of all cancers in males. Age distribution shows that prostate cancer mainly occurs in the elderly (85% of all cases occur in men aged 60 years or more) (Figure 2.6). The incidence rates are similar to those reported in Tunisia, Kuwait and other North African Registries (El Mistiri et al, 2010). Compared with Western Libya, the incidence in Western Libya was higher than the incidence in East Libya. In Western Libya, According to the Sabratha Cancer Registry during 2006, prostate cancer is the commonest male cancer and contributes to (17%) of all male cancer patients closely followed by cancer of lung (Sabratha Cancer Registry, 2006).

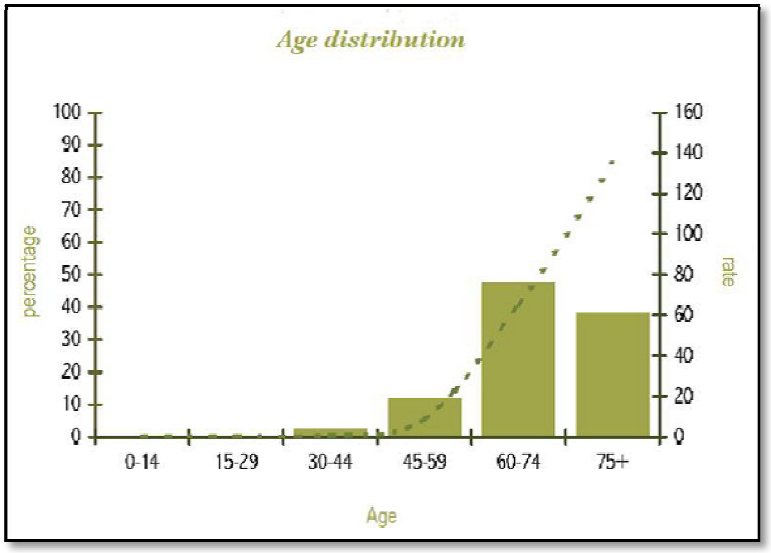

Figure 2.6. Age distribution of prostate cancer patients in Benghazi cancer registries (El Mistri et al, 2004).

2.4.2. Mortality

PCa is the most common cancer in men, accounting for 33% of all malignant tumors in men, and accounts for 9% of cancer deaths, the third highest in men after lung and colorectal cancers (Fletcher, 2007). In 2008, the American Cancer Society estimated 28,660 prostate cancer–related deaths in the United States, for an approximate annual rate of 23.3 per 100,000 population, representing a 41% decrease from the peak in 1991 (American Cancer Society, 2008). Furthermore, the mortality rate for PCa in white men in the United States has declined to a level lower than that observed prior to introduction of PSA-based screening in 1987 (Tarone et al, 2000).

In Europe in 2004, 85,000 men died of PCa, 8.9% of cancer deaths in men. The lifetime risk (0–74 years) of dying from PCa in the European Union was 1.1% in 2004 (Boyle & Ferlay, 2005). Mortality rates for cancer in general differ less between developing and developed countries than incidence rates. For men, total cumulative mortality for all cancers before age 65 is 18 higher in developed countries. Mortality as a result of PCa differs considerably around the world, but the differences are also much smaller than for incidence (Quinn & Babb, 2002). Survival for PCa is better in high-risk countries: 87% in the United States versus 45% in developing countries. These figures are modified by inflation of incidence through early detection programmes that may cause lead-time and length-time bias, and by treatment effects. Since PCa is a disease of the elderly, survival is impacted by co-morbidity with increasing age (Coebergh et al, 1999; Houterman et al, 2005). A Dutch study showed 51% co-morbid conditions in PCa patients (Coebergh et al, 1999). Cancer specific mortality is therefore variable by age. A Swedish study showed an 80% risk of dying of PCa if diagnosed before age 60 years, 63% risk if diagnosed between 60 and 69, 53% for ages 70 to 79 and 49% for ages 80 and older (Gronberg et al, 1997).

The average 5-year survival for PCa in Europe in the early 1990s was 67%. It varied across Europe from less than 40% to more than 80% with lowest rates in Eastern Europe, the United Kingdom, Denmark, Malta and Portugal and highest in Austria, Iceland, Germany and France (Coleman et al, 2003; Sant et al, 2003). In the United States relative 5-year survival for PCa increased to 99.8% in the period 1995–2001 (Surveillance, Epidemiology, and End Results Program 2006). Crude mortality rates for

PCa are an indication for the presence of invasive cancers in a population. Mortality rates are high in the Caribbean, Southern and Central Africa, Northern and Western Europe, Australia/New Zealand and North and South America. They are low in Asia and North Africa. In particular PCa mortality in the USA was not very different from levels in other developed countries despite the difference in incidence, suggesting that a large proportion of cancers in the USA have a good prognosis. However, mortality in Singapore, Japan, India and China was lower than in other countries and consistent with the pattern of incidence (Quinn & Babb, 2002).

2.4.3. Etiology and risk factors

Despite all research efforts the causes of PCa remain unclear. It is belived that PCa has multifactorial origin with enviromental as well as genetic factors, reflecting a complex pathogenesis.

2.4.3.1. Age

Age is an important risk factor for development of histological PCa, the disease being rare below 40 years and becoming increasingly common with rising age, according to postmortem studies (Reynard et al, 2006). The median age at diagnosis is 68 years, with 63% diagnosed after age 65 (Ries et al, 2011). At 85 years of age, the cumulative risk of clinically diagnosed PCa ranges from 0.5% to 20% worldwide, despite autopsy evidence of microscopic lesions in approximately 30% of men in the fourth decade, 50% of men in the sixth decade, and more than 75% of men older than 85 years (Sakr et al, 1993; Gronberg, 2003). PSA-based screening has induced an important age migration effect; the incidence of PCa in men 50 to 59 years of age has increased by 50% between 1989 and 1992 (Hankey et al, 1999), with important implications for deciding on the need for, type of, and complications after therapy.

2.4.3.2. Location

Incidence European rates of PCa tend to be higher in Northern and Central European countries than in Southern and Eastern countries (Ferlay et al, 2010). In the USA, the incidence of PCa is several times higher than in Japan. Also, US rates are 1.6 times higher among African–American men than among Caucasian men (Leitzmann & Rohrmann, 2012). Studies based on migration patterns show distinct changes in the incidence of PCa. For example, rates of PCa among Japanese migrants to Hawaii are intermediate between the rates in Japan and those for Caucasians in Hawaii. During the last two decades, changes have been observed in the incidence rates of PCa in the USA and other industrialized countries, while PCa mortality relatively stable (Hsing et al, 2000). The rise in incidence rates in the mid-1980s is largely due to an increasingly common use of PSA as a method for early detection of PCa. By 2001, 75% of American men aged 50 years old or older reported that they had undergone a PSA test at least once (Sirovich et al, 2oo3), while that statistic is lower in other countries, such as

Germany (Sieverding et al, 2008). The use of PSA testing in the USA to detect PCa in an early phase has shifted the spectrum of diagnosed cancers toward an increased diagnosis of moderately differentiated tumors (Gleason sum scores 5–7). PSA screening is less common in Germany than in the USA, but the procedure has altered the age distribution of PCa cases in Germany as well; the mean age at diagnosis has declined from 73 years of age in 1980 to 69 years in 2006 (Leitzmann & Rohrmann, 2012).

2.4.3.3. Family history and prostate cancer risk

A family history of PCa is one of the strongest known risk factors for this disease. It has been estimated that 5-10% of all PCa cases and 30-40% of early-onset cases (men diagnosed <55 years) are caused by inherited susceptibility genes (Bratt, 2002). Risk increases two to three times for men with a first-degree relative diagnosed with PCa (Johns & Houlston, 2003). If the relative is <60 years old at diagnosis or more than one relative is affected (at any age), the individual's risk is four times the average. These factors combine so that if more than one relative is affected by early-onset PCa, the risk is increased by seven-fold (Carter et al, 1992). A strong family history of breast cancer may also affect a man's risk of PCa, particularly if the family members were diagnosed under the age of 60 (Hemminiki & Chen, 2005). In particular, germline mutations in the breast cancer susceptibility gene, BRCA2, can predispose men to PCa, increasing the risk of developing PCa up to five times in men overall, and more than seven times in men aged under 65 (Breast Cancer Linkage Consortium, 1999). Mutations in the BRCA1 gene may increase the risk of developing PCa in men under the age of 65 by a small amount, and there doesn't appear to be an increased risk after this age (Thompson & Easton, 2002; Fachal et al, 2011).

Recently, genome-wide association studies have identified several genetic variants that each slightly increase PCa risk (Haiman et al, 2007; Eeles et al, 2008). However, because such genetic variants are common in the population, they may contribute to a significant proportion of all PCa cases. Current research in this area is likely to identify further variants in the next few years. Genetic profiling is being used to inform prostate screening and treatment.

2.4.3. 4. Hormones

Androgens significantly alter PCa growth rates, and progression of PCa from preclinical to clinically significant forms may result in part from altered androgen metabolism. Elevated concentrations of testosterone and its metabolite, dihydrotestosterone, over many decades may increase PCa risk, but results have been inconsistent. Hormone levels may be affected both by endogenous factors (e.g., genetics) and by exogenous factors (e.g. exposure to environmental chemicals that affect hormone activity) (Bostwick et al, 2004).

2.4.3.5.Body size

Epidemiologic studies have generally shown weak positive associations between measures of obesity and total PCa incidence. A meta-analysis of the relation of body mass index (BMI) to PCa included 55,521 cases from 31 cohort studies and 13,232 cases from 25 case-control studies. It yielded a relative risk of total PCa per 5 kg/m2 increment of BMI of 1.05 (95% confidence interval [CI] = 1.01–1.08) (MacInnis & English, 2006). Of note, the positive relation of BMI to prostate cancer was more pronounced for advanced disease (relative risk [RR] per 5 kg/m2 increment of BMI = 1.12; 95% CI = 1.01–1.23), whereas the association was null for localized disease (RR per 5 kg/m2 increment of BMI = 0.96; 95% CI = 0.89–1.03). In that meta-analysis, there was little evidence for a relation of central obesity to total PCa, with weakly positive but statistically non significant associations for waist circumference (RR per 10 cm increment = 1.03; 95% CI = 0.99–1.07) and waist to hip ratio (RR per 0.1 unit increment = 1.11; 95% CI = 0.95–1.30). The greater risk seen for advanced PCa and the lack of an association with obesity for non advanced PCa indicates that the biological mechanisms underlying the association between adiposity and PCa are complex. The most salient hypotheses relate to the imbalance of various metabolic and hormonal perturbations associated with adiposity. Certain metabolic alterations sustained in obese men, such as increased levels of insulin, insulin-like growth factor-1 (IGF-1), and leptin may increase PCa risk (Hsing et al, 2001; Chang et al, 2001). Other adiposity-related hormonal alterations, such as reduced concentrations of testosterone and higher levels of estrogen may decrease PCa risk (Gann et al, 1996). Further complexity is added by

the possibility that testosterone may differentially affect low-grade and high grade PCa (Leitzmann & Rohrmann, 2012).

2.4.3.6. Diet

Descriptive epidemiologic studies of migrants, geographic variations, and temporal studies suggest that dietary factors may contribute to PCa developmente (Bostwick et al, 2004). The incidence of latent prostate cancers is similar around the world, but the incidence of clinically manifest cancers differs, with Asians having the lowest rates of clinical PCa. Thus the most convincing evidence for the role of the diet and other environmental factors in modulating prostate cancer risk comes from migration studies showing an increased incidence of PCa in first-generation immigrants to the United States from Japan and China (Muir et al, 1991; Shimizu et al, 1991). These observations suggest that diet may play a role in converting latent tumors into clinically manifest ones. A strong positive correlation exists between PCa incidence and the corresponding rates of several other diet-related cancers, including breast and colon cancers (Bostwick et al, 2004).

More recently, the specific type of dietary fat was shown to be important, with saturated fat increasing the risk (Whittemore et al, 1995; Kristal et al, 2002) whereas polyunsaturated fats might have a protective effect (Bidoli et al, 2005). High levels of lycopenes and carotenoids which proved to have anti-oxidative capacity are contained in high levels in tomatoes and their high intake may correlate with a risk reduction for PCa development (Basu & Imrhan, 2007). However, a European prospective study was not able to show a relationship between plasma carotenoids, tocopherols and overall risk of PCa (Key et al, 2007). Other protective dietary components such as vitamen B12, folate, vitamen E and D, selenium and zinc were discussed but further research is demanded (Johansson et al, 2008).

2.4.3.6. Sexual activity/ Sexual Transmitted Diseases

Sexual activity has been hypothesized to expose the prostate to infectious agents, which may increase the risk of PCa, akin to the causal relationship between HPV and cervical cancer in women. Some studies have found a link between sexually transmissible

infections (STIs) and PCa risk (Fernandez et al, 2005; Sarma et al, 2006), although not consistently (Patel et al, 2005; Huang et al, 2008).

Studies have also suggested a protective association between PCa and frequency of ejaculation, with RR ranging from 0.66 to 0.89 (Giles et al, 2003; Leitzmann et al, 2004).

2.4.3.7. Alcohol and smoking

Two meta-analyses of alcohol consumption and PCa have been carried out. The largest study found no association (Bagnardi et al, 2001) whilst the other showed only small risk increases, although dose-related, of 5%, 9% and 19% with consumption of 25, 50 and 100 grams per day (Dennis, 2001). Findings since these meta-analyses have been inconsistent. A higher risk of fatal PCa in smokers compared to non-smokers has been shown in some studies (Rohrmann et al, 2007; Gong et al, 2008). However, no clear trends were shown with number of cigarettes smoked per day or between current, ex- and never-smokers. Two large studies concluded that smoking is not likely to be linked to either the incidence or mortality of PCa (Adami et al, 1996; Doll et al, 2005).

2.5. Molecular genetics and cytogenetics

PCa can be divided for practical purposes into three groups – hereditary, familial and sporadic. More than 85% of all PCa are sporadic and only 10–15 per cent cancers are genetically determined (Kral et al, 2011).

2.5.1. Hereditary prostate cancer

One characteristic of hereditary carcinoma syndromes is a combination of different cancers in a family caused by a single mutation of a cancer susceptibility gene. Examples of this include breast and ovarian carcinoma caused by mutations in the *BRCA*1 gene and the hereditary non polyposis colon carcinoma families with a combination of colorectal, endometrial, gastric, and ovarian carcinoma resulting from mutations in the DNA mismatch repair genes *MLH*1 or *MSH*2. However, the clinical features of hereditary PCa (HPC) are less known. HPC is proposed to be defined in a family with at least three first-degree relatives (FDRs) with PCa or in a family with two affected FDRs age <55 Years (Gronberg et al, 2000).

Hereditary transmission may be autosomal dominant – through the mother or the father – and even X-linked – through the mother to her sons who will not transmit the susceptibility to their own sons –, and by the last way the disease jumping regularly one generation with subsequent its under-estimation. Autosomal dominant high-penetrant gene-related transmission is usually associated with disease onset at younger age while that recessive chromosome X-linked is characterized by late-onset disease (Tassing & Cussenot, 2005; Fisher et al, 2008).

The first chromosome locus associated with hereditary PCa was 1q24-25 and its putative gene was named HPC1 (hereditary prostate carcinoma 1), which, in turn, was identified with RNaseL gene (Table 2.1.A), involved in interferon-activated apoptosis for virus-infected cells. Indeed, recent studies show that RNaseL gene mutations are responsible for PCa particularly in men with γ-retrovirus- mediated prostate infections, among which especially the xenotropic murine leukemia virus related γ-retrovirus (XMRV). Actually, forty percent of hereditary PCa patients homozigous for a mutation in RNaseL are positive for XMRV whereas this virus is rarely detected in sporadic PCa specimens, such finding meaning as a true are breakthrough in the pathogenesis of PCa.

Polymorphic variants within RNaseL gene associated with raised risk of hereditary PCa (Bratt, 2007; Fisher et al, 2008; Alberti, 2010).

Other strong candidate susceptibility genes areElaC2/HPC2 (locus 17p11.2) and MSR1(macrophage scavenger receptor 1) (Table 2.1.A). Also a mutation in a gene on 8q24 locus should appear to increase the risk of PCa by 60%, but it is more relevant to pathogenesis of familial and sporadic PCa. An indeterminate number of weak candidate susceptibility loci have been suggested to be involved in hereditary PCa (Table 2.1.B). However, PCa high risk alleles, that are able to drive a lifetime penetrance of at least 66%, have a frequency unlikely above 2-3% of the cases, whereas PCa low risk alleles may have a more frequent impact on sporadic PCa. With regard to PCa susceptibility locus 1q42.2-43 (PCAP, prostate cancer predisposing), the prostate carcinoma tumor antigen-1 (PCTA-1), that is located within such chromosomal region, is not a PCa high risk gene while it could make one's low risk contribution to sporadic PCa, but it must be throughly explored (Maier et al, 2002; Fromont et al, 2008).

Table 2.1. Genes involved in hereditary PCa (Alberti et al, 2010).

Gene	Locus	Encoding function	Encoded protein
A-Strong candidate susceptibility genes involved in hereditary prostate cancer			
RNase L	1q24-25 HPC1	Encodes RNaseL	.RNaseL, endoribonuclease located in cytoplasm and mithocondria. . Interferon-activated, it plays an antiviral and proapoptotic role
ElaC2	17p11.2 HPC2	Encodes a zinc phosphodiesterase (ElaC protein 2)	.Zinc phosphodiesterase, located in the nucleus .Displays tRNA 3'-processing endonuclease activity (removal of a 3' trailer from precursor tRNA), thus inducing tRNA maturation
MSR1	8p22-23 PG1	Encodes membrane glycoproteins	.Glycoprotein membrane, Macrophage scavenger receptor type-I and –II. .Involved in the arterial wall deposition of cholesterol during low density lipoproteins
B) Weak candidate susceptibility genes (low risk alleles)			
1p35-36 (CAPB), 1q42-43 (PCAP), 16q23, 17q22, 20q13 (HPC 20), Xq27-28 (HPCX)			

2.5.2. Sporadic PCa

The accumulation of clonal genetic changes is common to all solid tumors, including PCa (Figure 2.7). In sporadic PCa, initial studies found recurrent changes involving losses of genetic material at 6q, 7q, 8p, 10q, 13q, 16q, 17p, 17q, and 18q; however, in most cases, the precise genes involved have yet to be identified (Karan et al, 2003).

Sporadic PCa often shows heterogeneous patterns of oncogene activation, and is rarely associated with mutations in classic oncogenes or tumour suppressor genes; the investigation of oncogene expression profile correlated to disease development and progression is highly challenging. Recently, genome-wide tools (e.g. comparative genome hybridization, spectral karyotyping, SNP analysis) have provided insight into common PCa chromosomal alterations. These genes can be distinguished into those playing an active part in the early and those in the late phases of carcinogenesis (Benedettini et al, 2010).

Genes having a putative role in tumour initiation encode for:

- The tumour suppressor proteins PTEN, p27, Nkx3.1and Rb.
- The transcription factor MYC.
- Glutathione S-transferase-π (GSTP1) which has a role on damage-related stress Prevention.
- Hepsin (cell-surface serine protease).
- Alpha-methylacyl-CoA racemase (AMACR), an enzyme involved in β-oxidation of branched-chain fatty acids.
- KLF6, a zinc finger transcription factor, the polycomb protein EZH2 (enhancer of zeste homolog 2) and telomerase have also been implicated as well in the early phases of prostate carcinogenesis (Benedettini et al, 2010).

In contrast, genes involved in cancer progression and metastases include the AR, p53, Bcl2, ETV1 and ERG1 (Benedettini et al, 2010).

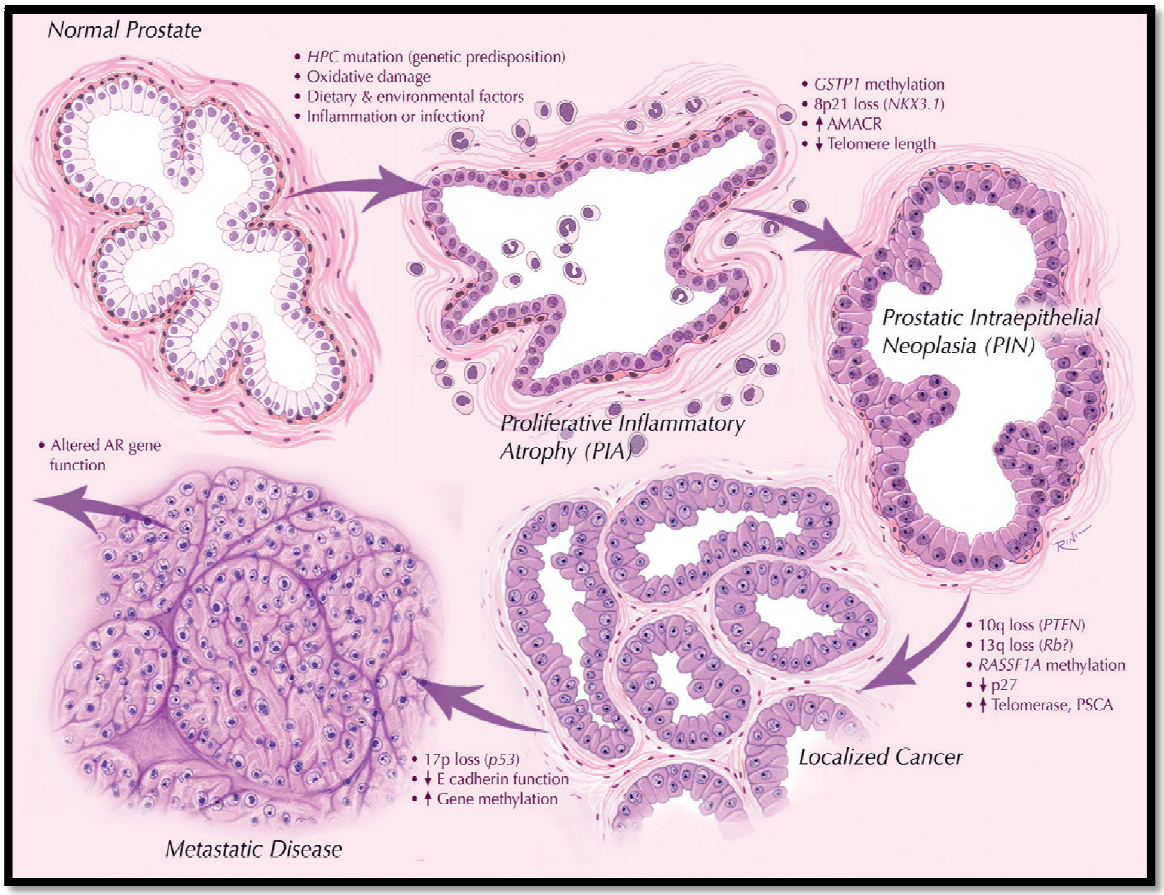

Figure 2.7. Contemporary model of prostate cancer progression. Genetic predisposition, oxidative damage, and inflammatory changes are associated with earliest steps of prostate cancer development. Downregulation of caretaker genes, such as *GSTP1,* by aberrant promotermethylation may increase potential for neoplastic transformation. Chromosomal loss and telomere shortening may also contribute to genetic instability and progression to invasive disease. Further methylation changes, loss of tumor suppressor gene function and additional mutational events are associated with metastases and androgen independence (Wein et al, 2012).

Changes in chromosome 8, typically loss of the p-arm and gain of the q-arm, or portions of these arms, are the most frequently observed genetic alterations. At least two to three separate regions are deleted on 8p, implying the existence of multiple tumor suppressor genes (TSG). 8p22 is commonly deleted, with frequencies of 32% to 65% reported in primary tumors and 65% to 100% in metastases. *MSR-1* lies in this region, and sequence variants in *MSR-1* have been found to be associated with increased disease risk; however, mutations in *MSR-1* have not been reported in sporadic PCa (Xu et al, 2002; Nupponen et al, 2004; Wiklund et al, 2009). Another promising candidate, *TSG*

on 8p, is the prostate-restricted homeobox gene *NKX3.1* at 8p21 (He et al, 1997). 8q gain, often involving the entire chromosomal arm, is the most common chromosomal abnormality found in advanced prostate cancer (e.g., hormone-refractory, lymph node metastases) and is correlated with disease progression and resistance to hormone deprivation or blockade (Alers et al, 2000; Isaacs, 2002; van Dekken et al, 2003).

Phosphatase and tensin homologue (PTEN), is a tumor suppressor gene located at chromosome 10q23 which is frequently changed (lost/mutated) in PCa. PTEN normally inhibits the phosotidy linositol 3'-kinase-protein kinase B (PI3K-Akt) signaling pathway responsible for cell-cycle progression and cell survival. In prostate, reduced PTEN levels correlate with high Gleasone grade and high stage (Li et al, 1997; McMenamin et al, 1999; Sun et al, 1999).

Inactivation or loss of CDKN1B (at 12p12-13) and its protein p27, a cyclin-dependent kinase inhibitor, is frequently found in high grade PIN and PCa (De Marzo et al, 1998). The reduced expression also correlates with poor prognosis either independently or together with the loss of PTEN expression (Cote et al, 1998; Halvorsen et al, 2003). It is also associated with Ki-67 determined proliferation (Halvorsen et al, 2003).

Glutathione-S-transferases belong to a superfamily of enzymes responsible for detoxification of a wide range of xenobiotics. These enzymes catalyze the nucleophilic attack of reduced glutathione on electrophilic compounds. Aberrant methylation of the CpG island at the glutathione-S-transferase P1 (GSTP1) locus is the most frequent somatic genome alteration reported in PCa (Lee et al, 1994; Jeronimo et al, 2001). Methylation of GSTP1 has been detected in greater than 90% of PCa and approximately 70% of PIN lesions, but is not present in normal prostate tissue or benign prostatic hyperplasia (Lee et al, 1994). In normal prostate tissue, expression of GSTP1 is limited to basal cells, but it can be upregulated in columnar epithelial cells exposed to oxidative stress. Increased levels of DNA methylation have also been associated with worse clinical outcomes in patients with PCa (Maruyama et al, 2002).

Recently a new gene fusion in PCa was discovered: the fusion between the androgen regulated gene TMPRSS2 (transmembrane protease, serine 2) (21q22.3) and one of the EST genes: ERG (v-ets erythroblastosis virus E26 homolog (avian)) (21q22.2), ETV1

(7q21.2) or ETV4 (17q21) (Tomlins et al, 2005). Among these, the **TMPRSS2-ERG** fusion is the the most common (Tomlins et al, 2006), occurring in up to 50% of clinically localized PCa in hospital-based cohorts (Perner et al, 2006). The high incidence of PCa probably makes this fusion the most common genomic alteration in human cancers so far described.

Since the discovery of a recurrent gene fusion between the androgen responsive gene ***TMPRSS2*** and ***ERG*** on chromosome 21, PCa are molecularly divided into "fusion-positive" and "fusion-negative" cancers (Brase et al, 2011). Although the ***TMPRSS2-ERG*** fusion is a critical early and common event in PCa development and progression (Kumar-Sinha et al, 2008; Carver et al, 2009), the clinical implications of the fusion are controversial (Wang et al, 2006; Hermans et al, 2009).

Similar to *BCR-ABL1* positive leukemias, colon cancers with microsatellite instability, or breast cancers with BRCA mutations, ETS gene fusions in PCa are associated with specific morphological features and prognoses, as well as specific molecular signatures. A particularly interesting picture is emerging in multi-focal PCa, where different tumor foci in a patient sample have different gene fusion status; however different sites of metastatic PCa from the same patient are uniformly fusion positive or negative (Kumar-Sinha et al, 2008).

TMPRSS2:ERG has been frequently but not unequivocally associated with more aggressive PCa and a poorer prognosis. TMPRSS2 gene rearrangement has been variously associated with high pathologic stage (Mehra et al, 2007) and higher rate of recurrence (Nam et al, 2007a), in independent cohorts of surgically treated localized PCa cases and the presence of gene fusion has been scored as the single most important prognostic factor (Nam et al, 2007a; Nam et al, 2007b). In a FISH based analysis of 445 cancer cases, not having an ERG fusion was found to be a good prognostic factor (90% survival at 8 years) compared to cancers with duplication of TMPRSS2:ERG in combination with deletion of 5'-ERG (2+Edel) that exhibited very poor cause-specific survival (25% survival at 8 years) (Attard et al, 2008).

2.6. Molecular definition of tumoural stem cells of the prostate

Stem cells are required for the maintenance of high-cell turnover– tissues where cells continually need to be replaced, and like most epithelial organs, the prostate is believed to contain stem cells capable of multilineage differentiation (Figure 2.3) (Wein et al, 2012).

The problem that arises in the PCa stem cell model is identifying which cells are the target of carcinogenics. It is possible that the early and late progenitors of the intermediate stem cells, rather than the stem cells themselves, justify the heterogeneity of PCa, both regarding the expression of androgen receptors and the phenotypic characteristics (Schalken, 2005). These cells probably represent aminimal percentage of the tumour mass (<0.01%). It is quite difficult to recognise them using the classical methods, and they present with a differential phenotype with high clonogenicity and therapeutic resistances (Fig. 2.8) (Maitland & Collins, 2005).

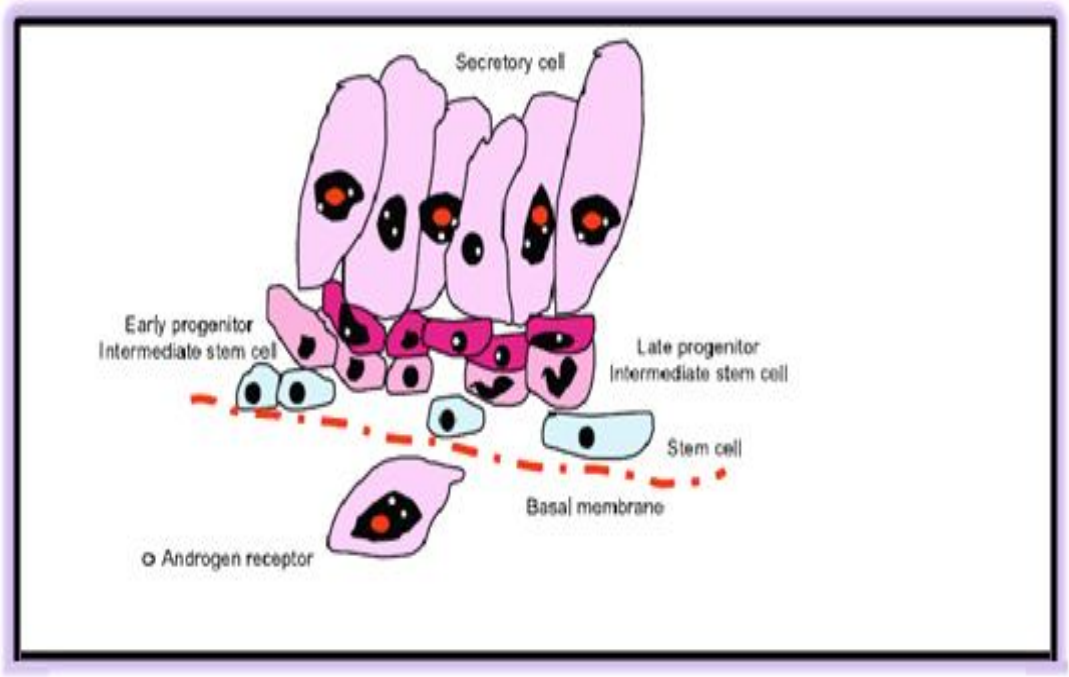

Figure 2.8. Model of malignant transformation of prostate acin (Algaba et al, 2007).

2.7. Pre-malignant changes of prostate gland

2.7.1. High-Grade Prostatic Intraepithelial Neoplasia (HPIN)

PIN is characterized by cellular proliferation within preexisting ducts and acini, with cytologic changes mimicking cancer, including nuclear and nucleolar enlargement (Figure 2.9). There is inversion of the normal orientation of epithelial proliferation from the basal cell compartment to the luminal surface, similar to adenomas in the colon (Bostwick & Cheng, 2012).

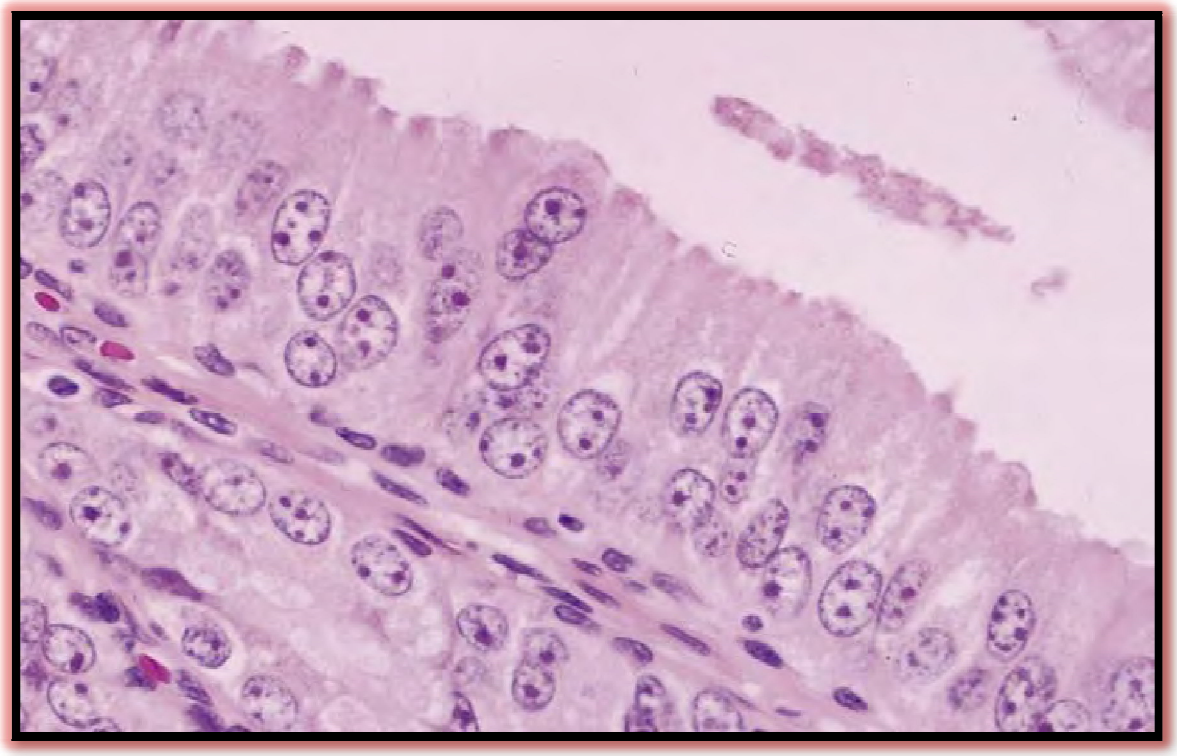

Figure 2.9. High-grade prostatic intraepithelial neoplasia. Nuclei are enlarged, with granular chromatin an nucleolomegaly (Weidner et al, 2009).

PIN was originally subdivided into 3 groups which have now been combined into low-grade (PIN I) and HGPIN (PIN II and PIN III). HGPIN differs from low-grade PIN in that cytologic atypia is more apparent, particularly the presence of prominent nucleoli, as observed using a 20x-power lens (200-fold magnification). Because of its lack of clinical significance, low-grade PIN should not be included in a pathology report to avoid confusion with HGPIN which does impact clinical management (Zynger & Yang , 2009).

Increasing grades of PIN are associated with progressive disruption of the basal cell layer, according to studies utilizing anti-keratin 34bE12 (Figure 2.10). Basal cell layer disruption is present in 56% of cases of high grade PIN, and is more frequent in acini adjacent to invasive carcinoma than in distant acini. The amount of disruption increases with increasing grades of PIN. Early invasive carcinoma occurs at sites of glandular outpouching and basal cell discontinuity in association with PIN (Bostwick &Cheng, 2012).

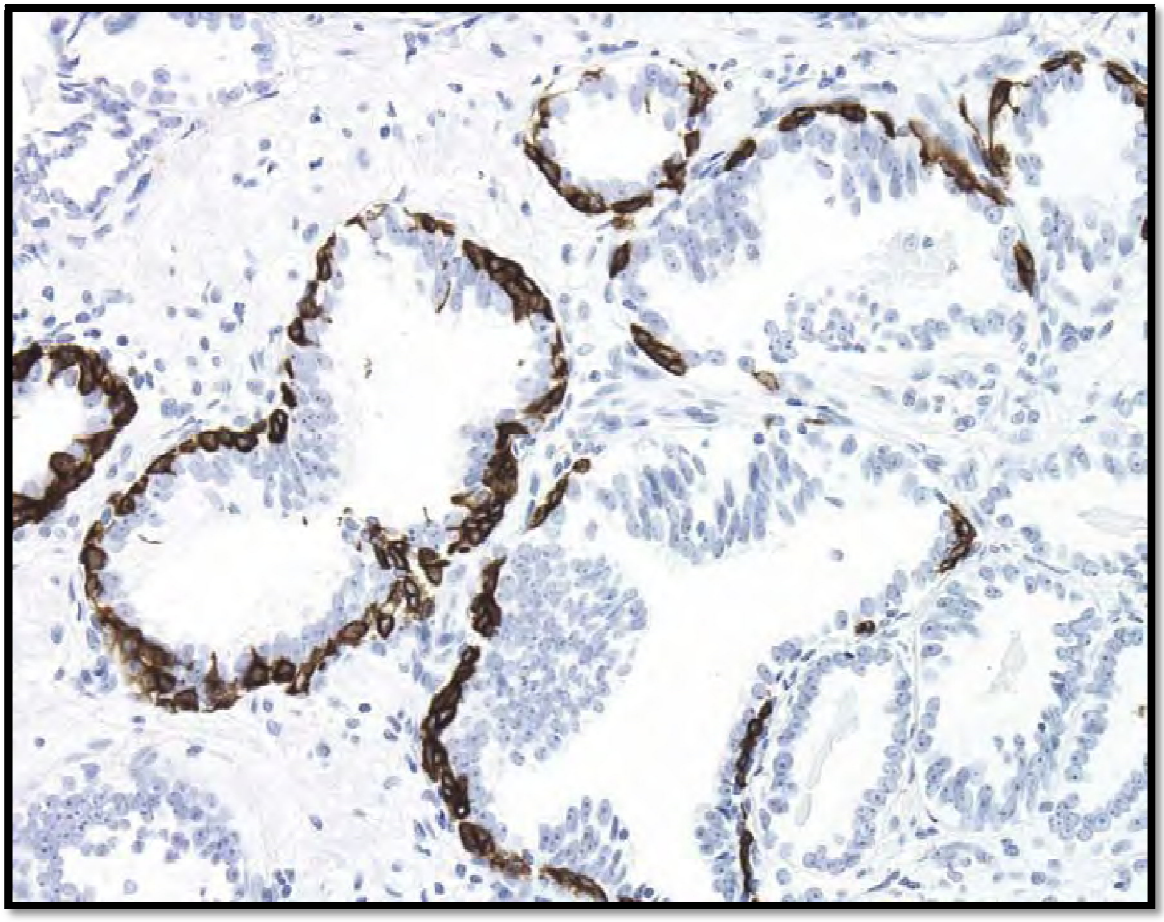

Figure 2.10. Keratin 34βE12 decorates the basal cells forming a discontinuous layer beneath the flat pattern of high-grade prostatic intraepithelial neoplasia (left and top); adenocarcinoma displays no immunoreactivity (center, right, and bottom) (anti–keratin 34βE12 immunohistochemical stain) (Weidner et al, 2009).

HGPIN currently is the only recognized premalignant precursor to prostatic adenocarcinoma. In 1969, McNeal was the first to describe HGPIN as a precursor to prostatic carcinoma. In 1986, McNeal and Bostwick further defined HGPIN (Dickinson, 2010).

HGPIN, the earliest accepted stage in carcinogenesis, possesses most of the phenotypic, biochemical, and genetic changes of cancer without invasion into the fibromuscular Stroma. The only method of detection is biopsy; PIN does not significantly elevate total and free serum PSA concentrations and cannot be detected by ultrasonography (Weidner et al, 2009).

The four main patterns of high-grade PIN are tufting, micropapillary, cribriform, and flat (Figure 2.11). The tufting pattern is the most common, present in 97% of cases, although most cases have multiple patterns. No known clinically important differences exist among the architectural patterns of high-grade PIN, and recognition of these patterns appears to be only of diagnostic utility (Weidner et al, 2009).

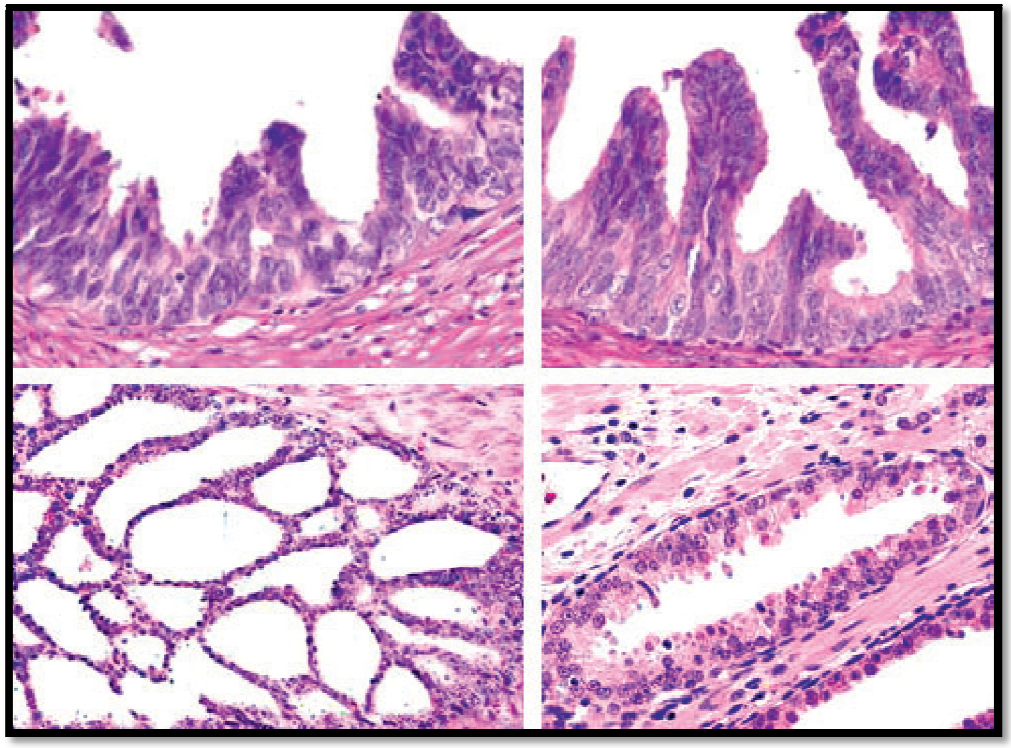

Figure 2.11. Examples of common morphologic patterns of PIN. Tufting pattern, top left; micropapillary pattern, top right; cribriform pattern, bottom left; flat pattern, bottom right (Bostwick & Cheng, 2012).

PIN distribution

PIN is found predominantly in the peripheral zone of the prostate (75-80%), rarely in the transition zone (10-15%), and extremely rarely in the central zone (5%), and this distribution parallels the freguency of zonal predilection for PCa. The frequency of high-grade PIN in needle biopsy series from 5% to 16% and in transurethral resection of the prostate (TURP) specimens between 2.3% and 4.2% (Fletcher, 2007).

Molecular markers of HGPIN

Prostate tumorigenesis is theorized to result from numerous genetic alterations. Currently, data reveals that both genotypically and phenotypically HGPIN exists in a spectrum between benign prostate and prostatic adenocarcinoma. As HGPIN is a precursor lesion, it is expected that some of the molecular abnormalities overlap with PCa or benign prostate while other abnormalities will be unique to HGPIN (Bostwick et al, 1996; Alcaraz et al, 2001; Bostwick & Qian, 2004).

Some of the aberrations which may be critical to the formation of HGPIN are increased expression of AMACR, loss of p27KIP1, PTEN, and RB activity, hypermethylation of the promoter region of GSTP1, and fusion of TMPRSS2-ERG genes. All of these alterations are also seen in prostatic adenocarcinoma (Zynger & Yang, 2009).

2.8. Histopathologic types of prostate cancer

2.8.1 Adenocarcinoma of prostate

Conventional adenocarcinoma of the prostate represents over 90% of the epithelial malignancies in this organ. The majority of cases exhibit an acinar or acinar/ductal growth pattern. The remining 10% comprise the variants of prostatic carcinoma (Fletcher, 2007).

Variants of usual acinar adenocarcinoma include, according to the 2004 World Health Organization (WHO) scheme; atrophic, pseudohyperplastic, foamy, colloid, signet ring, oncocytic and lymphoepithelioma-like carcinomas (Table 2.2). A recently characterized variant that can have atrophic and / or pseudohyperplastic features is microcystic adenocarcinoma. These variants have a wide incidence range, from exceedingly rare, such as the lymphoepithelioma- like and oncocytic carcinomas, to fairly common, such as foamy gland features in acinar adenocarcinoma (Humphrey, 2012).

Non-acinar carcinoma variants of account for a PCa bout 5–10% of carcinomas that are primary in the prostate. These histological variants or types, include, according to the WHO, sarcomatoid carcinoma, ductal adenocarcinoma, urothelial carcinoma, squamous and adenosquamous carcinoma, basal cell carcinoma, neuroendocrine tumours, including small-cell carcinoma, and clear cell adenocarcinoma (Table 2. 2) (Humphrey, 2012).

Recently described variants not present in the 2004 WHO list include PIN-like adenocarcinoma, large-cell neuroendocrine carcinoma, and pleomorphic giant cell carcinoma (Hameed et al, 2006; Tavora et al, 2008).

Table 2.2. WHO histological classification of tumors of the prostate

Epithelial tumors	**Mesenchymal tumors**
Glandular neoplasms Adenocarcinoma (acinar) Atrophic Pseudohyperplastic Foamy Colloid Signet-ring Oncocytic Lymphoepithelioms-like Carcinoma with spindle cell differention Carcinoma with spindle cell differention (carcinosarcoma, sarcomatoid carcinoma) Prostatic intraepithelial neoplasia (PIN) Prostatic intraepithelial neoplasia, grade III (PIN III) Ductal adenocarcinoma Cribriform Papillary Solid Urothelial tumors Urothelial carcinoma Squamous tumors Adenosquamous carcinma Squamous cell carcinoma Basal cell tumors Basal cell adenoma Basal cell carcinoma	Leiomyosarcoma Rhabdomyosarcoma Chondrosarcoma Angiosarcoma Malignant fibrous histocytoma Malignant peripheral nerve sheat tumors Hemangioma Chondroma Leiomyoma Granular cell tumor Hemangiopericytoma Solitary fibrous tumor **Miscellaneous tumors** Cystadenoma Nephroblastoma (Wilms tumor) Rhabdoid tumor Germ cell tumors Yolk sac tumor Seminoma Embryonal carcinoma and teratoma Choriocarcinoma Clear cell adenocarcinoma Melanoma
Neuroendocrine tumors	**Hematolymphoid tumors**
Endocrine differention within adenocarcinoma Carcinoid tumor Small cell carcinoma Paraganglioma Neuroblastoma	Lymphoma Leukemia
Prostatic stromal tumors	**Metastatic tumors**
Stromal tumor of uncertain malignant potential Stromal sarcoma	

2.8.1.1. Gross pathology

Gross identification of prostatic adenocarcinoma is often difficult in radical prostatectomy specimens, and definitive diagnosis requires microscopic examination. Adenocarcinoma tends to be multifocal, with a predilection for the peripheral zone. Grossly apparent tumor foci are at least 5 mm in greatest dimension and appear yellow-white with a firm consistency resulting from stromal desmoplasia. Some tumors appear as yellow granular masses that stand in contrast to the normal spongy prostatic parenchyma (Figure 2.12) (Weidner et al, 2009).

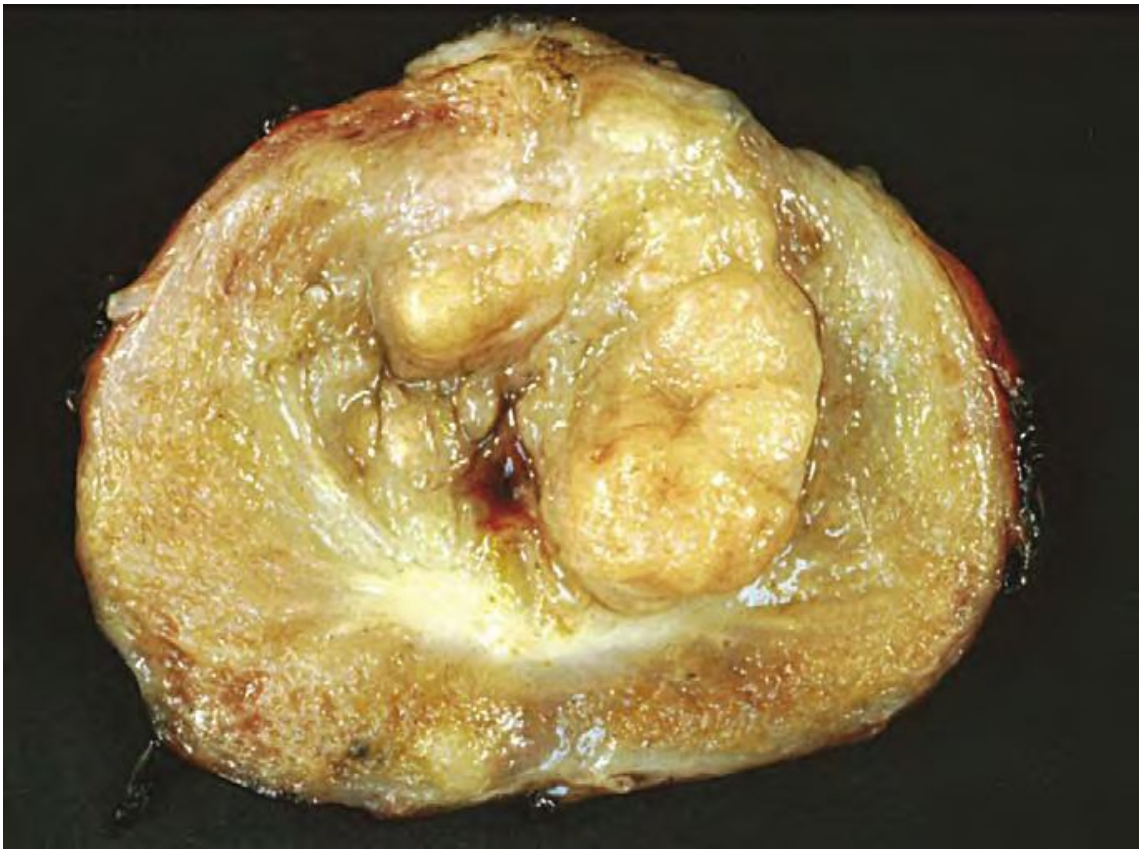

Figure 2.12. Prostate adenocarcinoma, gross appearance (Weidner et al, 2009).

2.8.1.2. Microscopic features

Most prostatic adenocarcinomas are composed of acini arranged in one or more patterns. The diagnosis relies on a combination of architectural and cytologic findings.

The light microscopic features are usually sufficient for diagnosis, but rare cases may benefit from immunohistochemical studies (Table 2.3) (Weidner et al, 2009).

Table 2.3. Differential diagnosis of prostatic adenocarcinoma (Weidner et al, 2009).

Atrophy
Postatrophic hyperplasia
Basal cell hyperplasia
Atypical adenomatous hyperplasia (AAH)
Sclerosing adenosis
Nephrogenic metaplasia
Verumontanum mucosal gland hyperplasia
Hyperplasia of mesonephric remnants
High-grade prostatic intraepithelial neoplasia (PIN)

Architecture

Architectural features are assessed at low- to medium-power magnification and include variation in acinar spacing, size, and shape (Figure.2.13). The arrangement of the acini is diagnostically useful and is the basis of Gleason grade. Malignant acini usually have an irregular haphazard arrangement, randomly scattered in the stroma in clusters or single acini, generally with variation in spacing except in the lowest Gleason grades (Weidner et al, 2009).

Stroma

The stroma in cancer frequently contains young collagen that appears lightly eosinophilic, although desmoplasia maybe prominent. One sometimes sees splitting or

distortion of muscle fibers in the stroma, but this feature is inconstant and unreliable by itself (Weidner et al, 2009).

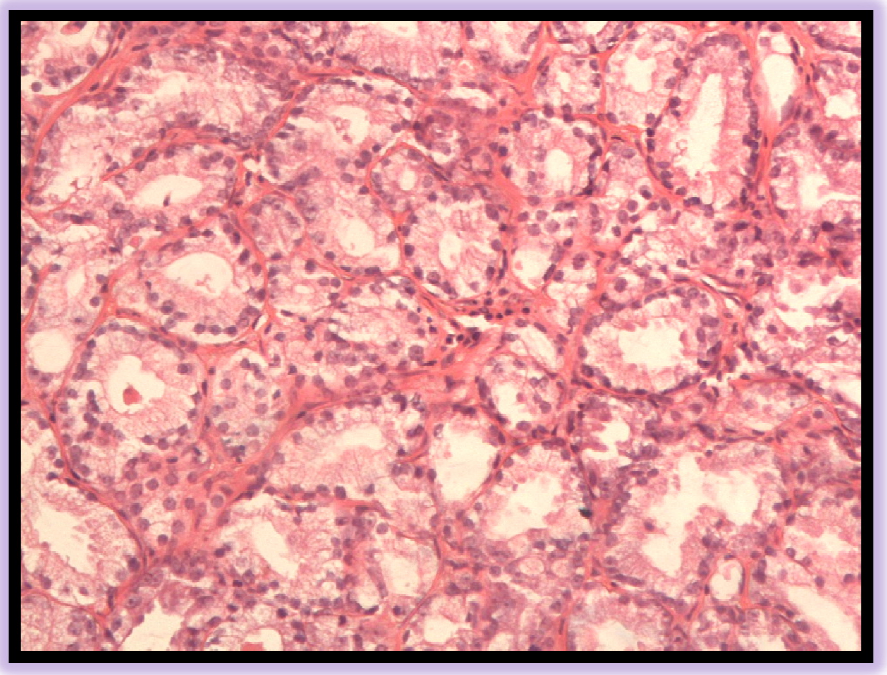

Figure 2.13. Gleason pattern 3 adenocarcinoma. Noted are greater variations in size, shape, and spacing of acini (H&E**X**40) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

Cytology

The cytologic features of adenocarcinoma include nuclear enlargement, irregularity of contour, hyperchromasia, and most important prominent nucleoli (macronucleoli, defined as measuring >1㎛ in diameter) (Figure 2.14). These nucleoli tend to be marginated and are often multiple. Mitoses are also of significance, but they are rarely found in well-differentiated tumors composed of either medium sized or small glands (Rosai & Ackerman's, 2011).

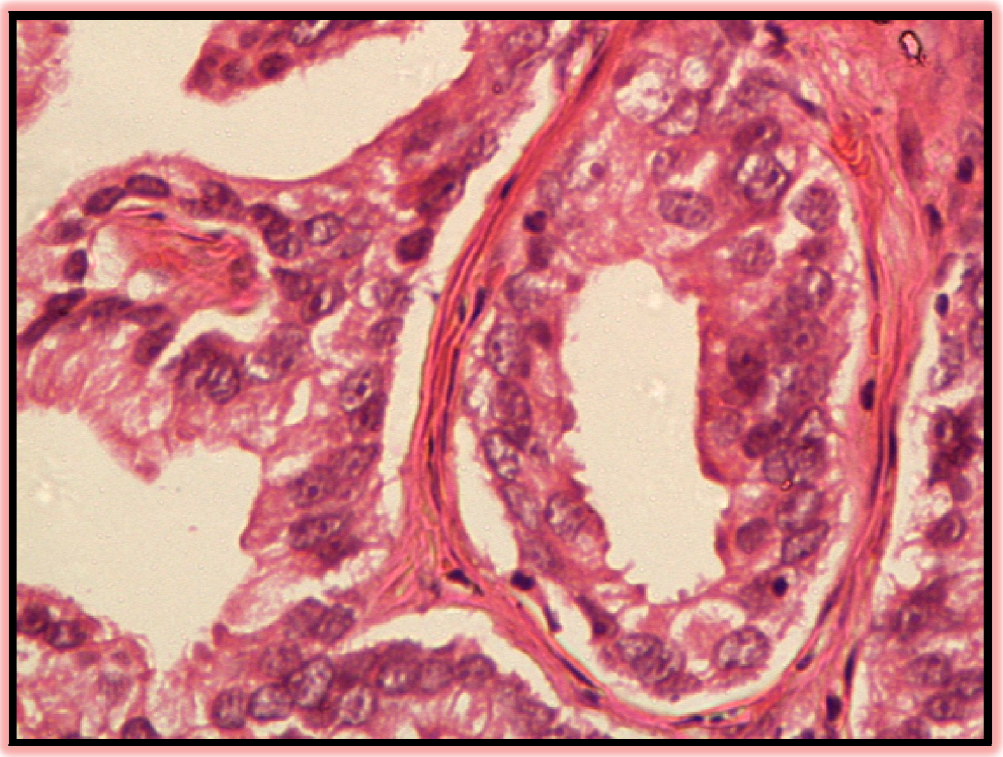

Figure 2.14. The malignant acini are lined by single cell lining (i.e., lack basal cell layer), with enlarged nuclei and prominent nucleoli (H&E**X**100) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

Intraluminal crystalloid, blue mucin, glomerulations, mucinous fibroplasias (collagenous micronodules), and perineural invasion (Figure 2.15) are also helpful findings that that should alert the pathologist to suspect a diagnosis carcinoma. Intraluminal crystalloids and blue mucin are not pathognomonic but they are freguently associated with carcinoma. However, mucinous fibroplasias, glomerulation, circumferential involvement of nerve, and glands in fat tissue are believed to be pathognomonic and allow an unequivocal diagnosis of prostate cancer (Fletcher, 2007).

Perineural invasion

Perineural invasion is common in adenocarcinoma and may be the only evidence of malignancy in biopsy specimens. This finding is strong presumptive evidence of malignancy but may rarely occur in benign acini. Complete circumferential growth, intraneural invasion, and ganglionic invasion are found only in cancer. This finding is probably not a useful predictive factor (Figure 2.15) (Weidner et al, 2009).

Vascular or lymphatic invasion

Microvascular invasion is a strong indicator of malignancy and its presence correlates with histologic grade, although it is sometimes difficult to distinguish from fixation associated retraction artifact of acini (Figure 2.16) (Weidner et al, 2009).

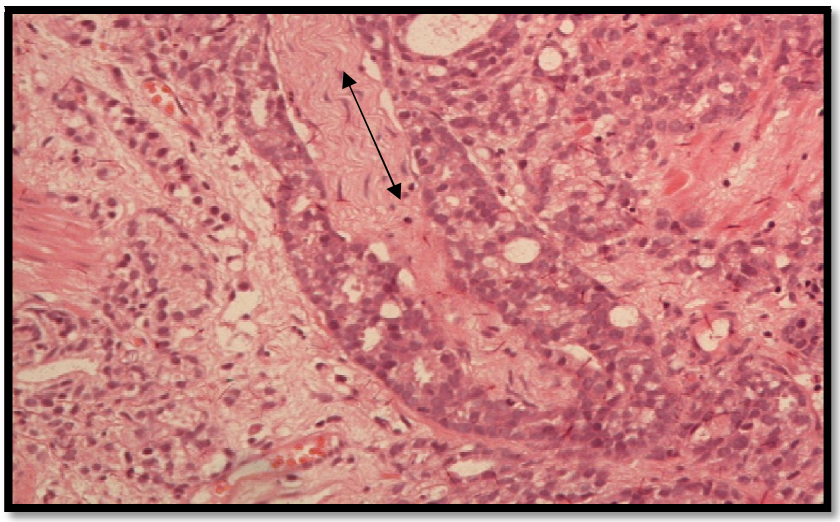

Figure 2.15. Perineural invasion of prostate cancer cells. Prostate cancer cells grow along the nerve branch (black arrow) (H&E**X**40) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

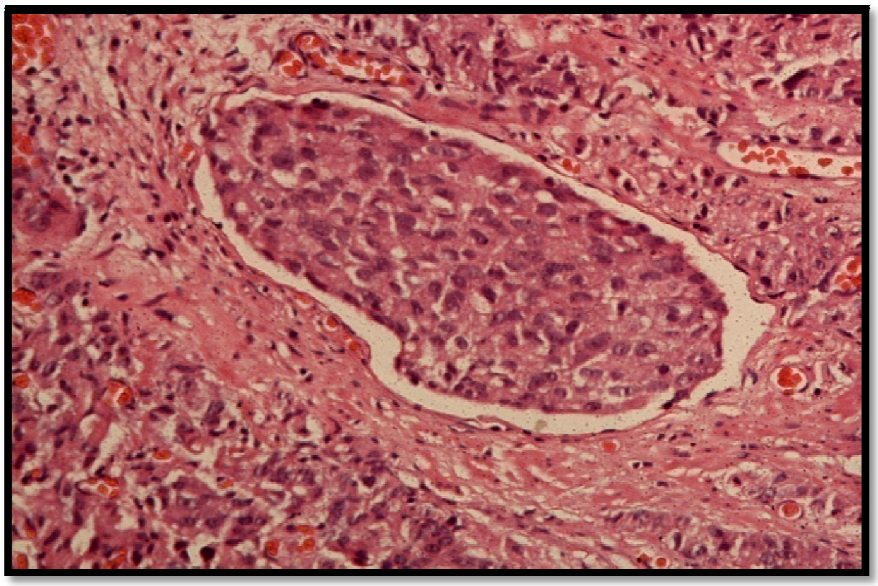

Figure 2.16. Tumour cells within confined vascular space; endothelial cells are inconspicuous (H&E**X**40) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

2.8.2. Ductal adenocarcinoma

Ductal adenocarcinoma of the prostate is a subtype of adenocarcinoma that has also been termed endometrioid, endometrial, papillary or papillary ductal adenocarcinoma (Lotan et al, 2009; Samaratunga et al, 2010; Amin & Epstein, 2011). Ductal adenocarcinoma is the most common histological variant of PCa. The incidence of ductal adenocarcinoma, including both pure ductal and mixed ductal–acinar adenocarcinomas, is 3.2% of all PCa. Mixed ductal–acinar carcinoma is more common than pure ductal carcinoma (Humphrey, 2012).

Clinically, men with prostatic ductal adenocarcinoma are typically aged 63–72 years (range 41– 89 years). Obstruction and haematuria are common clinical manifestations. The digital rectal examination is usually abnormal and often suspicious for malignancy. Most patients have an elevated serum PSA level. A substantial minority of men with ductal prostatic adenocarcinoma can present with 'metastatic' levels of serum PSA in the hundreds to thousands of ng / ml, bone pain, and skeletal metastases. Clinical stage is more often advanced than in standard acinar carcinomas. In the largest series to date, of 371 ductal cases, 12% of men with ductal adenocarcinoma presented with distant metastasis versus 4% of men with acinar adenocarcinoma (Morgan et al, 2010).

Microscopically, prostatic duct adenocarcinoma is characterized by pseudostratified columnar epithelium (Figure 2.17) (Epstein et al, 2011). The two most common growth patterns are papillary and cribriform. Solid cylinders and comedocarcinoma may also been seen, but it is not possible to classify these configurations as being ductal without an associated papillary and cribriform component. Single glands of ductal adenocarcinoma are distinctly unusual, and may assume the form of PIN like adenocarcinoma (Hameed & Humphrey, 2006; Tavora &Epstein, 2008). A particularly distinctive finding comprises rounded glandular profiles of cleared tumour cells with central eosinophilic luminal debris. Glands of ductal adenocarcinoma are often embedded within a fibrotic stroma, which is unusual for small acinar adenocarcinoma. Mitotic activity in ductal carcinoma is variable, but is higher than in most acinar adenocarcinomas, where it can be difficult to find any mitoses at all (Humphrey, 2012).

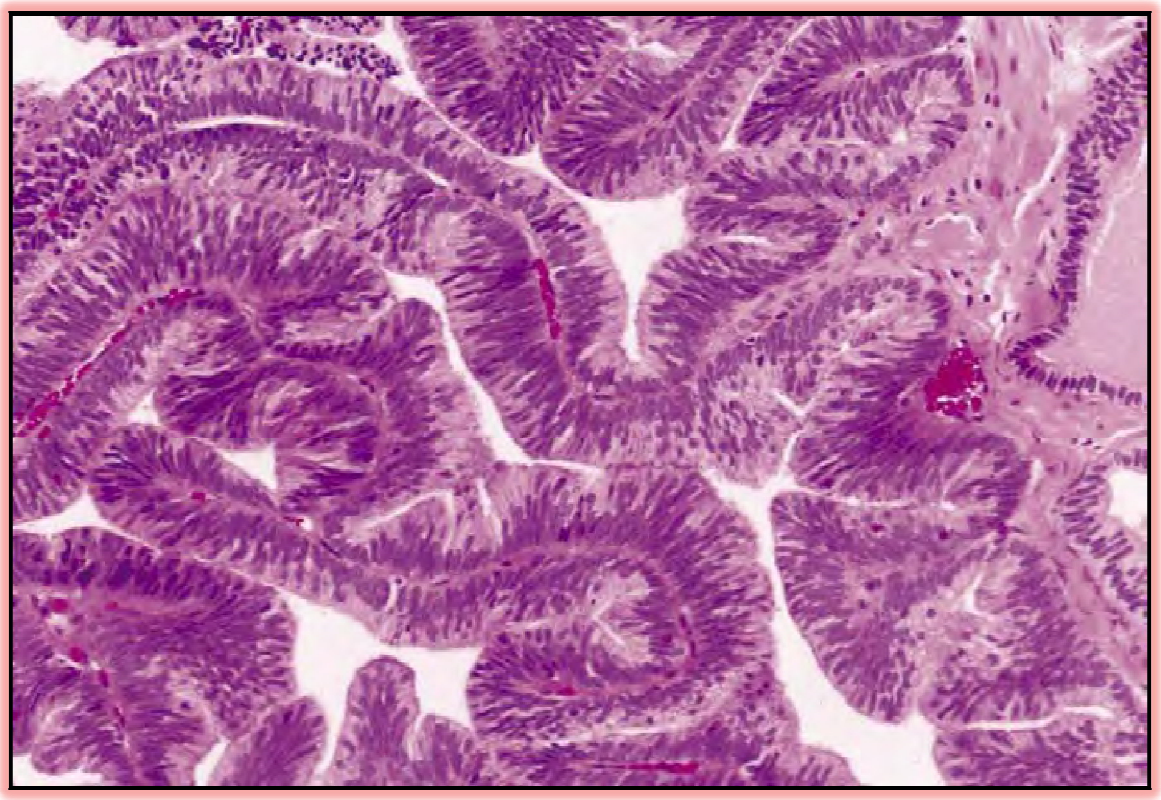

Figure 2.17. Ductal adenocarcinoma. This papillary proliferation filled the large periurethral prostatic ducts and protruded into the urethra (Weidner et al, 2009).

The histological grade of ductal adenocarcinoma is usually high-grade Gleason pattern 4, but, uncommonly, pattern 3 and pattern 5 can be seen. For mixed ductal–acinar adenocarcinomas, a single Gleason score should be given. The ductal component is usually of higher grade than the acinar proliferation. The immunophenotype of prostatic ductal adenocarcinoma is similar to that of acinar adenocarcinoma (Kelemen et al, 2006).

2.9. Diagnosis of prostate cancer

Digital rectal examination (DRE), serum PSA tests, and/or transrectal ultrasound (TRUS) can predict PCa. The diagnosis should be confirmed by biopsy. Each test identifies a proportion of cancers, with higher rates of detection when they are used in combination (Selley et al, 1997).

During the past decade two factors influenced significantly the increased detection rate of PCa in general and that of clinically insignificant PCa in particular: the widespread use of serum PSA as a screening tool to a large extent and to a lesser though significant extent the application of extended multiple core biopsy schemes (Master et al, 2005). In fact, 75% of men in the United States aged 50 years and older have been screened with the PSA test (Service et al, 2003).

Outside of the screening context, which is dealt with in depth in next topic, clinical suspicion of PCa is raised usually by abnormal DRE and/or by abnormal levels of serum PSA. Final diagnosis is achieved only based on positive prostate biopsies.

2.9.1. Serum PSA levels as a diagnostic tool

PSA is a 33 kDa glycoprotein, which belongs to the family of human kallikerin proteins and is a neutral serine protease. It has several isoforms with its isoelectric point ranging from 6.8 to 7.2 (You et al, 2010). Formation of PSA depends on the secretion of androgen and it is mostly found in prostatic tissue, although low concentrations of the protein can be found in other tissues such as kidney and endometrium (Clements et al, 1994). PSA is secreted by the prostatic epithelium and the epithelial lining of the acini and ducts of the prostatic tissue. It occurs in sperm and functions in liquefaction of the seminal fluids. PSA has the highest concentration in the prostatic lumen. In order to enter the blood circulation, PSA has to move through the prostatic basal membrane, stroma, capillary basal membrane and capillary endothelial cells (Lukes et al, 2001). Two forms of PSA are found in serum, free and bound to α 1-antichymotrypsin or to α 2-macroglobulin (You et al, 2010).

The only biomarker currently used for the detection and monitoring of treatment efficacy for PCa is the measurement of serum PSA levels and there is constant debate as to whether PSA actually aids in the management of PCa for the following reasons:

There are no distinct cut-off serum PSA levels that absolutely define if a patient does have PCa. Although a high serum PSA level is indicative of the presence of PCa cells, studies have shown that a proportion of men without PCa have high levels of serum PSA (Neal & Donovan, 2000) and about 22% of men with PCa have been found to have low serum PSA levels (Henrique & Jerónimo, 2004). This means that a proportion of men will undergo the unnecessary invasive procedure of a needle biopsy, while a proportion of men will have their PCa undetected.

PSA is not a PCa specific marker. An increase in serum PSA level may indicate the presence of other prostatic diseases such as BPH, which is also common in elderly men (75–90% incidence in men by the age of 80 years) and prostatitis (Roehrborn et al, 1999; Schatteman et al, 2000).

Serum PSA levels are not able to distinguish patients with indolent disease from those with aggressive PCa at the time of diagnosis. In addition, the current early detection of PCa results in most patients presenting with a low stage/grade PCa, making the clinical decision about whether and how to treat the patient difficult. Particularly in the case for elderly men with an expected life expectancy of less than 10–15 years, clinicians have to decide whether these patients will have a survival benefit from treatment or if watchful waiting is the best option (Chiam et al, 2012).

Using serum PSA levels to determine treatment efficacy requires monitoring over a period of time before a clinician can decide if the treatment is suitable for a patient. For instance in the case of chemotherapy, the clinician is not able to predict if a patient is responsive to the treatment until after a prolonged treatment period that may be accompanied by unpleasant side-effects (Chiam et al, 2012).

As a result of the specificity of the PSA test being challenged, various methods have been proposed to improve the test, which can be classified into two groups: PSA isoforms and PSA parameters. PSA isoforms consist of free PSA (fPSA), proPSA, complexed PSA (cPSA) and benign PSA (bPSA). PSA parameters on the other hand

involve looking at the percent free PSA (%fPSA), PSA density (PSAD), age specific PSA ranges, PSA velocity (PSAV) and PSA doubling time (PSA-DT) (You et al, 2010).

2.9.2. Digital rectal examination as a diagnostic tool

DRE is probably the most common diagnostic test in urological practice. It requires the insertion of a finger into the rectum to palpate the prostate gland for induration or abnormal masses. Suspected abnormalities can then be investigated further by ultrasound scan or biopsy (Selley et al, 1997).

Analyzing data from the Rotterdam section of the European Randomized Study of Screening for PCa, Schroder et al. evaluated the usefulness of DRE as a standalone screening test and in conjunction with measured serum PSA levels of 0–3.9 ng/ml and TRUS (Schroder et al, 1998). Although they showed that DRE has a poor performance in low PSA ranges with a calculated positive predictive value of DRE and TRUS at PSA 0 to 4.0 ng/ml of only 9.7% (Schroder et al, 2000), 17.3% of the cancers identified in their cohort would have remained undetected by PSA-based screening alone (Schroder et al, 1998).

Regardless of serum PSA levels, a DRE finding of a firm nodule or diffusely firmed prostate should promote prostate biopsy, as 5%, 14%, and 30% of men with PSA 0–1.0, 1.1–2.5, and 2.6–4.0 ng/ml, respectively, have PCa (Carvalhal et al, 1999). Carvalhal et al. found that the majority of cancer cases detected by DRE in patients with serum PSA of less than 4 ng/ml have features of clinically important and potentially curable disease (Carvalhal et al, 1999). Although for screening purpose DRE is fairly inferior to PSA, its role in combination with PSA for diagnosis is imperative, as it gives essential clinical information for staging.

2.9.3. Prostate biopsy

TRUS-guided systematic prostate biopsy is the standard test for PCa diagnosis. Prostate biopsy strategies have significantly evolved over the past decade. The current practice for initial biopsy using extended biopsy schemes (10–13 cores) including laterally directed biopsies has significantly reduced the false-negative rate of the previous dominant classic sextant biopsy. The increased diagnostic scheme of this state-of-the-art

approach not only results in lower detection rates of re-biopsies but was demonstrated to provide valuable staging information (Singh et al, 2004; Naya et al, 2004).

2.9.4. immunohistochemistry as a tool to diagnose primary carcinomas

The diagnosis of carcinoma on routine histological slides is sometimes extremely difficult and uncertain. The increase of biopsy measures, due to PSA testing and cancer screening, has lead to frequent finding of small suspicious foci on biopsy specimens. In addition to this, much smaller true carcinoma foci may now be found than before. The variety of benign mimickers, doubtful premalignant lesions and the often normal-appearing architere of small, well-differentiated carcinoma foci makes the diagnosis even more difficult. Therefore tissue markers with high sensitivity and specificity for malignancy would be of great value. The development of immunohistochemical techniques has offered pathologists new tools for the detection of cancer. Some of these are reviewed here.

2.9.4.1.Basal Cell–Specific Anti–Keratin 34βE12 (Keratin 903; High-Molecular-Weight Keratin)

High molecular- weight cytokeratin (HMCK) 34β E12 is a cytoplasmic marker that highlights intermediate cytokeratin (CK) filaments in glandular basal cells and is specific for basal cells in the prostate (Paner et al, 2008). Basal cell–specific anti–keratin 34βE12 stains virtually all the normal basal cells of the prostate; no staining occurs in the secretory and stromal cells (Weidner et al, 2009).

HMWCK when demonstrating a focal or diffuse basal layer, is helpful in designating glands which are ‘concerning for malignancy’ as benign in nature. However, HMWCK is a negative stain and there are well-recognized inherent problems with a negative stain. Assessment for the presence of a basal layer has several pitfalls. Benign mimics of prostatic adenocarcinoma (HGPIN, partial atrophy, postatrophic hyperplasia, and atypical adenomatous hyperplasia (AAH)/adenosis) often have a discontinuous basal cell layer. Prolonged formalin fixation has been shown to have a negative effect on detection of basal cell-specific keratins giving rise to false negative staining (Martens & Keller, 2006).

Recently, several studies have shown that the diagnostic power of 34βE12 can be facilitated by combination with other basal cell markers and/ or with Alpha-methylacyl CoA racemase (AMACR) (Zhou et al, 2003; Magi-Calluzzi et al, 2003; Jiang et al, 2004; Jiang et al, 2005). The most promising basal cell marker is p63, a p53 homologue, which is particularly advantageous as it stains the nuclei of prostate basal cells (Signoretti et al, 2000). Thus it can be used simultaneously with cytoplasmic antibodies. It might offer better sensitivity than 34Be12 in detecting benign glands (Shah et al, 2002).

2.9.4.2. Alpha-methylacyl-CoA racemase (AMACR), P504S protein

AMACR is a gene initially found to be associated with prostate cancer by cDNA library subtraction and cDNA microarray analysis on small number of samples (Xu et al, 2000). Later the characteristic expression of AMACR, also referred to as P504S protein, in prostate was confirmed through large scale gene expression profiling studies (Luo et al, 2001; Rubin et al, 2002) and subsequently, by immunohistochemistry (Jiang et al, 2001; Luo et al, 2002; Rubin et al, 2002).

AMACR is a mitochondrial and peroxisomal enzyme that is involved in beta-oxidation of branched chain fatty acids and in bile acid biosynthesis (Lloyd et al, 2008). It is expressed in normal tissues, e.g. hepatocytes, renal tubular epithelial cells and gall bladder mucosa, and also in a variety of dysplastic tissues or malignant tumours including colon cancer and papillary renal cancer (Oberholzer et al,2006; Dorer et al, 2006; Sonwalkar et al, 2010).

The highest rates of AMACR overexpression (>95% of cases) have been reported for, PCa which render it an applicable biomarker and, so far, it is the only one that has gained clinical acceptance. In combination with basal cell markers, AMACR can significantly increase diagnostic accuracy and help to avoid unnecessary rebiopsies (Zhou et al, 2004; Carswell et al, 2006; Herawi et al, 2007; Paner et al, 2008).

AMACR staining can be very reassuring, with impressive images of strongly positive atypical glands infiltrating absolutely negative prostatic parenchyma, making a straightforward diagnosis of cancer possible, where previously only ‘atypical glands’

would have been signed out. However, interpretation of an AMACR staining also requires experience, bearing in mind that AMACR expression can be heterogeneous, that a minority of PCa cases are AMACR-negative and that common benign mimicker lesions of PCa can display significant AMACR immunoreactivity (Murphy et al, 2007; Wang et al, 2008; Kristiansen et al, 2008; Kristiansen, 2012). Also, the discrimination of invasive glands adjacent to HGPIN from HGPIN outpouchings may be impossible, as HGPIN is also often AMACR-positive. Some authors even suggest AMACR as a marker of high grade PIN that is associated with non-sampled cancer foci, but this certainly needs further validation (Wu et al, 2004; Ananthanarayanan et al, 2005). The aforementioned drawbacks of AMACR clearly warrant the search for alternative diagnostic markers.

2.9.4.3. GOLM1 (GOLPH2, GP73)

GOLM1 is a 73 kDa Golgi phosphoprotein of as yet unknown function that had been described initially in liver disease, and particularly in hepatocellular carcinoma (Kladney et al, 2000; Kladney et al, 2002; Iftikhar et al, 2004; Marrero et al, 2005; Bachert et al, 2007). Overexpression of GOLM1 mRNA has also been reported in various profiling studies of PCa (Luo et al, 2002; Lapointe et al, 2004; Kristiansen et al, 2005). Later, three groups independently confirmed the strong overexpression of GOLM1 in PCa at protein level (Kristiansen et al, 2008; Wei et al, 2008; Varambally et al, 2008).

The largest cohort comprised of 614 cases that were concomitantly immunostained for p63 and AMACR as the gold standard and allowed for a casewise comparison of expression in tumour and adjacent normal tissue. First, this study confirmed the nearly universal overexpression of AMACR, but found 5% of tumours AMACR negative, another 5% partially negative and 45% were heterogeneously stained. In comparison, GOLM1 showed a lower rate of heterogeneity (25%) and was found up-regulated in 92.5% of cases. Importantly, GOLM1 was particularly helpful in the majority of AMACR-negative cases (84%), justifying considering its use as an additional ancillary marker for PCa (Kristiansen et al, 2008). A caveat is constituted from the ubiquitous expression of GOLM1 in benign tissues, which makes a comparison of the suspicious glands questionable, with adjacent clearly benign glands necessary to come to a

diagnostic conclusion. Also, a detailed analysis of typical benign mimicker lesions is still pending (Kristiansen, 2012).

2.10. Cancer grade

Although numerous grading system for PCa have been proposed in the literature, only the Gleason system has prevailed.

2.10.1. Gleason grading system

The microscopic grading system developed by Gleason in conjunction with the Veterans Administration Cooperative Urological Research Group (VACURG) is currently preferred (with the modifications) to the other grading systems that have been proposed over years. It is based on the degree of glandular architectural differentiation and the growth pattern of the tumor in relation to the stroma as evaluated on low-power examination (Rosai & Ackerman's, 2011).

Gleason noted that, in most cases, more than one histological pattern was present and he designated the predominant pattern as the primary pattern, while the subordinate pattern was designated the secondary pattern. If only one pattern was present then this was considered both the primary and secondary pattern for analytical purposes. In general, the grading of the cases in the series were based upon the largest specimen, such that if a patient had undergone radical prostatectomy then this would be graded in preference over a needle biopsy or transurethral biopsy from the same patient (Delahunt et al, 2012).

Gleason pattern 1 adenocarcinoma is uncommon and difficult to diagnose, particularly in biopsy specimens. It consists of a circumscribed mass of simple, monotonously replicated round acini that are uniform in size, shape, and spacing. Nuclear and nucleolar enlargement is moderate but allows separation from its closest mimic, AAH. Crystalloids are observed in more than half of cases (Figure 2.18) (Weidner et al, 2009).

Gleason pattern 2 is very similar to pattern 1 except for the lack of circumscription of the focus; this finding indicates the ability of the cancer to spread through the stroma. Slightly greater variation in acinar size and shape is observed, but the acinar contours are chiefly round and smoothly sculpted. Acinar packing is somewhat more than in pattern 1, and separation is usually less than one acinar diameter (Figure 2.18) (Weidner et al, 2009).

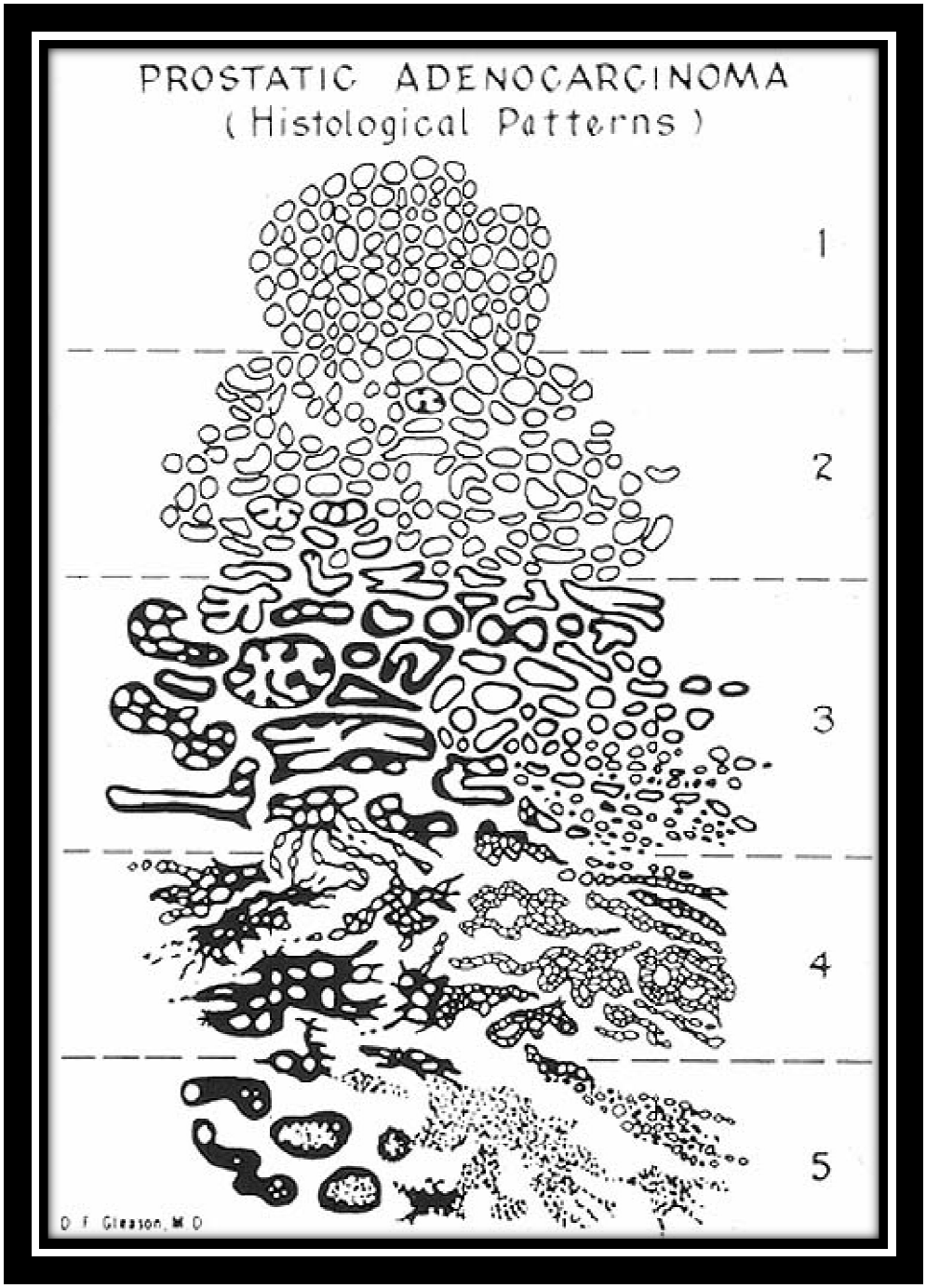

Figure 2.18. Gleason grading system of prostatic adenocarcinoma (Delahunt et al, 2012).

Gleason pattern 3 is the most common pattern of prostatic adenocarcinoma and encompasses a wide and diverse group of lesions. The hallmark of pattern 3 adenocarcinoma is prominent variation in size, shape, and spacing of acini (Figure 2.19, 2,20). Despite this variation, the acini remain discrete and separate, unlike the fused

acini of pattern 4 (see later). Acini are haphazardly arranged in the stroma, sometimes with prominent stromal fibrosis (Weidner et al, 2009).

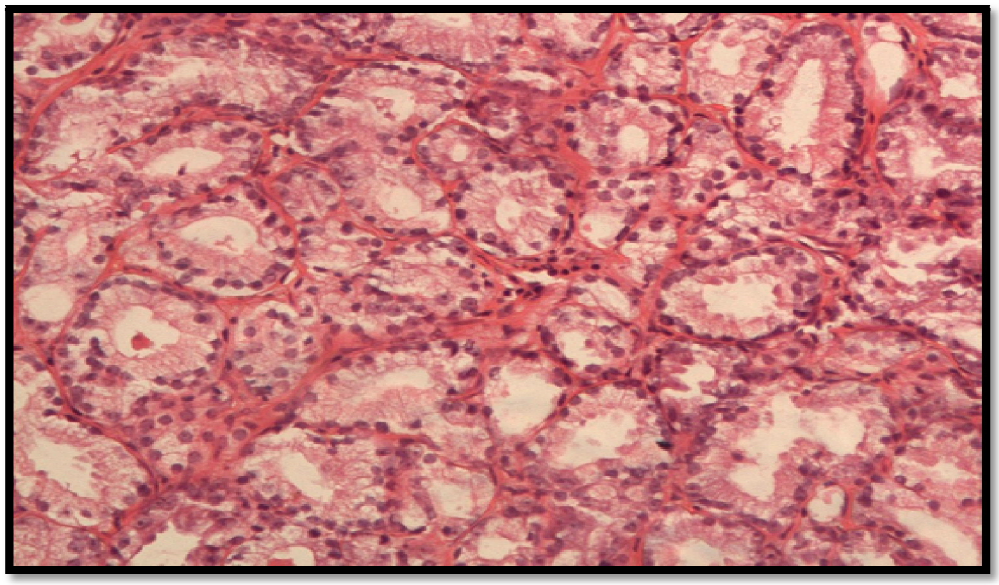

Figure 2. 19. Gleason pattern 3 adenocarcinoma, large acinar type, consisting of an irregular aggregate with angulated acini variability of size, shape, and spacing. The epithelium may separate from the adjacent stroma and create an artifactual space in some acini (H&EX20) (Department of Pathology, Faculty of Medicine, Benghazi University).

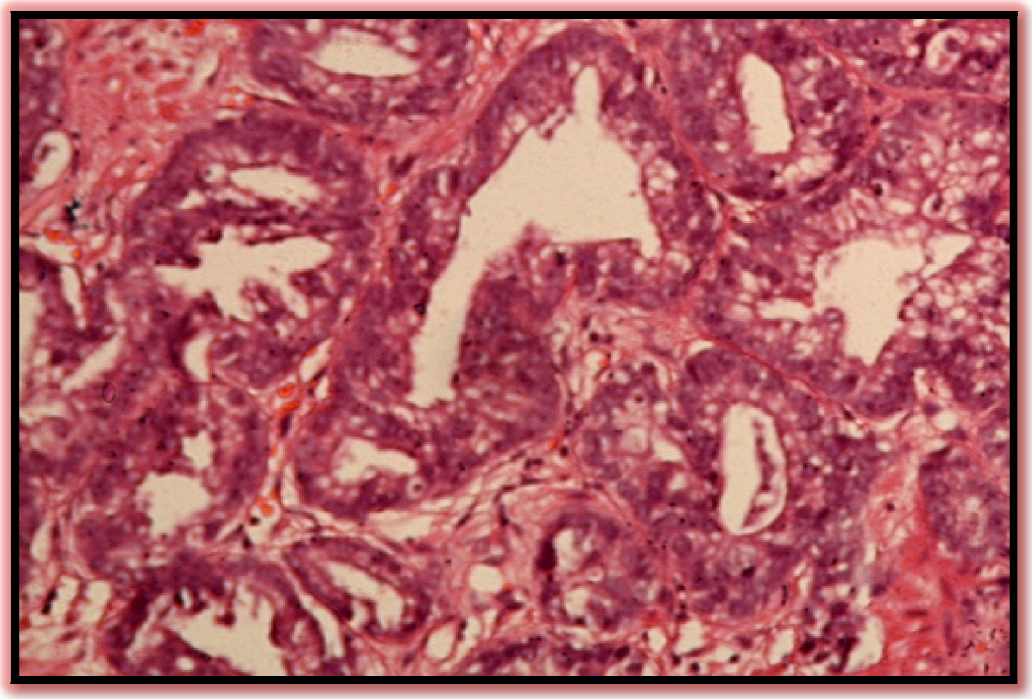

Figure 2.20. Gleason pattern 3 adenocarcinoma, large acinar type, consisting of an irregular aggregate with angulated acini (H&E X40) (Department of Pathology, Faculty of Medicine, Benghazi University).

Gleason pattern 4 characteristically shows fusion of acini, with ragged infiltrating cords and nests at the edges (Fig. 2.21, 2.22). Unlike the simple entwined acinar tubules of pattern 3, this pattern consists of an anastomosing network or spongework of epithelium. Pattern 4 adenocarcinoma is considered poorly differentiated and is more malignan than pattern 3 (Weidner et al, 2009).

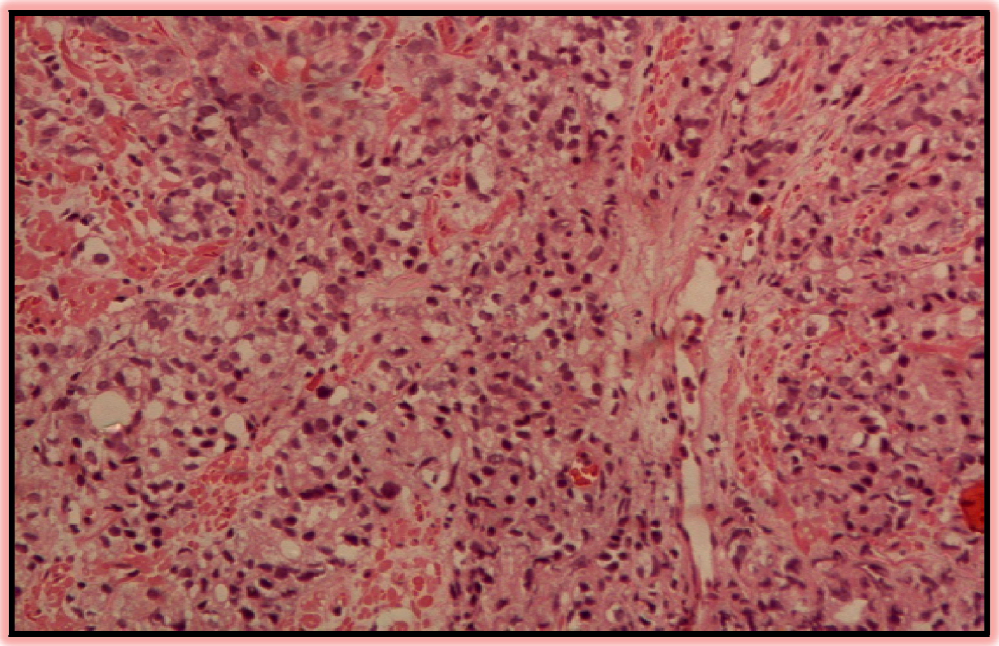

Figure 2.21. Gleason pattern 4 adenocarcinoma. Prominent fusion and close packing of acini are evident (H&EX20) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

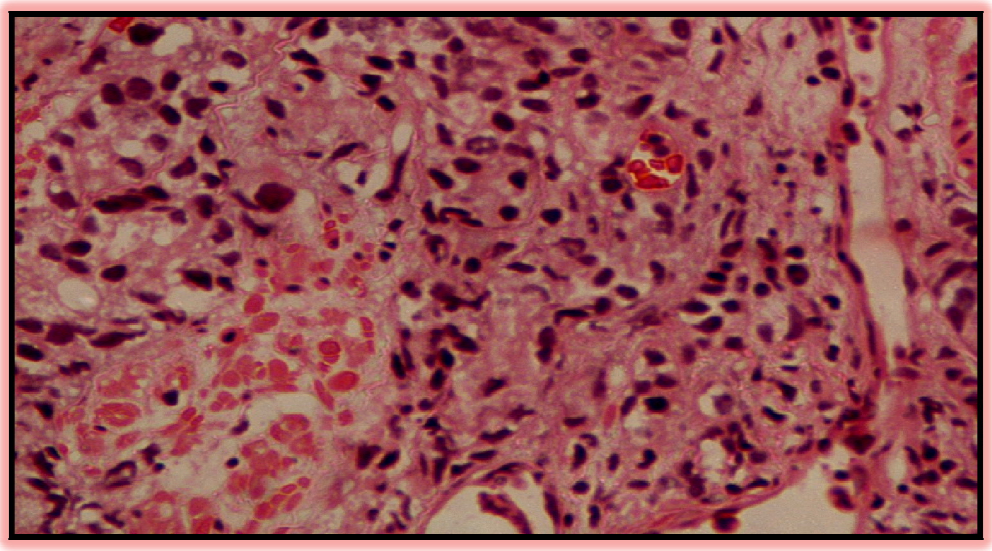

Figure 2.22. Gleason pattern 4 adenocarcinoma. The malignant acini are lined by cells with enlarged nuclei and prominent nucleoli (H&EX40) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

Gleason pattern 5 adenocarcinoma is characterized by fused sheets and masses of haphazardly arranged acini in the stroma, often displacing or overrunning adjacent tissues. In biopsy specimens, these cases raise serious concern for anaplastic carcinoma or sarcoma. Cases with scattered acinar lumens indicative of glandular differentiation are included in this pattern. Comedocarcinoma, an important subtype of this pattern, consists of luminal necrosis within an otherwise cribriform pattern. Pattern 5 also includes rare histologic variants such as signet ring cell carcinoma and small cell undifferentiated carcinoma (Figure 2.23, 2.24) (Weidner et al, 2009).

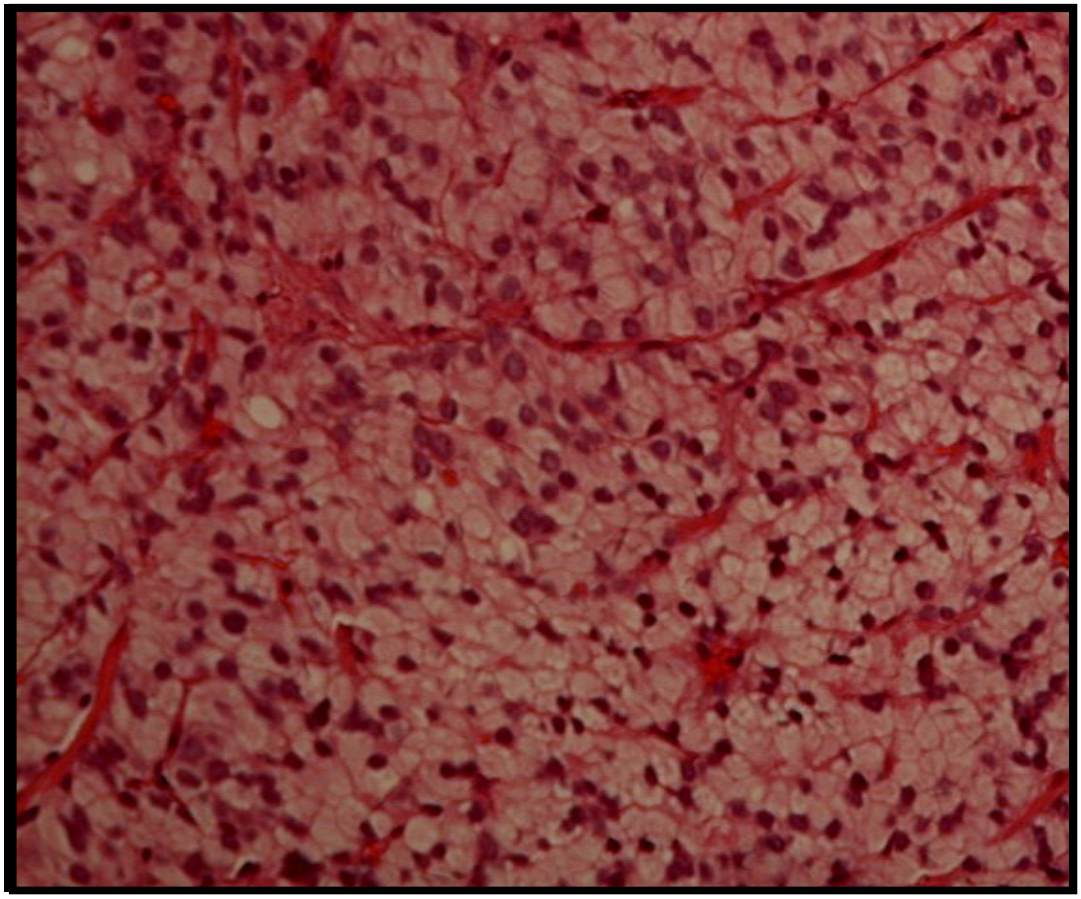

Figure 2.23. Gleason pattern 5 adenocarcinoma. Tumor cells are arranged in solid sheets with no attempts at gland formation (H&EX40) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

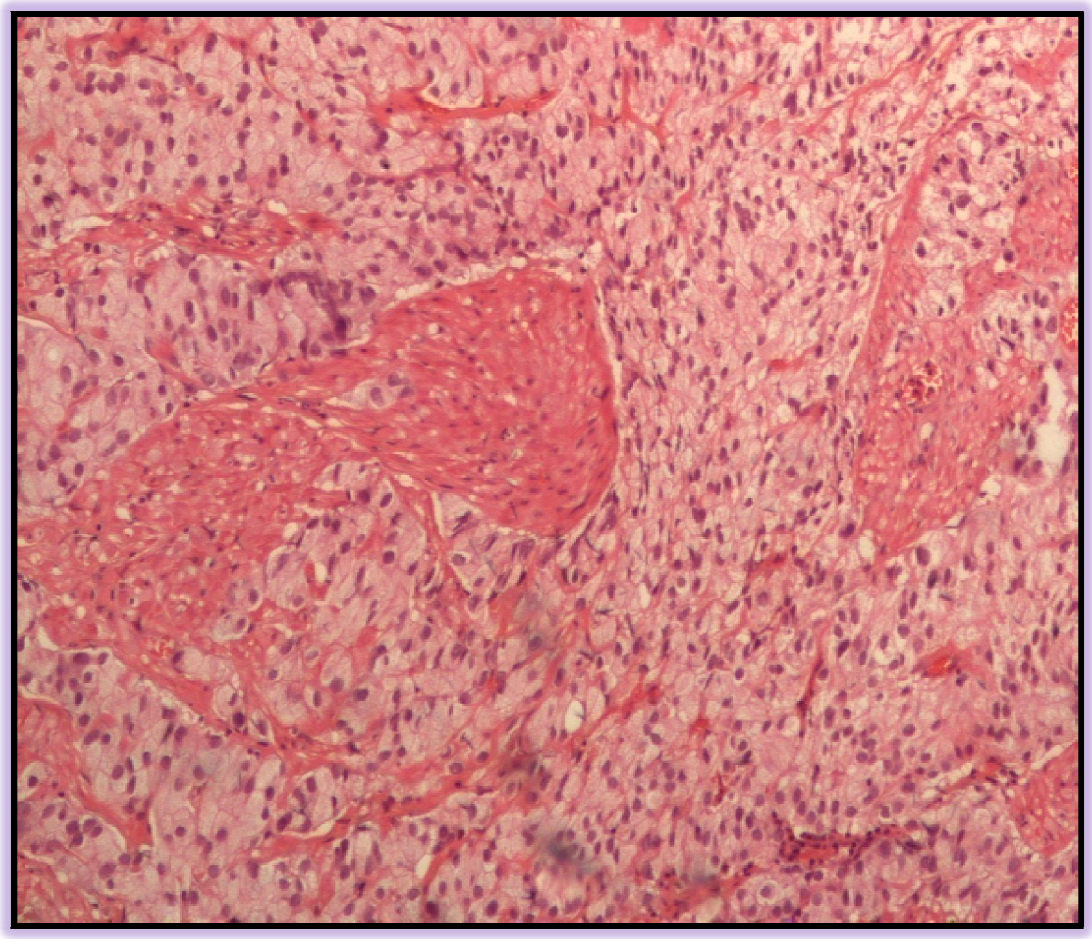

Figure 2.24. The Gleason pattern 5 tumor shows no glandular differentiation with either solid masses of cells or individually infiltrating cells (H&EX20) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

2.11. Staging of prostate cancer

Two systems are in common use for the staging of PCa. The Jewett system (stages A through D) was described in 1975 and has since been modified (Jewett, 1975). In 1997, the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer adopted a revised tumor, nodes, metastasis (TNM) system that employs the same broad T stage categories as the Jewett system but includes subcategories of T stage, such as a stage to describe patients diagnosed through PSA screening. This revised TNM system is clinically useful and more precisely stratifies newly diagnosed patients. The AJCC further revised the TNM classification system in 2002 and, most recently, in 2010 (Edge et al, 2010).

2.11.1. TNM staging

The TNM system is the international standard for prostatic adenocarcinoma staging. The Commission on Cancer of the American College of Surgeons has required it for accreditation since 1995. This staging scheme applies only to adenocarcinomas of the prostate, not to sarcomas and PCa variants (Weidner et al, 2009).

The 2010 AJCC made several changes in its staging of PCa from its 2002 version. These changes included extraprostatic extension and microscopic bladder neck invasion, both being included in the T3a category, Gleason score being recognized as the preferred grading system, and the prognostic factors of Gleason score and preoperative PSA being incorporated into stage grouping (Figure 2.25) (Table 2.6) (Cheng et al, 2012).

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Table 2.4. American Joint Committee on Cancer (AJCC) clinical TNM classification of prostatic tumours 2010 (Cheng et al, 2012)**.**

Primary tumour (T) clinical	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Clinically inapparent tumour neither palpable nor visible by imaging
T1a	Tumour incidental histological finding in < 5% of tissue resected
T1b	Tumour incidental histological finding in >5% of tissue resected
T1c	Tumour identified by needle biopsy (e.g. because of elevated PSA)
T2	Tumour confined within prostate
T2a	Tumour involves ≤ one-half of one lobe
T2b	Tumour involves >one-half of one lobe but not both lobes
T2c	Tumour involves both lobes
T3	Tumour extends through the prostate capsule
T3a	Extracapsular extension (unilateral or bilateral)
T3b	Tumour invades seminal vesicle(s)
T4	Tumour is fixed or invades adjacent structures other than seminal vesicles such as external sphincter, rectum, bladder, levator muscles, and/or pelvic wall
Regional lymph nodes (N) – clinical	
NX	Regional lymph nodes were not assessed
N0	No regional lymph node metastasis
N1	Metastases in regional lymph node(s)
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis
M1a	Non-regional lymph node(s)
M1b	Bone(s)
M1c	Other site(s) with or without bone disease

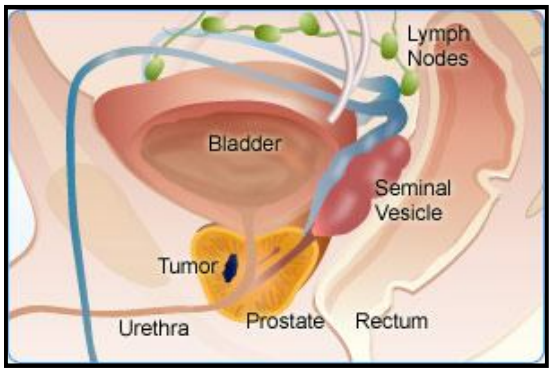
Table 2.5. American Joint Committee on Cancer (AJCC) pathological TNM classification of prostatic tumours 2010 (Cheng et al, 2012).

Primary tumour (pT) – pathological	
pT1	There is no pathological T1 classification
pT2	Organ confined
pT2a	Unilateral, one-half of one side or less
pT2b	Unilateral, involving more than one-half of side but not both sides
pT2c	Bilateral disease
pT3	Extraprostatic extension
pT3a	Extraprostatic extension or microscopic invasion of bladder neck
pT3b	Seminal vesicle invasion
pT4	Invasion of rectum, levator muscle, and/or pelvic wall
Regional lymph nodes (pN) – pathological	
pNX	Regional nodes not sampled
pN0	No positive regional nodes
pN1	Metastases in regional node(s)
Distant metastasis (pM) – pathological	
pM0	No distant metastasis
pM1	Distant metastasis
pM1a	Non-regional lymph node(s)
pM1b	Bone(s)
pM1c	Other site(s) with or without bone disease. When more than one site of metastasis is present, the most advanced category is used. pM1c is most advanced

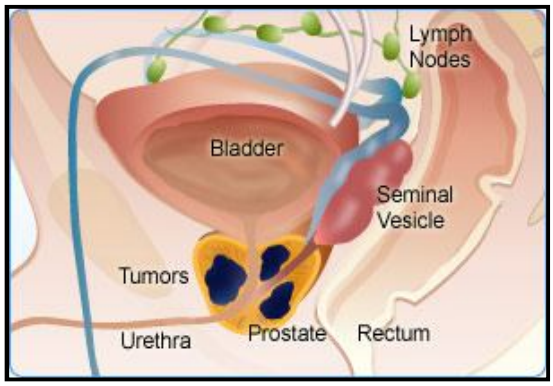
Table 2.6. American Joint Committee on Cancer (AJCC) stage grouping (2010 edition) (Cheng et al, 2012).

Stage	T	N	M	PSA(ng/ml)	Gleason score
I	T1a–c	N0	M0	<10	≤ 6
	T2a	N0	M0	<10	≤ 6
	T1–2a	N0	M0	X	X
II A	T1a–c	N0	M0	<20	7
	T1a–c	N0	M0	≥10 and <20	≤ 6
	T2a	N0	M0	<20	7
	T2b	N0	M0	<20	≤7
	T2b	N0	M0	X	X
II B	T2c	N0	M0	Any PSA	Any Gleason
	T1–2	N0	M0	≥20	Any Gleason
	T1–2	N0	M0	Any PSA	≥8
III	T3a–b	N0	M0	Any PSA	Any Gleason
VI	T4	N0	M0	Any PSA	Any Gleason
	Any T	N1	M0	Any PSA	Any Gleason
	Any T	Any N	M1	Any PSA	Any Gleason

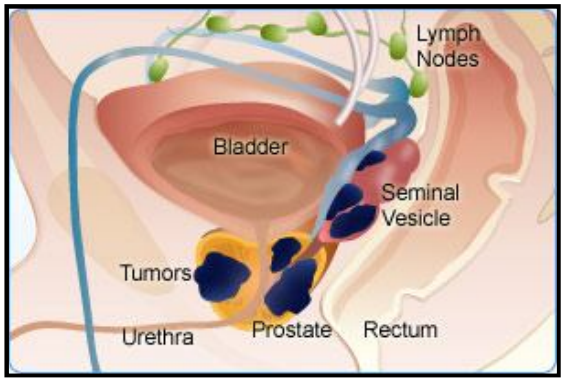
M, Metastasis; N, node; PSA, prostate-specific antigen; T, tumour; X, unknown.



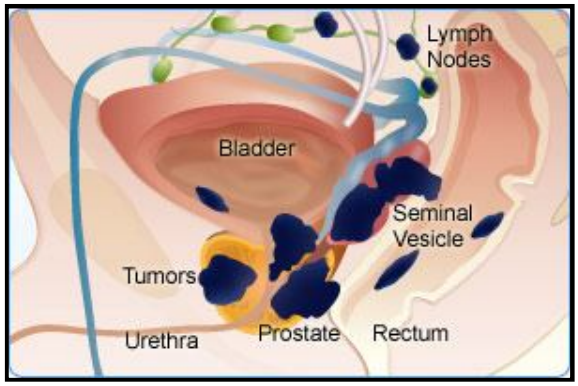
In stage I, cancer is found in the prostate only. It cannot be felt during a DRE and is not visible by imaging . It is usually found accidentally during surgery for other reasons, such as benign prostatic hyperplasia. Stage I prostate cancer may also be called stage A1 prostate cancer.



In Stage II, cancer is more advanced than in Stage I, but has not spread outside the prostate. Stage II prostate cancer may also be called Stage A2, Stage B1, or Stage B2 prostate cancer.



In Stage III, cancer has spread beyond the outer layer of the prostate to nearby tissues. Cancer may be found in the seminal vesicles. Stage III prostate cancer may also be called Stage C prostate cancer.



In Stage IV, cancer has metastasized (spread) to lymph nodes near or far from the prostate or to other parts of the body, such as the bladder, rectum, bones, liver, or lungs. Metastatic prostate cancer often spreads to the bones. Stage IV prostate cancer may also be called Stage D1 or Stage D2 prostate cance.

Figure 2.25. Stages of cancer prostate (www. cancerinformation.com).

2.11.2. Whitmore-Jewett staging system

Another popular staging system from a historical perspective is the Whitmore-Jewett staging system (Table 2.7) (Tanagho & McAninch, 2003).

Table 2.7. Whitmore-Jewett Staging System for Prostate Cancer

A1	≤3 foci of carcinoma and≤ 5% of tissue in resection for benign disease has cancer, Gleason sum < 7
A2	> 3 foci of carcinoma and > 5% of tissue in resection for benign disease has cancer, Gleason sum≥ 7
B1	Palpable nodule≤ 1.5 cm, confined to prostate
B2	Palpable nodule > 1.5 cm, confined to prostate
C1	Palpable extracapsular extension
C2	Palpable seminal vesicle involvement
D0	Clinically localized disease, with negative bone scan but elevated serum acid phosphatase
D1	Pelvic lymph node metastases
D2	Bone metastases
D3	Hormone-refractory prostate cancer

2.11.3. Transition of localized PCa to metastatic disease

Statistical data suggests that PCa is generally a slowly progressing disease. Despite our increasing understanding of the causes of PCa, the cellular and molecular mechanisms which enable localized PCa to invade and metastasize remain poorly understood. Moreover, it remains unknown how long it takes for an organ-confined primary tumor to develop into a highly invasive PCa (Semenas et al, 2012).

Indeed, it is not yet possible to diagnostically distinguish indolent localized prostate tumors, which possess little metastatic potential, from aggressive localized prostate tumors with high metastatic potential. Nevertheless, biochemical recurrence (BCR), defined by increased serum PSA levels following prostatectomy or radiation therapy for clinically localized PCa, has been shown to predict metastatic progression and PCa - specific mortality by a median of 8 years and 13 years, respectively. This suggests that it may take 8 to 13 years for a primary PCa to progress towards lethal metastatic disease (Roberts & Han, 2009).

It is now established that bone is the most common preferential site of PCa metastasis. A study by Coleman et al. have reported that in post-mortem examinations, approximately 70% of patients who have died from PCa complications show evidence of metastatic bone disease (Coleman et al, 2006) with common site for bone metastasis being in the axial skeleton (skull, vertebra, ribs and collar bone, scapula, and proximal femur) (Loberg et al, 2005).

Since bone is the most common site for PCa metastasis it is crucial to understand the underlying mechanisms that facilitate this preferential migration of circulating PCa cells to the bone. There is now compelling evidence which suggests that disseminated tumor cells (DTCs) migrate to the bone marrow using mechanisms similar to those that are commonly exploited by homing hematopoietic stem cells during bone marrow transplantation (Semenas et al, 2012).

Numerous investigations have been made to define the molecular transforming events occurring in prostatic epithelial cells and their local microenvironment that may contribute to PCa initiation and progression to locally invasive and metastatic disease stages as well as their acquisition of an androgen-independent (AI) phenotype in

humans. It has been shown that the sustained activation of epidermal growth factor receptor (EGFR), hedgehog, Wnt/β-catenin, hyaluronan (HA)/CD44, transforming growth factor (TGF)-β/TGF-βR receptors and stromal cell–derived factor-1 (SDF-1)/ CXC chemokine receptor 4 (CXCR4) frequently occurs during PCa progression to locally invasive and metastatic castration-resistant prostate cancers (CRPCs) (Figure 2.26) (Mimeault et al, 2008; Chae et al, 2011).

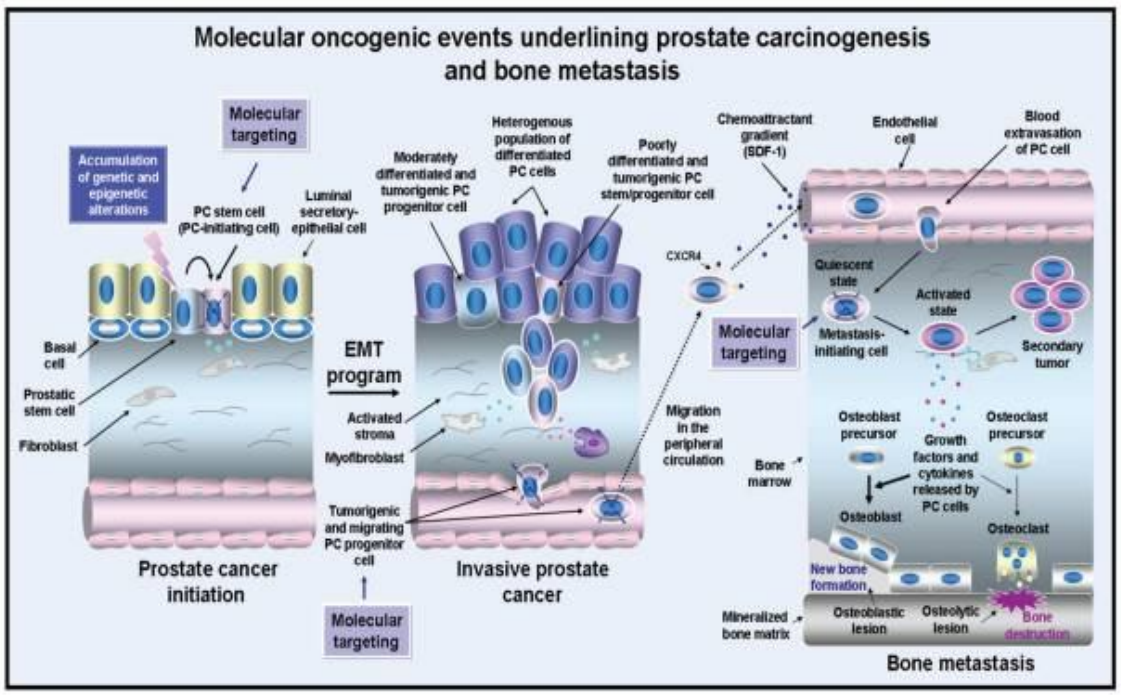


Figure 2.26. Molecular oncogenic events associated with PCa initiation and progression to a locally invasive disease stage and bone metastasis and novel targeting therapies. The scheme shows the PCa initiation through the accumulation of genetic and epigenetic alterations in prostate-resident adult stem cells resulting in their malignant transformation into tumorigenic PCa stem/progenitor cells also designated as PCa-initiating cells (Mimeault & Batra, 2011).

These tumorigenic cascades can account for the sustained growth, survival, invasion, metastases and treatment resistance of PCa cells. Moreover, the alterations leading to an enhanced expression and/or hypersensibility of AR also may occur in PCa cells (Yuan & Balk, 2009; Karlou et al, 2010). The majority of PCa patients also express diverse fusion genes resulting from the chromosomal rearrangements of the 5′-untranslated region of the androgen-regulated gene TMPRSS2 and v-ets avian erythroblastosis virus E26 transformation-specific (Ets) family genes including ERG, EVTL or ETV4 (King et al, 2009; Liu et al, 2011; Bismar et al, 2011). These fusion genes encode for

oncoproteins that can provide key roles for PCa progression and treatment resistance (Carver et al, 2009; Swanson et al, 2011). More specifically, it has been shown that the overexpression of a truncated form of transcriptional regulator ERG from the TMPRSS2-ERG fusion gene, which occurs in up to approximately 40% of PCa but is not detected in the normal prostate, may contribute to PCa development (Perner et al, 2007; Yoshimoto et al, 2008).

The truncated ERG oncoprotein can cooperate with the PTEN (phosphatase tensin homolog deleted on chromosome 10) downregulation- induced phosphatidylinositol 3-kinase (PI3K)/Akt activation and induce the PCa cell invasion and angiogenesis-like wild-type oncogenic ERG transcription factor (Carver et al, 2009). In addition, the changes in the tumor reactive stroma, including the release of different growth factors by activated myofibroblasts, typically take place during PCa progression under normoxic and hypoxic conditions and may promote the malignant transformation of PCa cells and neoangiogenesis (Giannoni et al, 2010; Giannoni et al, 2011).

2.12. Screening for prostate cancer

The rationale for screening is that early detection and treatment of asymptomatic cancers could extend life, as compared with treatment at the time of clinical diagnosis. Effective cancer screening requires an accurate, reliable, and easy-to-administer test that detects clinically important cancers at a preclinical stage and the availability of effective treatment that results in better outcomes when administered early, rather than after signs or symptoms of disease have developed (Richard, 2011).

For many years, the DRE was the primary screening test for prostate cancer. However, this test has considerable interexaminer variability (Smith & Catalona, 1995), and the majority of cancers detected by means of DRE are at an advanced stage (Richard, 2011). In the late 1980s, PSA testing, which was initially developed for prostate-cancer surveillance, was rapidly and widely adopted for screening; by 2001, a population-based survey in the United States showed that 75% of men 50 years of age or older had undergone PSA testing (Sirovich et al, 2003). The widespread use of PSA testing was based on its increased detection of early-stage cancer, as compared with DRE; there was no evidence that testing reduced the risk of death from PCa (Wolf et al, 2010).

2.12.1. Prostate-specific antigen (PSA)

Initially, PSA values above 4.0 ng per milliliter were considered abnormal, though lower cutoff levels have subsequently been proposed. Most abnormal PSA values are false positive results that can be caused by BPH, prostatitis or cystitis, ejaculation, perineal trauma, or the recent use of instruments for testing or surgery in the urinary tract. Moreover, a normal PSA value does not rule out PCa; in the control group in the PCa Prevention Trial, PCa was detected in 15% of men with normal results on DRE and PSA values of 4.0 ng per milliliter or less (and in 9% of men with normal results on DRE and PSA values $\leq$1.0 ng per milliliter) who underwent a prostate biopsy at the end of the study (Thompson et al, 2004).

Numerous approaches have been proposed to improve the diagnostic accuracy of the PSA test, including measuring PSA velocity (change over time), levels of free and protein-bound PSA, PSA density (the PSA level divided by the prostate volume), and

the use of cutoff values for PSA levels that are specific to the patient's age and race or ethnic group (Greene et al, 2009).

In the United States, approximately 90% of PCa are detected by means of screening (Hoffman et al, 2005). After the introduction of PSA testing, the lifetime risk of receiving a diagnosis of PCa nearly doubled, increasing from approximately 9% in 19855 to 16% in 2007 (Altekruse et al, 2010).

Two big randomized prospective studies are ongoing in the field of PCa screening, the European Randomized Study for Screening for PCa (ERSPC) and Prostate, Lung, Colorectal, and Ovarian Cancer Screening trial (PLCO), but for the results we will have to wait till the end of this decade (Eckersberger et al, 2009). The ERSPC included seven European countries with a total of 162,387 participants. With PSA cut-off at 3 to 4ng per ml and follow-up of nine years, the screening group was shown to reduce PCa mortality by 20% in the age group of 55 to 69 years (Schröder et al, 2009). The PLCO trial included ten US study centres with a total of 76,693 participants. With PSA cut-off at 4ng per ml and follow-up of ten years, there was no difference in PCa mortality between the screening and control groups, at the age group of 55 to 74 years. However, the control group was found to be contaminated with prior PSA screening in up to 50% of participants (Andriole et al, 2009).

Whereas early American Urological Association and American Cancer Society guidelines strongly supported routine, annual prostate-cancer screening, subsequent guidelines have taken into account the uncertainties regarding the outcomes of screening. Current American Urological Association and American Cancer Society guidelines, updated after the publication of the results of the ERSPC and PLCO trials, are summarized in (Table 2.8) (Richard, 2011).

Table 2. 8. Prostate-Cancer Screening Guidelines (Richard , 2011).

Recommendation	American Urological Association	American Cancer Society
Shared decision making between patient and clinician	Yes	Yes (consider use of decision aid)
Age to begin offering screening—— yr		
Average-risk patients	40	40
High-risk patients (black patients and those with first-degree relative with prostate cancer)	40	40-45
Discontinuation of screening	Life expectancy <10 yr	Life expectancy <10 yr
Screening tests	PSA, digital rectal examination	PSA, optional digital rectal examination
Frequency of screening	Annual (possibly less often for men in their 40s)	Annual (every other year when PSA <2.5 ng/ml)
Criteria for biopsy referral	Age, family history, race or ethnic group, findings on digital rectal examination, total PSA, free PSA, PSA velocity, PSA density, previous biopsy findings, coexisting conditions	PSA ≥4.0 ng/ml, abnormal digital rectal examination; individualized risk assessment if PSA is 2.5–4.0 ng/ml

2.13. Prognostic and predictive factors in prostate cancer

Prediction of prognosis is one of greatest challenges in tumor pathology (Burke et al, 2005). Despite attempts to introduce new biomarkers, histopathological grade remains the most important tissue-based predictor of prognosis for many cancer types in general and for PCa in particular. New markers with correlation to prognosis are described almost every month.

Prognostic factors, which predict relapse or progression independent of future treatment effects, can be stratified according to College of American Pathologists (CAP) into three different categories (Table 2.9) (Bostwick et al, 2000).

Table 2.9. Classification of prognostic factors for prostate cancer: recommendations from 1999 consensus conferences (Weinder et al, 2009).

Category 1: Factors that have been proven to be prognostic or predictive based on evidence from multiple published trials and are recommended for routine reporting
TNM stage Histological grade (Gleason score and WHO nuclear grade) Surgical margin status Perioperative PSA Pathological effects of treatment Location of cancer within prostate
Category 2: Factors that show promise as predictive factors based on evidence from multiple published studies but that require further evaluation before recommendation or are recommended despite incomplete data as diagnostic or prognostic markers
DNA ploidy Histological type Cancer volume in needle biopsy specimens Cancer volume in radical prostatectomy specimens
Category 3: Factors that have some scientific evidence to support their adoption as diagnostic or prognostic agents but are not currently recommended; also factors of uncertain significance
Prostate-specific membrane antigen Other serum tests (e.g., PSM, hK2, IGF) Perineural invasion Vascular or lymphatic invasion Microvessel density (shows promise, but insufficient data) Stromal factors, including TGF-beta, integrins Proliferation markers and apoptosis Nuclear morphometry and karyometric analysis Androgen receptors Neuroendocrine markers Genetic markers (show promise, but insufficient data) All other factors that do not appear in categories 1 or 2

2.13.1. Clinico-pathological prognostic factors

2.13.1.1. Age of patient

The role of the age of the patient per se as a significant prognostic factor in PCa is controversial (Austin & Convery, 1993; Gronberg et al, 1994). 567 patients completing external beam radiotherapy were examined by Herold et al. In addition to other factors, age of the patients greater than 65 years was a significant predictor of distant metastases at 5 years. They concluded that men over the age of 65 years were more likely to experience distant failure after radical radiation therapy than were younger men (Herold et al, 1998). Obek et al. also suggested that young age per se might be an independent favourable prognostic factor for disease recurrence after surgical radical prostatectomy (Obek et al, 1999). Also Freedland et al. found that young men had more favourable outcomes after surgical radical prostatectomy (RP) than older men, which made younger men suitable subjects in screening (Freedland et al, 2004).

2.13.1.2. Gleason grade

Gleason score is the strongest clinical predictor of PCa progression. Men diagnosed with Gleason grade 7 or higher tumors are at increased risk of extraprostatic extension, increased risk of recurrence after initial therapy, and more likely to die of their disease. In contrast, men diagnosed with well-differentiated Gleason 6 disease are at very low risk of cancer-specific death. In a multi-institutional radical prostatectomy cohort, the 15-year prostate cancer-specific mortality rates varied by the age of the patients at diagnosis ranging from 0.2 – 1 percent for pathological Gleason 6 or less, 4 – 6 percent for Gleason 3+4 tumors, 6 – 11 percent for Gleason 4+3 tumors, and 26 – 37 percent for Gleason 8 or higher cancers (Eggener et al, 2011).

The distribution of Gleason grades has shifted over time, and in the era of PSA screening, most men are now diagnosed with Gleason 6 or 7 tumors. As such, the accurate discrimination of prognosis among men with prostate cancer within this narrow range of Gleason scores is challenging. Some (Stark et al, 2009), but not all (Andrén et al, 2006), studies suggest that there is prognostic information among Gleason 7 tumors on whether the predominant pattern is Gleason 4 or 3. For example, in a population-based radical prostatectomy series, men with pathological Gleason 4+3 tumors had a 3-

fold greater risk of prostate cancer-specific mortality compared to men with Gleason 3+4 tumors (Stark et al, 2009). Still, prognostication among Gleason 7 PCa is far from accurate.

2.13.1.3. Clinical stage

This is a very important prognostic determinant, and it has become even more so with the incorporation of newer technology (Rosai & Ackerman's, 2011).

2.13.1.4. Pathologic stage

This represent the ultimate indicator of tumor extent and, as such, the most accurate predictor of prognosis currently available. Naturally, there is also a relationship between prognosis and the status of the individual factors that determine the stage, such as the prostate capsule, the seminal vesicles, and the lymph nodes. Thus, there is a strong association between the level of tumor invasion into or through the prostate capsule and the grade, volume, and rate of recurrence of the tumor. There is also an association between the radial distance of extraprostatic extension and PSA recurrence. Conversely, microscopic bladder neck involvement is not a significant prognostic factor. In cases with nodal metastases, the prognosis is worse when they are multiple rather than solitary, when they are detectable grossly rather than only microscopically, when their overall volume is large, and when they are accompanied by extracapsular extension. Their prognostic significance seems to be the same regardless of whether they are found in the usual pelvic location or around the prostate/seminal vesicles (Rosai & Ackerman's, 2011).

2.13.1.5. Tumor volume

Although tumour volume is an important factor in predicting prognosis in carcinoma of the prostate, direct and accurate estimation of tumour volume is not practical clinically. This is because the tumour may not always be palpable, and when palpable the volume cannot be evaluated in 3 dimensions. Transrectal ultrasound (TRUS) used as a tool for estimating the tumour volume either directly or as a guide for core biopsies has only limited ability to estimate PCa volume (Buhmeida et al, 2006).

Tumor volume is a significant predictor of pathologic stage, lymph node and distance metastesis, and overall disease outcome (McNeal et al, 1990; McNeal, 1992; Stamey et al, 1993). As described by McNeal et al. loss of differentiation and metastatic potential were strongly correlated with tumor volume (McNeal et al, 1986). However, it is controversial whether tumor volume is an independent prognostic factor. Epstein et al. show that Gleason grade, surgical margin, and tumor volume-independently from each other were strongly correlated with progression in univariate regression analysis. However, in multiple regression analysis, tumor volume did not provide independent prognostic information beyond that provided by GS and margin status (Epstein et al, 1993). Similarly, in a study on 1302 cases, Kikuchi et al. did not find tumor volume to be an independent prognostic factor (Kikuchi et al, 2004). The clinical importance of tumor volume has probably decrease in recent years because of stage migration. A high proportion of cancers are nowadays small when diagnosed and their volume is then less likely to discriminate between prognostic categories.

2.13.1.6. Perineural invasion

Perineural invasion is common in adenocarcinoma, present in up to 38% of biopsies, and may be the only evidence of malignancy in a needle core biopsy specimen. Only half of patients with intraprostatic perineural invasion evident in a biopsy specimen have EPE. In univariate analysis, perineural invasion was predictive of EPE, seminal vesicle invasion, and pathologic stage in patients treated by radical prostatectomy (Egan & Bostwick, 1997).

The prognostic significance of perineural invasion remain controversial. A recent study found independent significance only when the percentage of tumor on the needle biopsy cores was not considered (Rubin et al, 2000). In several studies perineural invasion did not predict tumor progression (Van den Ouden et al, 1997; Maru et al, 2001; Ito et al, 2003).

2.13.1.7. Vascular or Lymphatic invasion

Permeation of vascular channels as detected in whole – mount specimens of radical prostatectomy has been found to correlate with Gleason score, EPE, seminal vesicle involvement, and likelihood of tumor progression (Herman et al, 2000). Furthermore, peritumoral lymph vessel invasion is associated with an increased likelihood of regional lymph node metastases (Roma et al, 2006).

2.13.1.8. Location of cancer

The site of origin of cancer appears to be a significant prognostic factor. When cancer arises in the transition zone, it is apparently less aggressive than typical acinar adenocarcinoma arising in the peripheral zone. These adenocarcinomas are better differentiated than those in the peripheral zone, thus accounting for Gleason primary grade 1 and 2 tumors. The volume of low-grade tumors tends to be smaller than the volume of tumors arising in the peripheral zone, although frequent exceptions are seen. The confinement of transition zone adenocarcinoma to its anatomic site of origin may account in part for the favorable prognosis of clinical stage T1 tumors. Therefore, the prognosis of a patient with PCa depends more on the features of cancer in the peripheral zone than in the transition zone (Ohori et al, 2004).

The transition zone boundary may act as a relative barrier to tumor extension because malignant acini appear frequently to fan out along this boundary before invasion into the peripheral and central zones. The WHO recommends that prostate biopsy specimens be submitted separately, the anatomic site of each prostate biopsy be labeled at the discretion of the urologist, and that pathologists report each specimen separately (Bostwick & Foster, 1999). Thus, the anatomic site or sites of carcinoma within each prostate biopsy can be included in the pathology report and identified in the anatomic area specified by the urologist. The anatomic location or locations of carcinoma within total prostatectomy specimens should also be specified in the pathology report whenever possible.

2.13.1.9. Extraprostatic extension

The term extraprostatic extension was accepted at an International Consensus Conference to replace other terms, including capsular invasion, capsular penetration, and capsular perforation. Extension of cancer beyond the edge or capsule of the prostate is diagnostic of EPE. The three criteria for EPE, depending on the site and composition of the extraprostatic tissue, are (1) cancer in adipose tissue, (2) cancer in perineural spaces of the neurovascular bundles, and (3) cancer in anterior muscle (Weidner et al, 2009).

In patients treated by radical prostatectomy for clinically localized cancer, the frequency of EPE (stage pT3 cancer) is 23% (Theiss et al, 1995), 24% (Cheng et al, 1999), 41% (Bostwick, 1994), 43% (Ohori et al, 1995), or 52% (Zietman et al, 1994). A strong association of tumor volume with EPE and seminal vesicle invasion has been reported (Bostwick, 1999). Patients with EPE have a worse prognosis than those with organ-confine cancer (Epstein et al, 1996; Cheng et al, 1999). Cancer-specific survival 10 years after radical prostatectomy in patients with pT3 cancer is 54% (Schellhammer, 1988), 62% (Stein et al, 1992), 73% (Ward et al, 2005). Cancer-specific survival 10 years after definitive radiation therapy in patients with clinical stage T3 is 44% (Scardino , 1989), or 59% (Scardino et al, 1986).

2.13.1.10. Seminal vesicle invasion

In most recent studies, seminal vesicle invasion (SVI) is a poor prognostic parameter, with biochemical progression-free rates ranging from 5–60% (Buhmeida et al, 2006). The differences may be related to the definition of the seminal vesicle invasion. Some authors consider an intraprostatic portion of the seminal vesicle as true seminal vesicle, and as such consider its involvement by cancer as seminal vesicle invasion. Others call any seminal vesicle as extracapsular extension (Epstein et al, 1993). Some studies do not make any distinction between the seminal vesicles and the ejaculatory duct complex.

Seminal vesicle invasion is associated with high PSA failure rates (PSA levels not changed to normal) after radical prostatectomy, and subsequent distant metastases

(Bloom et al, 2004). Debras et al. evaluated the prognostic significance of SVI in radical prostatectomy specimens according to proximal or distal site of invasion. They concluded that the prognostic significance of SVI is not constant and depends on the site of invasion, in which patients with invasion extending to the free part of the seminal vesicles have poorer prognosis than those patients with invasion only limited to the proximal part of the seminal vesicles (Debras et al, 1998). 115 cases of established capsular penetration, 16 of periseminal vesicle invasion, and 45 of seminal vesicle invasion in-patients without lymph node metastases were evaluated by Epstein et al. They concluded that patients with SVI had a significantly worse prognosis than those with capsular penetration, and peri-seminal vesicle invasion was associated with an intermediate risk of progression (Epstein et al, 1993). The results of Freedland et al. revealed that patients with SVI had significantly higher PSA values, higher clinical stage, higher grade tumours, and were more likely to have concomitant extracapsular extension or a positive surgical margin. The study also identified a subset of men with low-grade disease, negative surgical margins, and older age, who – despite SVI – had an extremely favorable clinical course. The study concluded that SVI does not consistently suggest an unfavorable prognosis (Freedland et al, 2004).

2.13.1.11. Clinical risk groups and nomograms

Beyond individual factors, the combination of clinical and pathological factors represents a more powerful tool to aid in PCa prognostication. When combined together, the predictive power of the clinical and pathological features has consistently been shown to be greater than any single factor. There are multiple published studies that have developed tools in this regard, including the development of simple risk categories, risk calculators as well as clinical nomograms. The predictive utility of these combined clinical sets to risk stratify prostate cancer patients have been evaluated primarily in cohorts of patients following curative therapy, either radiation or prostatectomy. Moreover, most have relied on surrogate disease endpoints of PSA recurrence or biochemical failure. We present below examples of each type of categorization of features that are used clinically in a variety of settings (Martin et al, 2011).

Risk categories

Risk categories provide clinicians and patients a qualitative assessment of the likelihood of PCa progression after initial therapy. One example of risk categorization is the D'Amico Risk Classification that divides men into low risk, intermediate risk and high-risk categories of progression after radical prostatectomy, based on clinical stage, biopsy Gleason grade, and preoperative levels of PSA (Table 2.10). The risk grouping of an individual patient by the D'Amico classification system is determined by his most clinically advanced clinical feature, rather than a summary consideration of all three features. This risk classification system has been demonstrated in independent patient populations to provide accurate prediction of recurrence after radical prostatectomy (Boorjian et al, 2008).

Table 2.10. Prognostic risk groupings for localized/locally advanced prostate cancer categories (Sridharan & Warde, 2012).

Risk group	PSA ng/ml	Gleason score	UICC **T category**
Low (all of)	**≤10**	**≤6**	**≤T2a**
Intermediate (any of, if not low risk	**≤20**	**7**	**T1/T2**
High (any of)	**>20**	**≥8**	**≥T3**

Nomograms

Nomograms are chart-based tools using a scoring system of clinical characteristics to estimate individualized risk of recurrence and progression. The Kattan nomogram is one of the most widely used preoperative nomograms for the prediction of biochemical recurrence after radical prostatectomy. This nomogram uses information on clinical stage, Gleason grade on biopsy and pretreatment PSA levels to provide predicted probability of biochemical recurrence 5 years after radical prostatectomy. The nomogram was developed in a patient cohort with primarily clinically localized, low-risk disease. A recent study further tested the accuracy of the Kattan nomogram across high and low risk strata defined by the D'Amico risk classification (Korets et al, 2011).

In that study, the authors Korets et al. were able to confirm the nomograms predictive ability to estimate risk of recurrence for patients with high and low-risk prostate cancer. PSA recurrence is a good, but not perfect, predictor of development of distant metastases or cancer-specific mortality (Korets et al, 2011). Walz et al. examined the endpoint of recurrence within two years after surgery, given that early recurrence may better reflect the likelihood of micrometastatic disease. This nomogram reported that men with evidence of extraprostatic cancer were much more likely to experience an early recurrence, with a relative risk of early recurrence among men with tumors that had penetrated the capsule of 1.8, and for men with seminal vesicle involvement a relative risk of 3 (Walz et al, 2011).

2.13.2. Biological prognostic factors

2.13.2.1. Prostate specific antigen

Both the level of PSA and the velocity of rise prior to diagnosis have been explored as potential risk factors for poor outcome following definitive treatment (Martin et al, 2011). Level of PSA at diagnosis is a component of the standard risk stratification factors and remains a component of most clinical nomograms (Kattan et al, 1999; Walz et al, 2009). With the majority of men diagnosed today through PSA screening, the typical PSA level at diagnosis has decreased, reducing the sensitivity of this measure (Shao et al, 2009). A rapidly rising PSA prior to diagnosis has been identified as a strong predictor for poor outcome following surgery (D'Amico et al, 2004) or radiation (D'Amico et al, 2005), though other large studies have not found this relationship among men diagnosed during the PSA-era (Stephenson et al, 2009).

2.13.2.2. Microvessel density

Microvessel density (MVD) analysis offers promise for predicting pathologic stage and patient outcome in prostate cancer. Most of the prostatectomy studies found a positive correlation of MVD with pathologic stage (Wakui et al, 1992; Deering et al, 1995; Rogatsch et al, 1997). However, in one study, the important differences in MVD between stage pT2 and stage pT3 were observed only among low-grade cancers, whereas the reverse was true in another study. MVD in cancer detected in biopsy specimens showed a positive correlation with matched prostatectomies and was an independent predictor of EPE (Bostwick et al, 1996; Rogatsch et al, 1997). The bulk of evidence favors the relationship of MVD and cancer stage, although variance exists between methods and patient cohorts.

Generally good agreement exists about prediction of cancer recurrence based on MVD (Hall et al, 1994; Vesalainen et al, 1994; McNeal &Yemoto, 1996; Gettman et al, 1998). In studies in which patients were treated by surgery or external beam radiation therapy, MVD (Weidner et al, 1993; Fregene et al, 1993; Hall et al, 1994) and microvascular invasion (McNeal & Yemoto, 1996) predicted biochemical (PSA) failure. MVD did not correlate with biochemical failure after controlling for stage (pT2 or pT3)

and grade (Gleason grade 6 and higher) in patients treated by radical prostatectomy (Gettman et al, 1998).

2.13.2.3. Oncogens and Tumour suppressor genes

2.13.2.3.1. p53

The p53 protein, a tumor suppressor, functions in the transcription of growth inhibiting genes involved in apoptosis, cell cycle arrest and DNA repair (Gupta et al, 2012). The tumor suppressive function of p53 is mainly attributed to its role in one of two mechanisms: either promoting the repair and survival of damaged cells, or promoting the permanent removal of irreparably damaged cells through apoptosis (Brady & Attardi, 2010). p53 causes cell cycle arrest primarily by activating the transcription of a cyclin-dependent kinase inhibitor, p21/waf1, and induces apoptosis via transcriptional activation of the pro-apoptotic bcl2 family genes, Bax, PUMA and Noxa. An alternative and complementary signaling pathway that leads to programmed cell death includes the extrinsic death receptor pathway. The extrinsic pathway is initiated upon receptor ligation of FAS/CD95 ligand mediated by an adapter molecule FAS-associated death domain (FADD) that bridges the receptor with the downstream effector, caspase 8, resulting in the assembly of the death-inducing signaling complex (Gupta et al, 2012). The extrinsic and intrinsic apoptosis pathways are connected by the caspase-8-mediated cleavage of the proapoptotic bcl-2 family member Bid. Truncated Bid (tBid) translocates to mitochondria, where it induces the release of cytochrome C, followed by induction of apoptosis (Li et al, 1998).

More than 50% of human cancers, includingPCa, exhibit loss of normal p53 functions and/or defects in the p53 signaling pathway as well as missense mutations or deletions; these molecular alterations are associated with resistance to cell death (Keshelava et al, 2000; Ecke et al, 2007).

Mutant p53 expression is a late event in localized PCa (Hall et al, 1995; Mottaz et al, 1997), usually present in higher-grade cancer (Fan et al, 1994) and elevated in untreated metastatic cancer (Heidenberg et al, 1995; Moul et al, 1996), hormone refractory cancer (Hall et al, 1995; Heidenberg et al, 1995), and recurrent cancer (Moul et al,

1996). Inactivation of p53 is associated with late progression of prostate cancer and may be a marker of survival in stage T2-3N1-3M0 (Qian et al, 2002).

Protein expression of p53, Ki-67, and bcl2 were evaluated in archival paraffin-embedded radical prostatectomy specimens from 162 patients of clinically localized cancer by Moul et al. to determine the clinical use of p53, Ki-67, and bcl2 immunohistochemical protein expression in the primary tumour as combined predictors of disease progression. The study concluded that p53, Ki-67, and bcl2 have potential as biomarkers to predict recurrence in patients with clinically localized PCa after radical prostatectomy. All three markers were clearly correlated with recurrence estimates at 6 years (Moul et al, 1996). The same conclusion was obtained by (Bauer et al, 1996).

Grignon et al. studied 471 patients to assess the prognostic value of identifying abnormal p53 protein expression in tumours of patients with locally advanced PCa who were treated with either external beam radiation therapy alone, or total androgen blockade before and during the radiation therapy. Statistically significant associations were found between the presence of abnormal p53 protein expression and increased incidence of distant metastases, decreased progression-free survival, and decreased overall survival. Among patients receiving both radiation therapy and hormone therapy, those with tumours exhibiting abnormal p53 protein expression experienced a reduced time to the development of distant metastases (Grignon et al, 1996).

2.13.2.3.2. Her2/neu (ERBB2)

The Her2/neu protein is a notorious proto-oncogene that has been implicated in a number of different cancers, particularly in breast cancer and the target of a number of current and experimental therapies (Baxevanis et al, 2010). Her2/neu is a transmembrane tyrosine kinase that is important in assisting differentiation and cell growth. Despite its major role in the diagnosis and treatment of breast cancer, Her2/neu plays an important role in the understanding of prostate adenocarcinoma oncogenesis (Dasgupta et al, 2012).

Although Her2/neu is not necessarily correlated with a Gleason's score (Mofid et al, 2007), patients suffering from metastatic PCa were more likely to have higher levels of

serum Her2/neu versus those with nonmetatstatic or localized disease (Osman et al, 2005) suggesting that Her2/neu may be an important marker for advanced disease (Okegawa et al, 2006) or clinically worse outcomes (Neto et al, 2010).

Similar to the other major oncogenes discussed so far, Her2/neu is capable of activating the androgen receptor in the androgen independent stage. Her2/neu can promote survival of LNCaP cells through the Akt pathway, even in the absence of androgens. Interestingly, this effect can be halted by the addition of Dn-Akt, an inhibitor of Akt. Additionally, Her2/neu can provide androgen independent activation of the AR via a pathway modulated by both MAPK and c-Jun, which is also important in stabilizing the AR (Mukherjee et al, 2011). This interaction between Her2/neu and the AR is regulated by an miRNA, miR-331-3p, the addition of which can inhibit both the downstream activation of PI3K/Akt signaling, in addition to reducing the AR-regulated PSA expression (Epis et al, 2009). Additionally, Her2/neu can, via PYK2, help facilitate the cell adhesion that allows for the tumor's metastatic potential (Yuan et al, 2007). Her2/neu's relationship with the AR, however, is not universally accepted as LNCaP cells have decreased AR mRNA in addition to decreased AR and AR regulated PSA (Cai et al, 2009).

Her2/neu may also play an important role in the metastasis of PCa into the bone. In patients with bone metastases, Her2/neu over expression is associated with a poorer prognosis (Nishio et al, 2006).

C-MYC

one of the most commonly studied oncogene in PCa pathogenesis is MYC, a regulator gene that codes for transcription factor. MYC is thought to regulate 15% of all genes in humans and is located in the human genome on chromosome 8q24 amplicon that is frequently amplified in PCa patients (Dasgupta et al, 2012).

The C-MYC protein is a nuclear transcription factor that regulates a number of cellular processes including cell cycle progression, metabolism, ribosome biogenesis, protein synthesis and mitochondrial function (Gurel et al, 2011). C-MYC is over-expressed in a large variety of tumor types, often associated with somatic genetic alterations such as

translocations and gene amplification (Nesbit et al, 1999). FISH analysis identified MYC overexpression in ~9% of primary prostate tumors but ~75% in advanced PCa patients. In a separate study, using comparative genomic hybridization investigators detected gain of the 8q region in 72.5% of cases whereas only 29% of them had genomic amplification as identified by FISH (Dasgupta et al, 2012). MYC overexpression has also been correlated with FOXP3 downregulation, and deletion of FOXP3 in human primary prostate cells resulted in concomitant increased MYC mRNA and protein level. At molecular level, FOXP3 binds to the promoter region of MYC and repress its transcription, and hence loss of FOXP3 increased MYC expression in PCa patients (Wang et al, 2009).

In PCa, there is evidence that C-MYC is involved in disease progression since a region encompassing the MYC locus (8q24) is somatically amplified at low levels in a subset of patients (Sato et al, 1999; Nesbit et al, 1999; Jenkins et al, 1997), and the presence of amplification in this region correlates with both high histological grade and worse prognosis (Ribeiro et al, 2006; Ribeiro et al, 2007). Whether there is amplification of MYC in high grade PIN is controversial since MYC amplification has been reported in up to 50% of HGPIN lesions (Qian et al, 1997), but more recent experiments revealed a lack of MYC amplification in such lesions (Bethel et al, 2006).

2.13.2.4. Oncogenic transcriptional coactivators

2.13.2.4.1. Androgen receptors (AR)

The dependence of PCa cells on androgen stimulation was first described in a seminal article by Huggins and Hodges (1972). Androgen binds to the AR and translocates to the nucleus, where the binding of this complex to androgen responsive elements affects the transcription of androgen-regulated genes (e.g., PSA) and ultimately stimulates proliferation and inhibits apoptosis of PCa cells. Therefore, androgen- deprivation therapy by chemical and surgical castration has been the mainstay of the treatment for early metastatic PCa. However, all patients invariably will progress at some point during the course of their disease as their tumor adapts to the androgen-deprived environment and becomes ‘‘castrate- resistant’’ (Jin et al, 2011). The AR gene is localized on chromosome X and it contains a series of CAG trinucleotide repeats. The

length of CAG repeats varies among individuals and this polymorphism is believed to be related to the transcriptional activity of AR. Fewer CAG repeats are associated with increased risk of developing tumour as well as more aggressive forms of PCa and breast cancer of women (Buhmeida et al, 2006).

Mechanisms that enhance AR signaling in androgen-depleted conditions include: AR gene amplification, AR mutations, changes in the balance of AR cofactors, increases in steroidogenic precursors, and activation via "outlaw" pathways. Along with AR signaling, various other AR-independent "bypass" pathways have been shown to operate aberrantly during androgen independence. Changes in the epigenetic signatures and micro RNA concentrations have also been implicated in the development of androgen- independent prostate cancer (AIPC) (Figure 2.27) (Saraon et al, 2011).

AR overexpression has been implicated in many AIPC cases, both in vitro and in vivo. Together, gene and protein expression data show that the AR is overexpressed at the mRNA and protein levels, respectively (Brown et al, 2002; Edwards et al, 2003). Studies have revealed that approximately 25%–30% of androgen-independent tumors have AR amplifications (Koivisto et al, 1997). Interestingly, AR amplification has not been found in any untreated PCa samples, suggesting that AR amplification is one by-product of hormone therapy leading to AIPC. Gene amplifications of the AR loci have also been found in many clinical PCa samples that were in an androgen-independent state, indicating that gene amplification may lead to AR protein overexpression, and subsequently to increased AR signaling. Recently, it was found that increased AR expression sensitized PCa cells to lower-than-normal concentrations of androgens (Waltering et al, 2009).

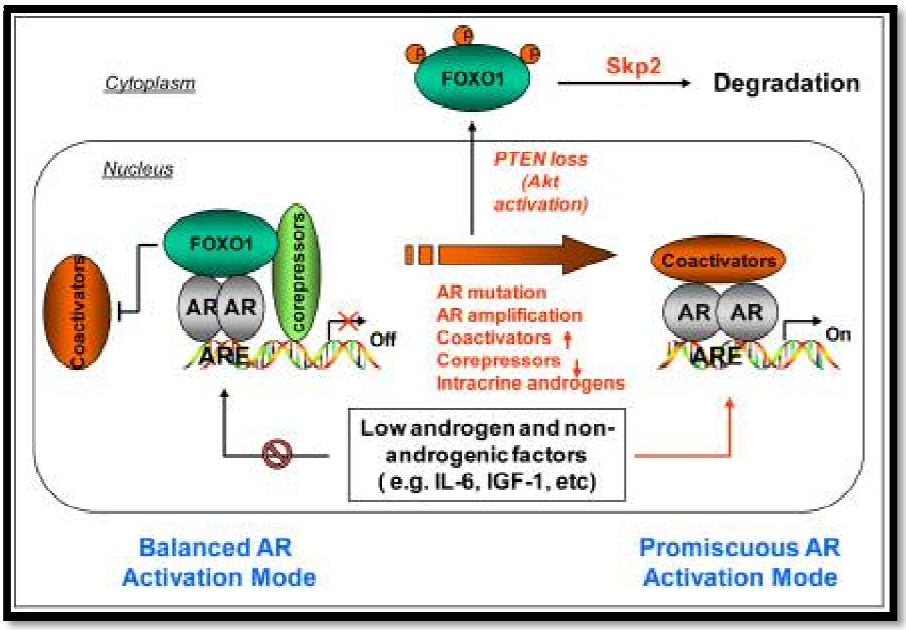


Figure 2.27. Possible molecular mechanisms underlying the shifting of the AR activation from the balanced mode in the normal prostate to the promiscuous mode in prostate cancer, especially the castration-resistant disease (Jiang & Huang, 2010).

High AR protein expression was found to help cancer cells survive and continue proliferating in environments with minimal androgen concentrations, a finding that may explain the evolution of AIPC during androgen deprivation (Waltering et al, 2009). Furthermore, AR overexpression at the mRNA and protein level has also been observed in the absence of AR gene amplification, which suggests the existence of gene amplification–independent regulators such as epigenetic and miRNA factors (Powell et al, 2004). It appears that tumors have selective pressures for continued AR signaling to allow for survival and further evolution, and therefore therapies that are more efficient at blocking this crucial signaling pathway are potentially promising approaches to prevent cancer progression.

AR mutations are another means for PCa cells to gain androgen-independent properties. The AR gene is located on the X chromosome, and a loss of function of the gene results in androgen-insensitivity syndrome. The frequencies of genetic mutations in the AR loci are typically rare in early stage prostate tumors (0%–4%) (Newmark et al, 1992), but are more frequent in advanced and recurrent tumors (Taplin et al, 1995). AR mutations have been reported in 10%–20% of patients with androgen independent tumors,

strengthening the model that particular mutations in the AR gene help cells to survive and proliferate in androgen-deprived conditions (Taplin et al, 1995).

2.13.2.5. Growth factor receptors

2.13.2.5.1. Insulin-like growth factor (IGF)

A number of growth factors have been shown to be implicated in the development of PCa. One of the most studied growth factors in the process of promoting oncogenesis in PCa is insulin-like growth factor (IGF). Although the IGF functions as an endocrine hormone, being predominantly secreted by the liver, it can also act as an autocrine and paracrine hormone, whose local secretion may be a possible stimulus for cell growth in neoplasms (Pollak et al, 2004).

IGF-I and IGF-II work via the same receptor, a transmembrane glycoprotein with tyrosine kinase activity, IGF1R (Sayeed et al, 2012). Increased expression of IGF1 and IGF-II has been shown via immunohistochemistry to be a positive correlation with serum PSA over 10. Additionally, the same study discovered that IGF-II has a positive statistically significant correlation with Gleason score (Liao et al, 2005).

Mita et al. found that IGF-II and IGF binding protein2 (IGFBP2) play a role in PCa progression and their increased expression is a prognostic indicator in hormone- treated PCa patients (Mita et al, 2000). The results of the study by Figueroa et al. indicate that the higher expression of IGFBPs in human PCa correlates with the Gleason score, and the expression of certain IGFBPs may be used as markers of aggressive clinical behavior (Figueroa et al, 1998).

2.13.2.5.2. Epidermal growth factor receptor (EGFR)

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that is related to the ErbB2, and participates in several signaling cascade including Akt, MAPK, and STAT, whereby it plays an important role in tumor cell growth. Overexpression of EGFR is correlated with time to biochemical relapse (Peraldo-Neia et al, 2011) and the interference of EGFR with miRNA 28, does allow for increased apoptosis of prostate tumor (Addepalli et al, 2010).

Additionally immunohistochemically, speaking, higher association of EGFR was statistically correlated with a higher serum PSA. Additionally, the relevance of EGFR to PCa oncogenesis can be further revealed by the fact that specimens with a diagnosis of Gleason scores above 7 were significantly more likely to have co-expression of EGFR with an association Her2, c-erb-2 (Di Lorenzo et al, 2002).

EGFR seems to display a rather complicated interaction with androgens and AR. Normally androgens are responsible for the down regulation of EGFR. In the cancer cell, however, the introduction of androgens may increase the levels of EGFR mRNA, and antibody mediated inhibition of EGFR prevented androgen mediated proliferation, although this remains debatable as another study revealed that EGFR was shown to have increased ubiquitination and degradation following activation of the androgen receptor (Mukherjee & Mayer, 2008).

2.13.2.6. Heat shock proteins 27, 26 and 70 (HSPs)

HSPs were first discovered as a cohort of proteins that are powerfully induced by heat shock and other chemical and physical stresses in a wide range of species (Lindquist & Craig, 1988). HSP are a subset of the molecular chaperones, best known for their rapid and abundant induction by stress. HSP genes are activated at the transcriptional level by heat shock transcription factor 1 (HSF1). During the progression of many types of cancer, this heat shock transcriptional regulon becomes co-opted by mechanisms that are currently unclear, although evidently triggered in the emerging tumor (Ciocca et al, 2013).

HSP 27 expression has been associated with poor prognosis in ovarian, gastric, liver and PCa, and osteosarcomas. In contrast, HSP 27 expression has been associated with good prognosis in endometrial adenocarcinomas, oesophageal cancer, and in malignant fibrous histiocytomas. Although there are fewer studies in other cancers, the data suggest that HSP27 has no prognostic value in head and neck squamous cancer, bladder and renal cancer, and leukemia (except when associated with other markers) (Ciocca & Calderwood, 2005).

HSP70 expression is correlated with poor prognosis in breast cancer, endometrial cancer, uterine cervical cancer, and transitional cell carcinoma of the bladder. This is consistent with the HSP70 associations with poor differentiation, lymph node metastasis, increased cell proliferation, block of apoptosis, and higher clinical stage, which are markers of poor clinical outcome. In contrast, high HSP70 expression was correlated with good prognosis in oesophageal cancer, pancreatic cancer, renal cancer, and melanoma. HSP70 expression showed no correlation with prognosis in ovarian cancer, oral cancer, head and neck squamous cancer, gastric and PCa, and leukemia (Ciocca & Calderwood, 2005).

It is suggested that HSPs might also be of interest as prognostic markers for PCa (Cornford et al, 2000; Lebret et al, 2003; Kurahashi et al, 2007). Other studies show that certain HSPs inhibited apoptosis and may therefore serve as independent survival factors, especially in androgen independent PCa (Thomas et al,1996; Bostwick, 2000; Gibbons et al, 2000).

2.13.2.7. DNA ploidy

DNA ploidy or chromosome complement is a crude measure of genomic instability, a hallmark of tumorigenesis, and in most cases has been correlated as a biomarker portending worse prognosis for prostate cancer (Tran et al, 2012).

Patients with diploid tumors have a more favorable outcome than do those with aneuploid tumors. Among patients with lymph node metastases who are treated with radical prostatectomy and androgen deprivation therapy, those with diploid tumors may survive 20 years or more, whereas those with aneuploid tumors die within 5 years (Zincke et al, 1992). However, the ploidy pattern of PCa is often heterogeneous, creating potential problems with sampling error. Analysis of multiple biopsy specimens is important for correct preoperative ploidy estimation (Haggarth et al, 2005). A good correlation exists between DNA ploidy and histologic grade, and DNA ploidy adds clinically useful predictive information for some patients (Lorenzato et al, 2004; Bantis et al, 2005).

Most low-stage tumors are diploid and high-stage tumors are nondiploid, but numerous exceptions occur (Tribukait et al, 1991). The 5-year cancer-specific survival is approximately 95% for patients with diploid tumors, 70% for those with tetraploid tumors, and 25% for those with aneuploid tumors (Deitch et al, 1992).

2.13.2.8. Proliferation index

2.13.2.8.1.Ki-67

The Ki-67 protein is well known and widely used to assess the tumour proliferation rate. It is one of the several cell-cycle-regulating proteins, which can be demonstrated by immunohistochemistry. It is a DNA-binding protein that is expressed in all phases of cell cycle but undetectable in resting cells (Berney et al, 2009). The Ki-67 labeling index of PCa has been said to predict tumor-specific mortality both in cases of limited disease and in cases associated with lymph node metastases (Masuda et al, 1998). The combined determination of Gleason score and proliferation index constitutes a particularly powerful prognostic tool (Chiusa et al, 1997).

In a 6-year study involving 808 patients diagnosed with PCa, an immunohistochemical assessment of Ki-67 expression was evaluated for its relationship to the specificity of the cancer and overall survival. Compared to information from the Gleason score and PSA, Ki-67 provided additional prognostic information (Khatami et al, 2009; Berney et al, 2009). In another study of a group of men treated with radiotherapy and androgen deprivation for PCa, Ki-67 expression levels in conjunction with MDM2 were found to be correlated to distant metastasis and survivability (Khor et al, 2009). Nevertheless, further studies will be needed to validate these results and explore the possibility of combining Ki-67 with existing prognostic tools as a powerful biomarker for localized PCa (Jhavar et al, 2009).

2.13.2.9. Cellular adhesion and adhesion molecules

Cell-cell and extracellular matrix interactions are becoming major targets for understanding how the phenotype of a cell is regulated. Transmembrane receptors on the cell surface extend out through the plasma membrane and form a bridge directly connecting the cytoskeleton with proteins and receptors located within the extracellular matrix or on neighboring cells (Rokhlin & Cohen, 1995).

Under normal conditions, cell-cell adhesion molecules maintain epithelial cell integrity and cellular architecture. The process of tumor invasion and metastasis is associated with alterations in the functions of several adhesion molecules. In general, tumor cells lose their capacity for normal adherence, which facilitates their detachment from their site of origin (Ahmad & Hart, 1997; Elzagheid et al, 2008).

Cell adhesion is responsible for the three-dimentional organization, stability and viability of tissue in mammals. Through extracellular protein interaction as well as intracellular and anchoring motifs, cell adhesions provide a construct for many necessary interaction. Communicating junctions such as gap junction, anchoring junctions such as desmosomes and adherens junction, and sealing junctions such as zoula occludens or tight junctions all provide the robust scaffold for epithelial architecture. It is this construction of the anchoring of cells to the basement membrane that is the most crucial for the function and integrity of various tissue (Cress & Ngle, 2006).

Cell adhesion is essential in all aspects of cell growth, cell migration and cell differentiation in vertebrate cells. Cellular adhesion molecules (CAMs) are important participants in cell- cell interactions and interaction between cells and components of the extracellular matrix. These molecules have been implicated in a wide variety of cellular functions including signal transduction, cellular communication and recognition, embryogenesis, inflammatory and immune responses, and apoptosis (Cohen et al, 1997). For metastatic tumor cells, they must enter into the blood or lymphatic circulation, which presumably involves the loss of intercellular adhesion and make CAMs likely participants in the development of metastatic disease. Evidence to

date suggests that the CAMs may be associated with invasion and metastasis in a variety of human malignancies (Okegawa et al; 2004).

The majority of adhesion molecules can be grouped into families. In the case of cadherins, integrin, selectins and syndecans, this grouping is based both structural and functional similarities of the family members. The immunoglobulin superfamily (IgSF) represent a more diverse group of protein that have structural similarity but a variety of different functions, and consequently subfamilies with functional similarity are arising. Finally, a large number of adhesion molecules do not fit into any of these families and these have been listed as others. Some of the others adhesion can be divided into subfamilies (Isake & Horton, 2000).

2.13.2.9. 1. THE CADHERIN-CATENIN COMPLEX

E-cadherin

E-cadherin is one of the most important molecules in cell-cell adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in regions of cell-cell contact known as adherens junctions (Penina-Slaus, 2003). As a member of a large family of genes coding for calcium-dependent cell adhesion molecules (CAMs), the cadherin glycoproteins are expressed by a variety of tissues, mediating adhesion through homotypic binding (Barth et al, 1997).

The classical cadherins include E-, N-, and P-cadherin (Nelson, 2008). Epithelial (E-) cadherin (also called uvomorulin, L-Cam, cell-Cam 120/80, or Arc-1) was the first to be identified and constitutes the prototypic member of the classical cadherin family. E-cadherin is a 120 kDa glycoprotein and is found in almost all epithelial tissues. Other members include P-cadherin (placental cadherin) and N-cadherin (neuronal cadherin). P-cadhering was originally found to be highly expressed in mouse placenta throughout pregnancy and is restricted to the basal or lower layers of adult stratified epithelium, whereas N-cadherin is expressed by neuronal and muscle cells in human embryo and adult tissues (Gama & Schmitt, 2012).

E-cadherin gene and protein structure

The human epithelial E-cadherin gene CDH1 maps to chromosome 16q22.1. isolated the full length gene by using recombinant lambda phage, cosmid and P1 phage clones. The gene they cloned encompasses 16 exons and spans a region of ~100 kb. The exons range from 115 to 2245 bp. Further analysis of the gene showed 15 introns ranging from 120 bp (intron 4) to 65 kb (intron 2). The intron-exon boundaries are highly conserved in comparison with other "classical cadherins", and in intron 1 a 5' high-density CpG island was identified that may have a role in transcription regulation. This island covers the region from exon 1 to exon 2 of the human E-cadherin gene, while other exons lacked such features, including the biggest (exon 16 of 2245 bp). The chromosomal location of CDH1 on 16q22.1 was later confirmed by fluorescent in situ hybridization (FISH) analysis. All classical cadherin genes analyzed so far have 16 exons separated by 15 introns (Figure 2.28) (Pećina-Slaus, 2003).

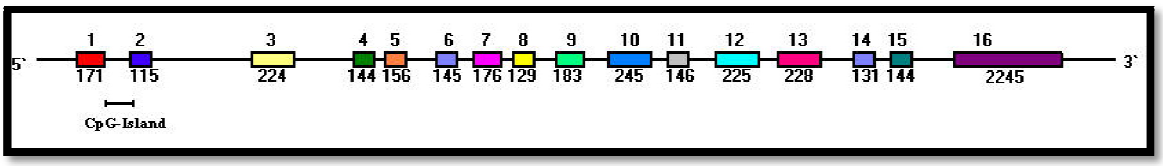


Figure 2.28. Genomic organization of the human E-cadherin gene (Pećina-Slaus, 2003).

The mature E-cadherin molecule, with approximate molecular mass of 120 KDa, comprise a single transmembrane domain, a cytoplasmic domain (C-terminal) of about 150 aminoacids, and an ectodomain of about 550 amino acids comprised of five repeated domains (EC1 to EC5). Crystal structures and mutagenesis studies support a model in which the first extracellular domain (EC1) is involved in binding to an opposing cadherin. However, recent evidence suggests that extracellar domains in addition to EC1 regulate the cadherin function. The binding between the extracellular domains of cadherin is weak, but strong cell-cell adhesion occur during lateral clustering of cadherin, and through interaction between the E cadherin cytoplasmic domain and catenins (Figure 2.29). β-catenin and plakoglobin (γ-catenin) interact directly with a core region of 30 amino acids within the C-terminal of cadherin

cytoplasmic domain. N-terminal of both β-catenin and γ-catenin interact with α-catenin, which links the cadherin to the cytoskeleton. In fact, α-catenin has been show to associate with actin-binding proteins such as vinculin and EPLIN. Another catenin, P 120-catenin, interacts with the highly conserved juxtamembrane domain of cadherins, preventing the entrance of E cadherin into degradative endocytic membrane pathways (Pinho et al, 2011).

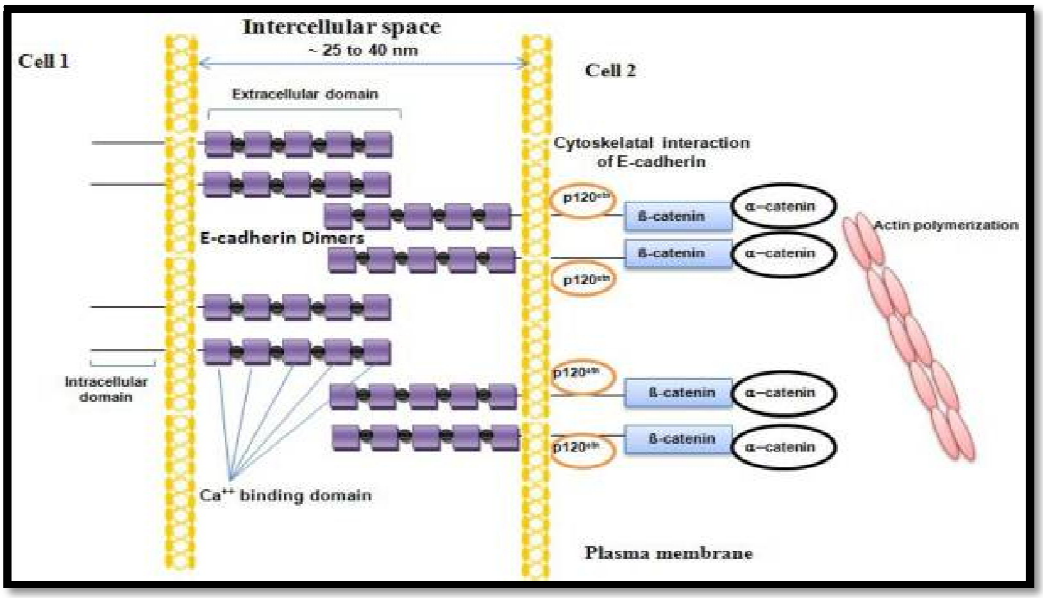


Figure 2.29. Schematic illustration of E-cadherin in adherens junction formation.E-cadherin forms homodimer in the extracellular domain in a Ca^{2+} dependent manner, while cytoplasmic domain binds with catenin and in turn regulates actin reorganization (Baranwal & Alahari, 2009).

The role of E-cadherin in malignancy

Progressive accumulation of somatic mutations in a number of different genes characterizes the process of tumorigenesis. Many genes involved in the process of tumorigenesis are components of one of a great many signal transduction pathways through which signals traffic via molecular networks. It is now apparent that epithelial malignancy can in certain aspects be explained by alterations in the adhesive properties of neoplastic cells (Pećina-Slaus, 2003).

Malignant carcinoma cells are characterized in general by poor intercellular adhesion, loss of the differentiated epithelial morphology and increased cellular motility. Down regulation or a complete shutdown of E-cadherin expression, mutation of the Ecadherin gene, or other mechanisms that interfere with the integrity of the adherens junctions, are observed in carcinoma cells. In human tumors, the loss of E-cadherin mediated cell adhesion correlates with the loss of the epithelial morphology and with the acquisition of metastatic potential by the carcinoma cells (Baranwal & Alahari, 2009).

The disruption of E-cadherin function has been documented in many human solid tumours. Downregulation of E-cadherin has been observed in many epithelial cancers and specific gene mutations have been detected. Particularly interesting is the association between E-cadherin dysfunction and the invasive/ infiltrative growth pattern of neoplastic cells. In poorly cohesive, infiltrative tumours, such as lobular carcinoma of the breast and diffuse gastric carcinoma, E-cadherin dysfunction and/or mutation is seen (Becker et al, 1994).

Work on lobular breast cancer has shown a loss of membranous expression of E-cadherin in these tumours, which was associated with mutations in the cadherin gene in most cases. This loss of expression of E-cadherin on cell membranes, often with relocation to the cytoplasm, has been observed in many malignancies and is an indicator of abnormal cadherin function (De Leeuw et al, 1997). Similarly, studies in gastric carcinoma have shown that in tumours of mixed intestinal and diffuse morphology only the diffuse component of the tumour was found to have mutations in the E-cadherin gene. Further evidence implicating E-cadherin in these particular tumours has come from work identifying a germ line mutation in the E-cadherin gene in one particular family with a strong history of gastric carcinoma (Machad et al, 1999).

In PCa It has been demonstrated that tumor tissues exhibit decreased levels of E-cadherin (Drivalos et al, 2011). Clinically, decreased or absent E-cadherin expression in PCa is associated with high tumor grade, advanced clinical stage, and poor survival (Cheng et al, 1996). The expression of this cadherin was significantly correlated with histological differentiation and bones metastasis, but not with lymphatic or vascular invasion (Pontes et al, 2010). In a some study it has been demonstrated that there is a significant decrease of membrane expression of E-cadherin/β-catenin complex and an

increase of cytoplasmatic and nuclear location of the same complex, in high Gleason score PCa (Aaltomaa et al, 1999).

A low E-cadherin to high N-cadherin expression switch has been correlated with progression of PCa and high mortality. N-cadherin is not expressed in normal prostate tissue; however, in PCa it has been detected especially in poorly differentiated areas (Contreras et al, 2010).

Because in PCa cell lines, mutational inactivation of α-catenin can be the cause of the impaired E-cadherin function, (Umbas et al, 1997) studies the relationship between E-cadherin and α-catenin expression. The results suggest that loss of α-catenin expression could be one of the mechanisms responsible for the loss E-cadherin mediated cell-cell adhesion in human PCa and might in some cases provide prognostic information. The same was concluded by Aaltomaa et al. who studied the expression of α-catenin in locally PCa. They found that α-catenin had prognostic significance in the early phases of cancer progression (Aaltomaa et al, 1999).

Catenin

Catenins are peripheral cytoplasmic proteins, which were first identified in association with the epithelial cell adhesion molecule E-cadherin (uvomorulin) in immunoprecipitation experiments with anti-E-cadherin antibodies (Ozawa et al, 1989; Nagafuchi & Takeichi, 1989). Analysis of various truncated E-cadherin polypeptides expressed in mouse L cells led to the definition of a 72 amino acid region within the cytoplasmic domain of E-cadherin that mediates the interaction with catenins (Ozawa et al, 1990). Additional deletion and point mutations within this 72 amino acid domain delimited the catenin-binding site to a 30 amino acid peptide (E-cadherin, amino acid positions 677-706) (Stappert & Kemler, 1994).

The catenin family comprises α-(120 KDa; chromosome 5q21-22), β-(92 KDa; chromosome 3p22) and γ-(plakoglobin; chromosome 11q11) catenin, with β- and γ-catenin sharing the greatest homology. β-catenin and γ-catenin bind directly to cytoplasmic tail of E-cadherin in mutually exclusive manner; α-catenin then links the bound β- or γ catenin to the actin microfilament network of the cytoskeleton. Recently, another catenin-like molecule, P120, has been identified in association with E-cadherin at the cell-cell junction, although this complex does not appear to form a link with the actin cytoskeleton. Originally identified as one of the several substrates of tyrosine kinase PP60 Src, P120 also associates with β-catenin and E-cadherin (Wijnhoven et al, 2000). It has been show that P120 acts as an inhibitory regulator of cadherin function in colon carcinomas (Aono et al, 1999).

β-catenin

β-catenin is a multifunctional protein that is involved in cellular structure and the Wnt/β-catenin signaling pathway. Wnt/β-catenin signaling is believed to be an inducer of cell proliferation in different tumors However, in certain physiological contexts β-catenin also promotes apoptosis. High levels of β-catenin are found in a number of cancer cell types. Recent studies have shown that β-catenin may be correlated with carcinogenesis (Li et al, 2012).

Unlike the multitude of cadherins and several tissue-specific α-catenin variants, a single β-catenin protein is present in vertebrates and insects. The protein is highly conserved, with, for example, only six amino acids different between the human and Xenopus proteins, and it is 67% identical to the Drosophila homolog armadillo. The primary structure of the 781amino acid β-catenin consists of an amino terminal region of about 150 amino acids, a central _520 residue domain composed of 12 armadillo (arm) repeats, and a carboxy terminal 100 residue region. E-cadherin binds to the arm domain, whereas α-catenin binds to residues 118-149, just before the start of the arm domain (Shapiro & Weis, 2009). The arm domain is an elongated super helical structure formed by the successive packing of helical arm repeats (Huber et al, 1997). The super helix features a groove that forms part of the binding site for β-catenin ligands (Choi et al, 2006). The entire β-catenin arm domain interacts with cadherin. For convenience, we divide the cadherin sequence into five β-catenin interaction regions. Region I includes a β strand that pairs with region III and forms several direct polar contacts with β-catenin. Cadherin region II includes a helix that interacts with the carboxy-terminal arm repeats (Roura et al, 1999).

Studying β-catenin function

Particular interest of scientists is focused on a multifunctional protein β-catenin. Along with E-cadherin it forms adherent junctions mediating epithelial cell adhesion and it is the key protein in canonical Wnt signaling pathway (MacDonald et al, 2009). In the absence of Wnt ligands, β-catenin abundantly occurs in adherent complexes, while its level in the cytoplasm is very low. Free cytosolic β-catenin is phosphorylated in a complex formed by adenomatous polyposis coli (APC), Axin, glycogen synthase kinase 3β (GSK3β) and casein kinase I (CKI). Phosphorylated β-catenin is ubiquitinated by ubiquitin ligase protein (βTrCP) and then degraded in the proteasom. Binding one of the Wnt ligands to the receptors FzD/Lrp5/6 triggers signal inactivating the degradation complex. Then β-catenin is stabilized and goes to the nucleus, where it binds T-cell factor/lymphoid enhancer factor (TCF/Lef) and activates gene expression (Figure 2.30) (Stanczak et al, 2011).

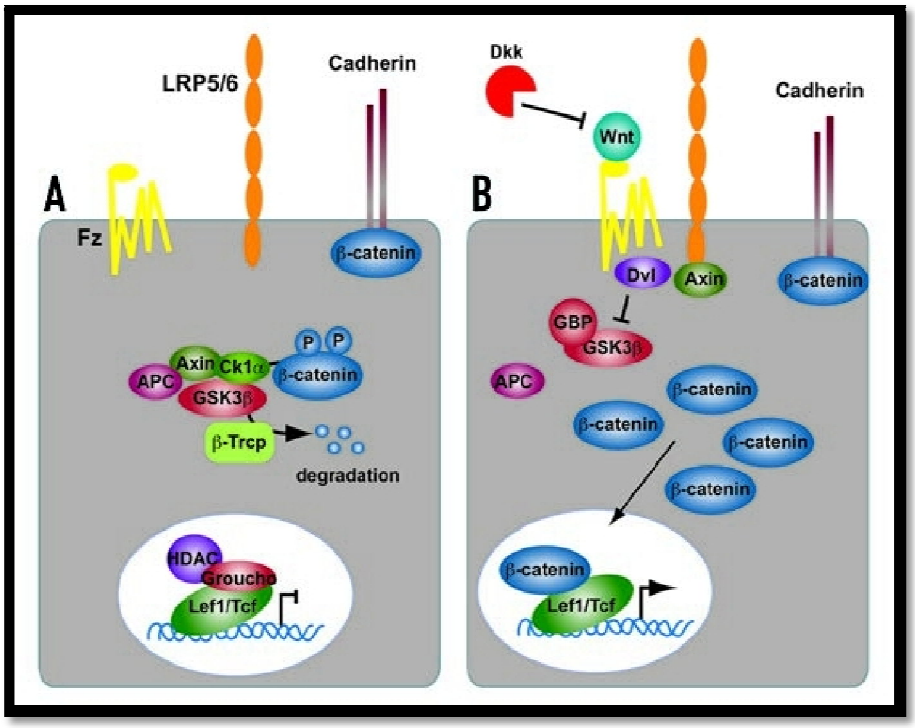


Figure 2.30. Summary of the Canonical Wnt signaling cascade. (A) In the absence of a Wnt ligand, (B) Wnt proteins bind to the Frizzled/Lrp receptor complex at the cell surface (Boras-Granic & Wysolmerski, 2008).

Disruption of cadherin-mediated cell adhesion, for example, can lead to β-catenin release and activation of Wnt signalling. Moreover, among the Wnt/β-catenin target genes are the transcription factors Twist-related proteins 1 and 2 and zinc finger protein SNAI2 (also known as neural crest transcription factor Slug), which inhibit E-cadherin gene expression. Together, these events can induce epithelial- mesenchymal transition, which is thought to promote the invasive behaviour of tumour cells. Importantly, cadherins and TCF/ LEF-1 proteins compete for binding to β-catenin, which provides an opportunity for further crosstalk between cell adhesion and the Wnt/β-catenin signalling pathway (Kypta & Waxman, 2012).

One recently identified protein that can interact with and co activate the AR is β-catenin, which binds to the 5α –dihydrotestosterone (DHT) liganded AR LBD via a site that is distinct from the hydrophobic cleft that mediates binding of LXXLL motifs found in many other coactivator proteins (Singh et al, 2012). However, the biological role of AR interactions with β-catenin has not been established and may be complex given further direct interactions between AR and Tcf4 as well as between AR and amino-terminal enhancer of split (a Tcf corepressor and member of the Groucho/TLE

family) (Cheng et al, 2006). Although β-catenin can function as an AR coactivator and may selectively regulate a subset of AR-responsive genes, another function for the AR–β-catenin interaction in normal prostate epithelium may be to sequester nuclear β-catenin and thereby suppress β-catenin/Tcf4 signaling, consistent with AR functioning in normal prostate epithelium to suppress growth and stimulate terminal differentiation (Mulholland et al, 2003; Shah et al, 2003; Song et al, 2003). The vitamin D and retinoic acid receptors can similarly bind to β-catenin and interfere with Tcf4 co activation by β-catenin (Chen et al, 2006).

β-catenin was first linked to cancer through its association with APC, a key tumour suppressor in colorectal cancer. This discovery led to a model in which *APC* mutations promote cancer progression by disrupting cadherin-dependent cell adhesion. Additionally, a mutation in β-catenin was first studied in the context of cadherin-dependent cell adhesion, as such mutations were thought to perturb cell adhesion and the cytoskeleton in cancer cells. These observations were interpreted as being consistent with the cellular changes that take place during metastasis (Behrens et al, 1996; Molenaar et al,1996).

β-catenin in prostate cancer

Currently, the function of β-Catenin in human PCa is unclear (Kypta & Waxman, 2012). CTNNB1 mutations in PCa occur rarely, in only 5% of cases (Francis et al, 2013). This low mutation rate of ~5% was corroborated in a separate study of 138 tumour samples (Bova & Isaacs, 2000). In smaller studies, mutated β-catenin was found in two of six locally advanced PCa (Gerstein et al, 2002), but not in eight tumours from patients with CRPC (de la Taille et al, 2003). a more common pathway toward β-catenin activation in PCa is via methylation of APC, the gene that codes for the colon cancer suppressor that complexes with β-catenin in the cytoplasm and mediates phosphorylation and ubiquitination to modulate the intracellar levels of β-catenin (Henrique et al, 2007). In addition, GSK3β is inactivated in advanced PCa (Mulholland et al, 2006). GSK3β is a key kinase for the β-catenin NTD that activates ubiquination on proteosomal degradation (Rubinfeld et al, 1996). In advanced PCa, calpain cleaves β-catenin causing an N-terminal truncation. This is potentially, another mechanism for β-catenin activation during late stage disease (William et al, 2010).

PTEN is frequently altered in PCa, with mutations and/or deletions found in 30% of primary cancers and 63% of metastatic prostate tumours. PTEN is a phosphatase that negatively regulates the phosphatidylinositol-3-kinase/Akt (PI3K/Akt) pathway. PTEN loss promotes phosphorylation of Akt through PI3K, which in turn phosphorylates multiple targets including GSK3β. Activation of this pathway results in an increase in cell proliferation, cell survival and protein synthesis (Song et al, 2012). Evidence suggests that β-Catenin can interact with the PI3K/Akt pathway following PTEN loss, through the inactivation of GSK3β and stabilization of β-Catenin. PTEN null PCa cells have increased nuclear β-Catenin expression, TCF promoter activity and expression of the β-Catenin regulated gene Cyclin D1, which are suppressed upon re-expression of wild type PTEN (Francis et al, 2013).

There have been a number of contradictory IHC studies of β-catenin expression in PCa (Table 2.11). It has been observed that β-Catenin expression and localization change during human PCa progression, however, results are inconsistent (Francis et al, 2013). Aberrant expression of all main three catenin types (α, β- and γ-catenin) has been associated with extra prostatic extension (van Oort et al, 2007). dela Taille et al. who observed that increased cytoplasmic and nuclear expression in 29% of PCa overall: 21% of tumours with Gleason score <7; 26% of tumours with a Gleason score of 7; 37% of tumours with Gleason score >7; 38% of CRPCs (de la Taille et al, 2003).

Jaggi et al. demonstrated that Gleason grade > or =7 cancers showed significantly lower expression of E-cadherin and β-catenin compared to Gleason grade <7 PCa. In addition, β-catenin was down regulated in 4 of 5 (80%) specimens with identifiable HGPIN and had demonstrable nuclear staining in higher grade PCa (P =0.0001). However, E-cadherin and β-catenin membranous or nuclear expressions were not significantly associated with final pathologic stage of the specimens (P values>0.05). Overall, the expression of E-cadherin and β-catenin is significantly down regulated in PCa compared to surrounding benign appearing prostate, which correlates with increasing Gleason grade. Furthermore, nuclear localization of β-catenin in high grade PCa may be a useful biomarker for aggressive PCa (Jaggi et al, 2005).

A some studies have demonstrated that the membranous overexpression of β-catenin is significantly associated with the metastatic PCa cells in the bone and that the high

frequency of expression suggests its involvement in the intercellular adhesion of the metastatic cells in the bone. Furthermore, studies have demonstrated that δ-catenin is overexpressed in PCa and is correlated positively with increasing Gleason scores (van Oort et al, 2007). The same observation was demonstrated by Saha et al. who observed that the membranous overexpression of E-cadherin and β-catenin are significantly associated with the metastatic prostate cancer cells in bone and that the high frequency of expression suggest their involvement in the intercellular adhesion of the metastatic cells in bone (Saha et al, 2008).

Table 2.11. Studies of β-catenin expression and / or localization in prostate cancar.

Study*	Participants (*n*)	Overall change in expression	Change in localization
de la Taille et al. (2003)	212 (90 with CRPC)	Increased	Increased cytoplasmic and nuclear expression in 29% of prostate cancers overall: 21% of tumours with Gleason score <7; 26% of tumours with a Gleason score of 7; 37% of tumours with Gleason score >7; 38% of CRPCs
Horvath et al. (2005)	252 (20 with D2 disease and pelvic lymph node metastases)	Reduced	Decreased membrane and nuclear expression, but cytoplasmic expression unchanged. In men with advanced cancer, nuclear expression is reduced compared with hyperplastic prostatic tissue and localized prostate cancer. Low-risk patients with localized tumours, preoperative PSA levels <10 ng/ml and in whom <10% of prostate cancer cells expressed nuclear β-catenin demonstrated reduced relapse-free survival compared with other patients in this group.
Bismar et al. (2004)	101 (3 with stage IV disease)	No change	88% diffuse membrane expression, 12% negative for membrane, nuclear and cytoplasmic expression
Whitaker et al. (2008)	170 (23 with CRPC, 80 with BPH)	Increased	High intensity in membrane, cytoplasmic and nuclear compartments: 18% of BPH samples; 15% of tumours with Gleason score <7; 22% of tumours with a Gleason score of 7; 44% of tumours with Gleason score >7. Nuclear expression: 37% of BPH samples; 14% of tumours with Gleason score <7; 9% of tumours with a Gleason score 7; 5% of tumours with Gleason score >7; no significant change in nuclear expression in CRPC
Kallakury et al. (2001)	112	Reduced	Loss of overall expression was observed in 4% of tumours, with loss more frequent in tumours with Gleason score ≥7
Chen et al. (2004)	67 (23 with metastatic disease)	Increased	Cytoplasmic and nuclear staining detected in 43% of tumours with Gleason score ≤7 and 78% of tumours with Gleason score >7. Loss of membrane staining in 100% of tumours with Gleason score >7. Increased overall staining density in tumours with Gleason score >7 and in lymph node and bone metastases
Jaggi et al.(2005)	17 carcinomas	Increase in Gleason 4-7 ,decrease with Gleason 8–10	Increase nuclear staining Gleason 7–10.

The canonical wnt/β-catenin signaling pathway

Wnts are a large family of 19 secreted glycoproteins that control many essential biological processes such as embryogenesis, organogenesis and tumorigenesis) (Rajalin & Aarnisalo, 2011). Wnt signaling is currently known to include two major pathways: 1) the canonical or Wnt/ β-catenin pathway (Fig.2.30), and 2) the non-canonical pathways which do not involve β-catenin stabilization. There is also a pathway which controls the orientation of mitotic spindles in Drosophila and Caenorhabditis elegans but this has not yet been found in vertebrates (Kharaishvili et al, 2011).

Canonical Wnt signaling, which regulates β-catenin protein levels within cells, is initiated upon engagement of a member of the Frizzled family of seven transmembrane receptor proteins in combination with either Lrp5 or Lrp6 (low density lipoprotein related proteins 5 and 6). Lrp5 and Lrp6 are members of a larger family of low density lipoprotein related receptors and most reports have focused specifically on their role in mediating Wnt signal transduction. However, roles for other members of this family, including LRP and Lrp4, in controlling Wnt signaling have also been reported (Hendrickx & Leyns, 2008; Choi et al, 2009). The formation of this ligand-receptor complex results in the activation of kinases which induce phosphorylation of serine residues in the cytoplasmic tail of Lrp5 and/or Lrp6 (Niehrs & Shen, 2010). A number of putative specific kinases have been reported to phosphorylate these residues, and the process has also been shown to be associated with activation of heterotrimeric G proteins and the cytoplasmic Dishevelled protein family. New evidence has emerged showing that the phosphorylation and activation of Lrp6 (and potentially Lrp5) requires endocytosis and subsequent acidification of the compartment containing the endocytosed receptor. This process requires the Prorenin receptor and a vacuolar H+-ATPase (George et al, 2007; Cruciat et al, 2010). Finally, binding of Wnt ligands to these receptor complexes is regulated by a number of proteins that either bind to the receptor component (such as DKKs, SOST, or Wise/SOSTDC1) or to the Wnt ligand itself (for example, SFRPs) (Kenneth et al, 2011).

The phosphorylation of the cytoplasmic tail of Lrp6 leads to the recruitment of the scaffolding protein Axin to the receptor complex. This recruitment is facilitated by the phosphorylation of multiple copies of this phosphorylated proline-rich serine motif in

each Lrp6 molecule and via potential clustering of multiple Lrp6 receptors upon activation (Bilic et al, 2007; MacDonald et al, 2008). Axin is a component of a multi-protein complex that, in the absence of an upstream signal, is responsible for inducing the degradation of the β-catenin protein. Other components of this complex include the colon cancer tumor suppressor protein APC, and the serine/threonine protein kinase glycogen synthase kinase 3 (GSK3). In un-stimulated cells, Serine-45 in the β-catenin protein is constitutively phosphorylated within the cytoplasm, which creates consensus sites for GSK3 to mediate further phosphorylation of β-catenin. This hyperphosphorylated form of β-catenin is then targeted for ubiquitin-dependent proteolysis. In addition to the Wnt-induced pathway that inhibits degradation of β-catenin via inhibition of GSK3 activity, a parallel pathway is induced by the receptor complex which facilitates nuclear entry of β-catenin from the cytoplasm. One report indicates that a PI3K-dependent pathway acting via Rac1 and JNK2 is necessary for nuclear entry of β-catenin (Wu et al, 2008). Another report found that activation of the Ras signaling pathway can also facilitate this process (Phelps et al, 2009).

Once β-catenin enters the nucleus, it interacts with members of the TCF/Lef family of DNA binding proteins to bind to specific promoter targets. β-catenin TCF/Lef complexes have been shown to interact with a variety of nuclear factors to control specific transcriptional targets. Examples of such proteins include p300, CBP, Hrpt2, Foxo, bcl9-2, reptin, pontin, Grouchos, Prmt2, and CtBP. One result of such interactions is the reorganization of chromatin near the transcriptional initiation site of target genes (Mosimann et al, 2009).

Wnt antagonists and agonists

Several secreted protein families antagonize or modulate Wnt/ β-catenin signaling (Figure 2.31). Wnt antagonists are of two main classes—those that associate directly with Wnts, such as the five members of the secreted frizzled-related protein (sFRP) family and Wnt inhibitory factor 1 (WIF-1), and those that associate with Wnt receptors, such as members of the Dickkopf-related protein (Dkk) family that bind to Lrp-5 and Lrp-6 (Bovolenta et al, 2008). Although sFRP family members and WIF-1 have the potential to inhibit all Wnt signals, Dkk family members that associate with Lrp-5 or Lrp-6 (notably, Dkk-3 does not) are predicted only to inhibit Wnt/β-catenin

signals. Furthermore, although downregulation of sFRP-1, sFRP-4 and WIF-1 has been observed in PCa, this does not preclude other sFRP and Dkk family members from involvement, and might simply reflect a paucity of data implicating other members in the disease (Kypta &Waxman, 2012). Indeed, a recent study reported downregulation of sFRP-2 in PCa (O'Hurley et al, 2011).

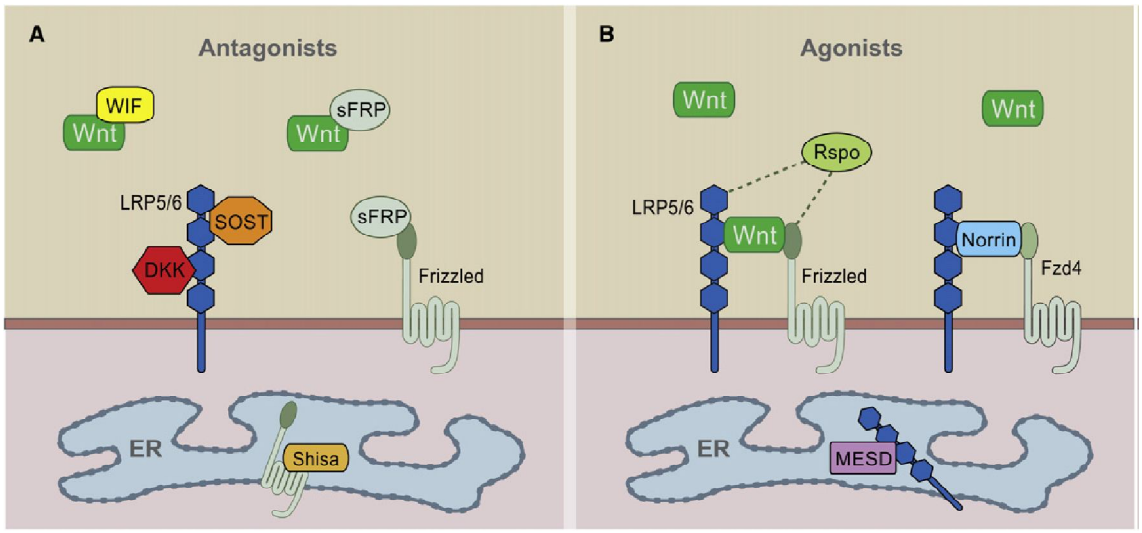


Figure 2.31. Secreted Wnt Antagonists and Agonists. (A) Antagonists. WIF and sFRP bind directly to secreted Wnts and/or Fz. DKK and SOST/WISE proteins bind LRP5/6 to prevent Fz-LRP6 complex formation. Shisa proteins trap Fz in the ER. (B) Agonists. Wnts are the primary agonists and form a complex with LRP5/6 and Fz to activate signaling. Norrin acts similarly to Wnt, but binds specifically to FZD4. R-spondin proteins (Rspo) act via and may bind to LRP5/6 and/or Fz receptors. In the ER, the chaperone MESD is needed for LRP5/6 maturation.

Th Wnt-binding property suggests that sFRPs and WIF may also regulate Wnt stability and diffusion/distribution extracellularly beyond just Wnt inhibitors. Some sFRPs have been shown to act in Wnt-independent roles such as regulators of extracellular proteinases (Bovolenta et al, 2008).

WISE and SOST constitute another family of Lrp5/6 ligands/antagonists (Li et al, 2005; Semenov et al, 2005). Like DKK1, SOST is able to disrupt Wnt-induced Fz-LRP6 complex in vitro (Semenov et al, 2005). Both DKK1 and SOST are strongly implicated in human diseases. Shisa proteins represent a distinct family of Wnt antagonists that trap Fz proteins in the ER and prevent Fz from reaching the cell surface, thereby inhibiting Wnt signaling cellautonomouslyn (Yamamoto et al, 2005). Shisa proteins also antagonize fibroblast growth factor (FGF) signaling by trapping FGF receptors in

the ER. Other Wnt antagonists with multivalent activities exist. Xenopus Cerberus binds to and inhibits Wnt as well as Nodal and bone morphogenetic protein (BMP) (Piccolo et al, 1999), and IGF binding protein-4 (IGFBP-4) antagonizes Wnt signaling via binding to both Fz and Lrp6, in addition to modulating IGF signaling (Zhu et al, 2008).

Norrin and R-spondin (Rspo) proteins are two families of agonists for Wnt/β-catenin signaling (Figure 2.29). Norrin is a specific ligand for FZD4 and acts through FZD4 and Lrp5/6 during retinal vascularization (Xu et al, 2004). Rspo proteins exhibit synergy with Wnt, Fz, and Lrp6 (Wei et al, 2007), and show genetic interaction with Lrp6 during embryogenesis (Bell et al, 2008), but their mechanism of action is controversial. Results that Rspo binds to both Fz and Lrp6 (Nam et al, 2006), to Lrp6 primarily (Wei et al, 2007), or to neither (Kazanskaya et al, 2004) have been reported. Another model suggests that Rspo is a ligand for Krm and antagonizes DKK/Krm-mediated Lrp6 internalization (Binnerts et al, 2007), but this seems unlikely given that Krm1 and Krm2 double knockout mice are viable and do not exhibit Rspo mutant phenotypes, and that Rspo activates β-catenin signaling in cells lacking both Krm genes (Bell et al, 2008; Ellwanger et al, 2008). Rspo genes are often coexpressed with, and depend on, Wnt for expression (Kazanskaya et al, 2004), and may represent a means of positive feedback that reinforces Wnt signaling. Mutations in Norrin and Rspo genes cause distinct hereditary diseases .

Wnt signaling in prostate cancer and cancer stem cells

Wnt ligands are up-regulated in PCa, and their expression often correlates with aggressiveness and metastasis. Hall et al. determined that 15 of the 19 Wnts are expressed in four PCa cell lines (Hall et al, 2005). Elevated expression levels of Wnt1, Wnt5a, Wnt7b, and Wnt11 have also been correlated to PCa aggressiveness (Li et al, 2008; Uysal et al, 2010; Yamamoto et al, 2010). In addition, DKK1 expression increases during PCa initiation but decreases during metastasis (Hall et al, 2008). The correlation of Wnt activation and skeletal metastasis may be important for therapy; there is currently no cure for metastatic PCa. Other Wnt pathway members are dysregulated in PCa. Frizzled-4 (FZD4, a Wnt receptor) is co-expressed in human prostate tumor samples with the ETS-related gene (*ERG*) (Gupta et al, 2010). Further experimentation has shown that FZD4 overexpression decreases E-cadherin expression

in ERG-positive PCa and leads to an epithelial-to-mesenchymal transition (EMT), which is a crucial step in metastasis initiation. Other studies have shown that Wnt inhibitory factor-1 (WIF1) is down-regulated in PCa (Wissmann et al, 2003), and induced overexpression of WIF1 reverses EMT in PCa cell lines and decreases their invasive capacity *in vitro* and *in vivo* (Yee et al, 2010). Also, when PCa cell lines were transfected with WIF1, they were more sensitive to chemotherapy and had reduced phosphorylation of Akt (a key effector of PI3K signaling which is frequently phosphorylated in PCa) (Ohigashi et al, 2005).

2.13.2.9. 2.Integrins

Integrins are transmembrane glycoprotein receptors for extracellular matrix (ECM) proteins. They are composed of two subunits α and β, the combination of which gives them a different specificity and function. Currently, 26 members of the family of integrins have been described (18 α and 8 β subunits). The extracellular domain of integrins has binding sites and upon interaction with the ECM, they form links between cells and ECM components (Drivalos et al, 2011).

Integrin signaling plays a key role in the alteration of cellular growth and tumor progression through the regulation of gene expression, apoptosis, cell adhesion, proliferation, migration and angiogenesis, as well as proteinase expression (Goel et al, 2009).

High Gleason score has been correlated with low and/or negative expression of integrin subunit α3 (Schmelz et al, 2002). The expression of α6 subunit diminishes with increasing histological grade, especially at sites of contact with the basement membrane (King et al, 2008). PCa demonstrates decreased positive staining for subunit α7, with a further decrease in metastatic disease (Ren et al, 2007). Other α subunits that have been found down-regulated include α2, α4, α5, and αv (Ramsay et al, 2007). Interestingly, αIIβ subunitis expressed only in PCa and not in normal tissue (Trikha et al, 1998).

Among the β subunits, β1, β3, and β6 are upregulated, while β1C and β4 are downregulated in human prostate cancer. No reports are available for β5, β7, and β8. Two variants, β1C and β1A, are shown to be expressed in benign prostatic epithelium.

β1C is expressed at both protein and mRNA levels in benign prostatic epithelial cells, but is markedly downregulated in adenocarcinoma (Drivalos et al, 2011).

2.14. Immunohistochemistry as adjunct tool in biomedical research

Immunohistochemistry (IHC) is an umbrella term that encompasses many methods used to determine tissue constituents (the antigens) with the employment of specific antibodies that can be visualized through staining. When used in cell preparations it is called IHC, a term that some authors use for all methods entailing the immunological search of cell antigens, even when this involves tissue slices (Matos et al, 2010).

History and background

The history of IHC dates back to 1941 when Coons and colleagues labeled an antibody with fluorescent dye and used it to identify an antigen in tissue sections. Since the 1970s the use of IHC techniques has taken off exponentially, in parallel with the development of specific molecular markers. Since the mid 1980s the use of microwave techniques has been widely used for antigen retrieval from formalin-fixed, paraffin-embedded archived material and has had an enormous impact on the field of IHC (Teruya Feldstein, 2010).

The immunohistochemistry technique

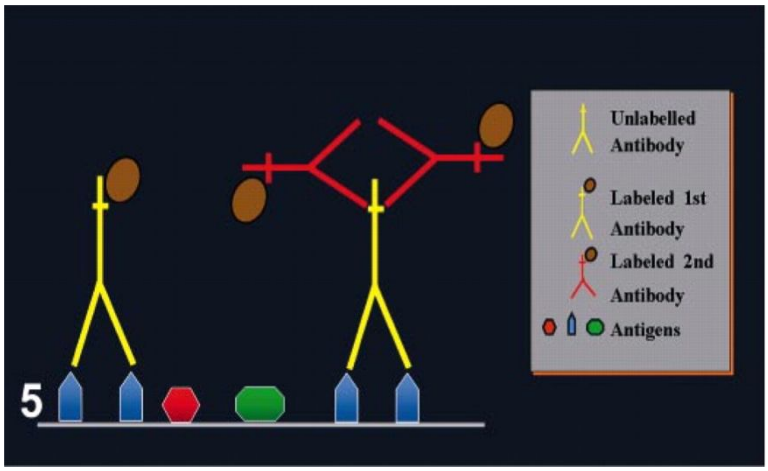
The technique comprises two phases: (1) slide preparation (specimen fixation and tissue processing) and stages evolved for the reaction (in order: antigen retrieval, non-specific site block, endogenous peroxidase block, primary antibody incubation, and the employment of systems of detection, revealing and counterstaining and also slide mounting and storage); (2) interpretation and quantification of the obtained expression (Matos et al, 2010).

The Ag-Ab reaction cannot be seen with the light microscope unless it is labeled. Therefore, labels (reporter molecules) are attached to the primary, secondary, or tertiary Abs of a detection system to allow visualization of the immune reaction. A variety of labels have been used, including fluorescent compounds, enzymes, and metals. The most commonly used labels are enzymes (e.g., peroxidase, alkaline phosphatase, glucose oxidase). Enzymes in presence of a specific substrate and a chromogen will produce a colored precipitate at the site of the Ag-Ab reaction. Selection of a detection system is very important, considering that the sensitivity of an immune reaction will

depend mostly on the detection system used. Detection systems are classified as direct or indirect methods.

Direct methods

This is the simplest of the immunocytochemical methods. The reaction is a one-step process with a primary Ab conjugated with a reporter molecule (label). Different labels have been used, including fluorochromes, enzymes, colloidal gold, and biotin. The method is quick but lacks sufficient sensitivity for the detection of most Ags in routinely processed tissues (Ramos-Vara, 2005).



Indirect methods

The need for more sensitive Ag detection prompted Coons et al. (1955) to develop a two-step method. The first layer of Abs is unlabeled, but the second layer, raised against the primary Ab, is labeled. The sensitivity of this method is higher than a direct method because 1) the primary Ab is not labeled, retaining its activity and resulting in a strong signal and 2) the number of labels (e.g., peroxidase) per molecule of primary Ab is higher, increasing the intensity of reaction. The result is the ability to detect smaller amounts of Ag or to increase the dilution of the primary Ab because at least two labeled Igs can bind each primary Ab molecule. These methods are also more convenient than the direct method because the same secondary Ab can be used to detect different primary Abs, provided the latter are raised in the same species (Ramos-Vara, 2005).

Uses of immunohistochemistry

Diagnostics

IHC assists the pathologist in areas of tumor classification, multilineage differentiation, molecular correlates, and infectious etiologies. In the clinical diagnostic arena, lymphoma subclassification has led the way in enhancing the number of antibodies in use. The National Comprehensive Cancer Network guidelines for lymphoma workup show up to 8 pages of suggested algorithms for B- and T-cell neoplasms. In addition, immunophenotypic panels and algorithms for diagnostic workup of malignant neoplasms with indeterminate morphology and carcinomas of unknown primary site are increasing (Teruya-Feldstein, 2010).

Prognosis

IHC is commonly used to detect prognostic markers to indicate indolent or aggressive biology. For example, in diffuse large B-cell lymphoma, one of the most common lymphoma subtypes, IHC surrogate prognostic marker studies are refined and used in combination with the clinical international prognostic indices to help predict how patients will respond to chemotherapy. However, an international collaborative project recently recommended the importance of harmonization of techniques, uniformity of scoring criteria, and centralized consensus review in multicenter clinical trials before proceeding to broad clinical application. IHC can also inform us of the biologic behavior and prognosis of a tumor. An example is shown of a case in which combined imaging, morphology, immunophenotyping, and cytogenetics were used for an aggressive plasma cell neoplasm that relapsed. Combined features, such as the extent of disease, as seen on imaging studies, the immature plasma cell morphology, high MIB-1 proliferative index, strong p53 reactivity, and multiple cytogenetic abnormalities including del(13q), predicted an aggressive relapse of disease. Active research is ongoing for optimal multimodality use of prognostic biomarkers in lymphoma subtypes and has been a topic of international meetings (Teruya-Feldstein, 2010).

Recent advances and future directions

"Genogenic IHC" heralds a new era in IHC, and identification of the underlying molecular changes by IHC is being used both for diagnosis and therapy. Markers to monitor drug resistance include P-glycoprotein, the product of the mdr gene (multidrug resistant); N-MYC and tumor suppressor genes such as p 53; retinoblastoma

susceptibility suppressor gene; putative suppressor genes - BRCA-1 gene, DNA repair genes (microsatellite instability) are all examples of genogenic IHC.The genetic mutations such as loss of E-cadherin protein in lobular carcinoma of the breast, ALK over-expression to recognize the t(2;5) translocation in anaplastic large cell lymphoma, FLI-1 over-expression for the t(11;22) translocation of PNET/ES; WT-1 over-expression for the t(11;22) translocation of DSRCT are the newer examples of genogenic IHC markers.

For targeted therapy, trastuzumab, a monoclonal antibody to HER-2neu gene (Cerb2) (breast cancer); rituximab, an anti-CD20 monoclonal antibody (B-cell NHL); and imatinib against C-kit positive tumors (GIST) have demonstrated success. Research and trials are in vogue for monoclonal antibodies against several growth factor-related receptors such as vascular endothelial growth factor (VEGF), platelet-derived growth factors (PDGFR-B), and epidermal growth factors (EGFR) for the treatment of cancers of breast, colon, lung; and renal cancers.

Newer technology for the development of more specific antibodies from recombinant antibody fragments has paved the way for molecules with ultra-high affinity, high stability, and increased potency, which is almost unattainable by the traditional immunization methods. Automation in IHC has been advocated for carrying out the procedures for consistency in performance. Methods using automated computerized image capture and analysis systems as opposed to the traditional subjective observations of IHC stains are being introduced. The emergence of tissue microarrays (TMA) as a high-throughput technique for examining hundreds of marker molecules in histological microarray sections comprising between 100 and 1,000 core tissues on a single glass slide enables economical evaluation in terms of sample utilization and reagent costs. In future, TMA will be an increasingly sought-after tool for evaluating the expression of proteins by IHC and thus validating the findings of DNA microarrays. This technique holds the promise of better understanding of the genetically heterogeneous groups of diseases, such as lymphomas, which have shown different response to treatment despite identical international prognostic index (IPI). The results obtained by these high-throughput methods can be analyzed by automated Although a relatively simple technique, IHC has some particularities and its outcome depends on many factors. The usefulness and contributionof IHC in solving problems in pathological image analyzers.

Finally it is of interest that IHC has a newfound role to detect agents of bioterrorism, and it is also of value in the field of veterinary pathology (Jambhekar et al, 2008).

Limitations, difficulties and problems

anatomy is directly proportionate to the experience of the hands that perform the reactions and also the eyes that interpret the results. Therefore, even though very simple in concept, immunostaining methods requires rigor of execution and may present significant bias. Hence, its outcomes must be interpreted with caution.

the main bias that may follow the analysis of IHC reactions are didactically divided into reaction bias (examples: specimen fixation, tissue processing, antigen retrieval and detection system) and interpretation bias (examples: selection of antibody panels, sensitivity of the chosen panel, choice of antibody types and clones, results and literature interpretation) (Matos et al, 2010).

Due to their flexibility and relatively low cost, the most used protocols currently (such as the ABC method, for example) are indirect and therefore require many stages of incubation. High sensitivity could be obtained with the application of immunological principles, enzymatic amplification reactions and/or the employment of avidin-biotin complex, however the various steps required must be rigorously followed in order to avoid non-desirable interactions. It is fundamental that, on technical planning, all reagents follow the sequence rigorously established, where the employment of work flow charts for such stages are very useful in avoiding false results. Making notes of all reaction stages and pattern of each antibody are equally important and are suggested in patterning technique programs (Matos et al, 2010).

CHAPTER III

PATIENTS & METHODS

3. Patients and Methods

Clinicopatholgical features and follow up data

Archival samples of **40** prostatic adenocarcinoma were examined in the present study: All the tumor samples were collected from Pathology Department, Faculty of Medicine, Benghazi University between January 2006 to December 2011 based on availability of representative paraffin blocks.

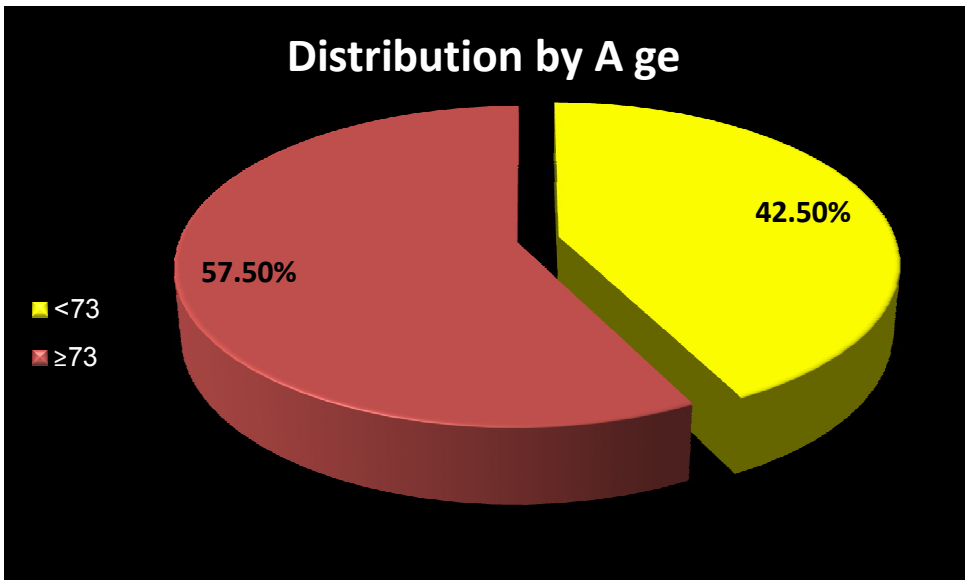
The patient's clinical files were read in the hospital archives in order to collect the appropriate clinical information and follow up data for current study. For each patient, we obtained the following information: age, histological diagnosis, grading, staging, pre-treatment PSA level, date of diagnosis, treatment, cause and date of death. All the patients were followed up until death or when last seen alive at their clinical visit (Dec-2012) with the median follow up-time of months (range:6-72 month, mean: 25 month). The duration of follow-up was determined for each patient from hospital and clinic charts. Clinical stages were determined according to the International Union Against Cancer (UICC) classification of 2009. Clinical staging routinely included abdominal and pelvic computerized tomography (CT), chest radiograph or thoracic CT, isotope bone scanning, and extended/extensive prostate biopsy, as described elsewhere. PSA levels at diagnosis ranged between 0.1 and 500 (mean: 113 ng/ml), and Gleason score at biopsy ranged between 6 and 10.

An experienced pathologist confirmed all diagnosis, and the following histopathological features were recorded included; histological type, histologic grading determined in accordance with the Gleason grading system, lymphovascular invasion, perineural invasion. All tumors were classified using the histopathological criteria of WHO classification. The key clinicopathological data of patients are summarized in Table 3.1.

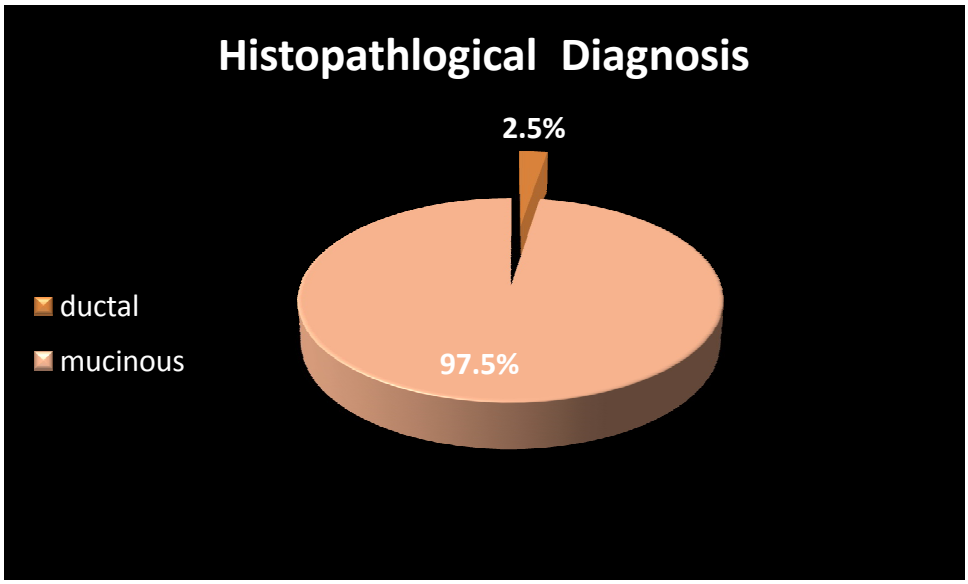
Table 3.1. Clinicopathological characteristics of the patients with PCa

Characteristic	No. of the patients	(%)
Age (yrs)		
≥74	**23**	**(57.5%)**
<74	**17**	**(42.5%)**
Type of biobsy		
Core biopsy	**31**	**(77.5%)**
TURP	**8**	**(20%)**
Radical prostatectomy	**1**	**(2.5%)**
Histopathological type		
Acinar adenocarcinoma	**39**	**(97.5%)**
Ductal adenocarcinoma	**1**	**(2.5%)**
Pre-treatment PSA level		
Mean (range)	**112**	**(0.1-500)**
Gleasone score		
6	**2**	**(5%)**
7	**12**	**(30%)**
8	**8**	**(20%)**
9	**11**	**(27.5%)**
10	**7**	**(17.5%)**
Histological grade		
Gr II (5-7)	**14**	**(35%)**
Gr III (8-10)	**26**	**(65%)**
Perineural invasion		
Yes	**9**	**(22.5%)**
No	**31**	**(77.5%)**
Lympho/vascular invasion		
Yes	**3**	**(7.5%)**
No	**37**	**(92.5%)**
Clinical stage		
II-III	**7**	**(17.5%)**
IV	**33**	**(82.5%)**
Metastasis		
Yes	**33**	**(82.5%)**
No	**7**	**(17.5%)**
Site of distant metastasis		
Bone	**29**	**(87.87%)**
Liver	**2**	**(6.06%)**
Bone and others (lung , liver, pancrease)	**2**	**(6.06%)**
Biological recurrence		
Yes	**14**	**(35%)**
No	**19**	**(47.5%)**
Unknown	**7**	**(17.5%).**
Status at end point		
Death	**21**	**(52.5%)**
Alive	**12**	**(30%)**
Unknown	**7**	**(17.5%)**

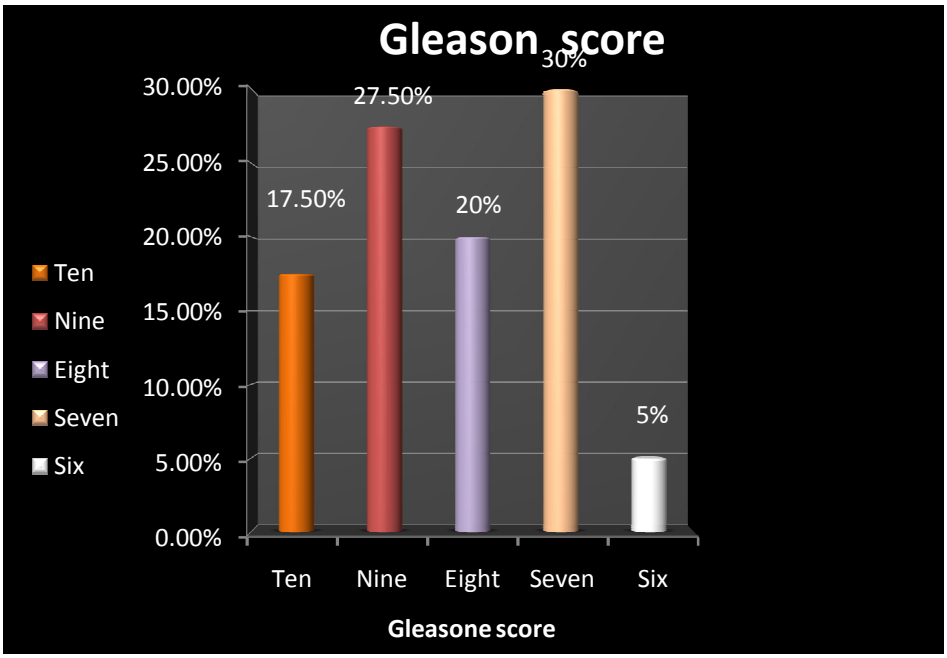
Among 40 cases in current study, 23 patients (57.5%) were 73 years or older while 17 patients (42.5%) were younger than 73 years, the mean age was 73 years.



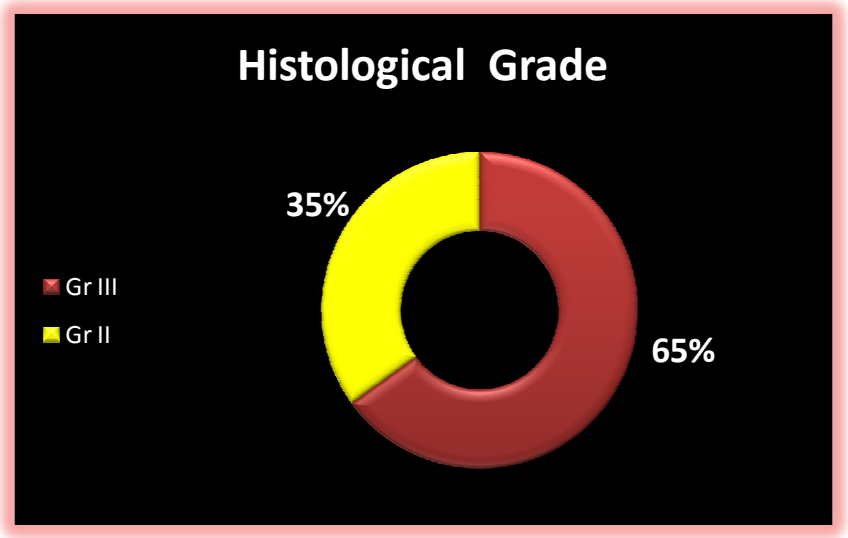
All patients have adenocarcinomas, 39 (97.5%) patients were acinar adenocarcinomas, only one case (2.5%) was diagnosed as ductal adenocarcinoma.



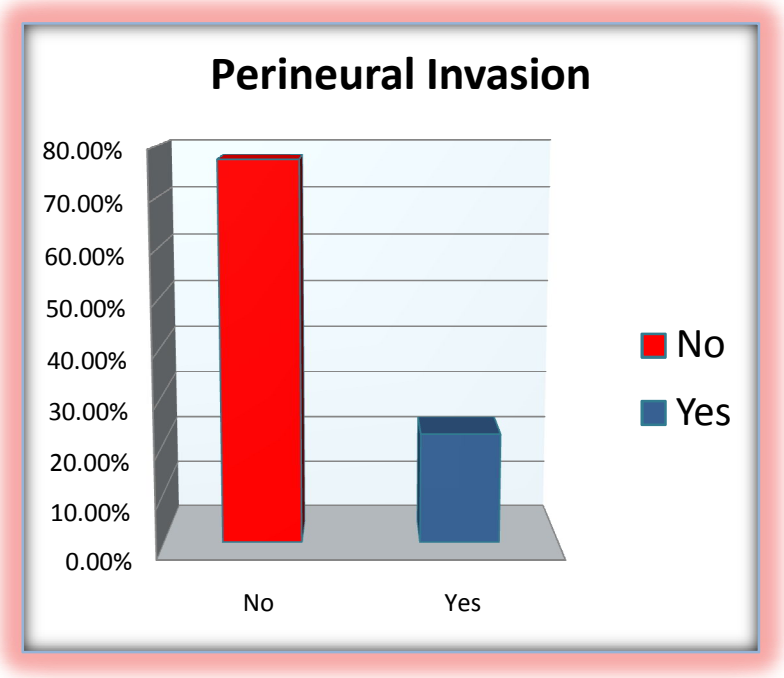
Among the 40 patients, 12 of them (30%) were of Gs 7, 11 (27.5%) of Gs 9, 8 (20%) of Gs 8, and only two patients of Gs 6 (5%).



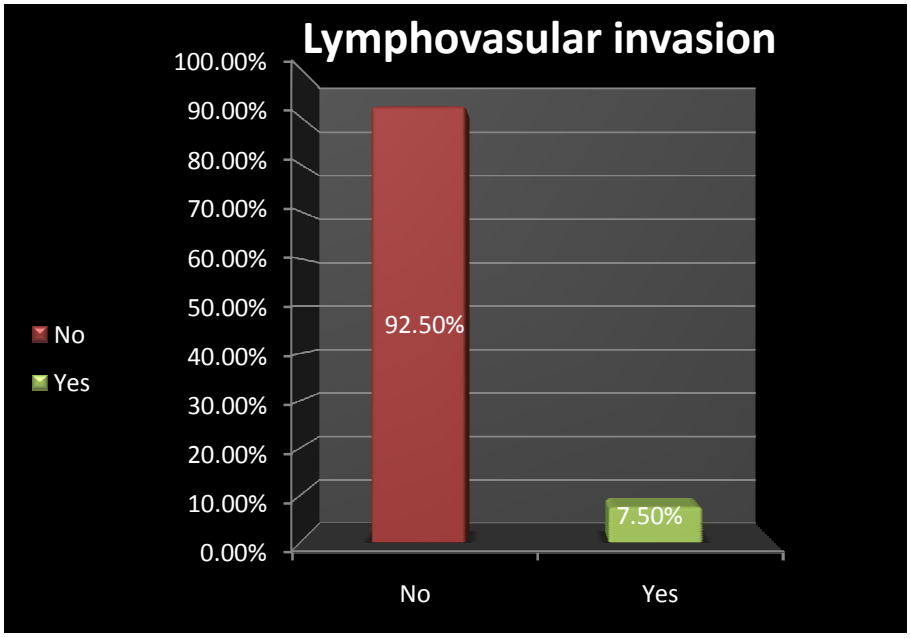
Among the 40 patients, 26 of them (65%) were poorly differentiated adenocarcinomas (Gs 8-10) by WHO grading system, 4 (35%) were moderately differentiated adenocarcinomas (Gs 5-7).



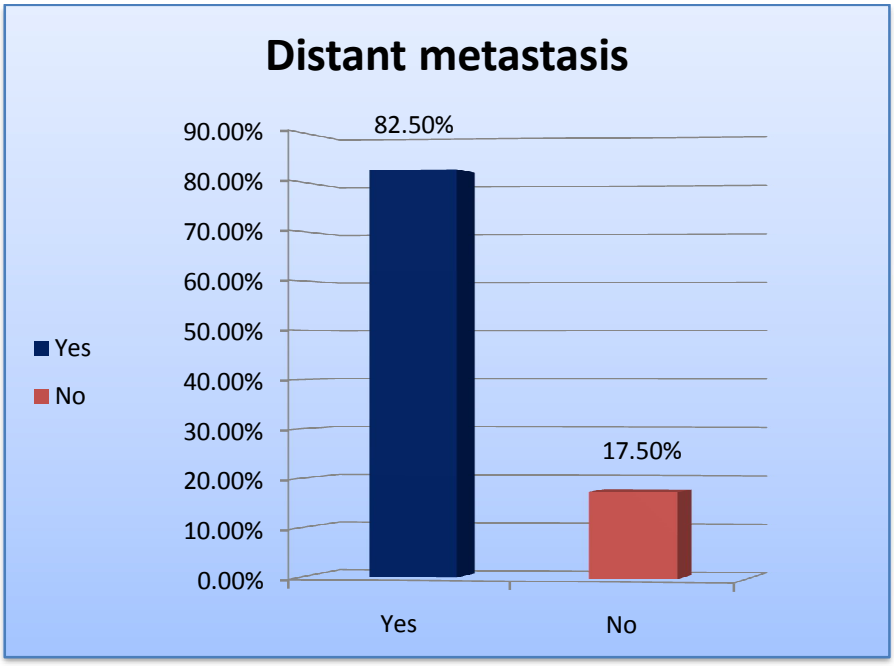
Regarding perineural invasion, 22 .5% of patients have perineural invasion whereas, 77.5% of them were free.

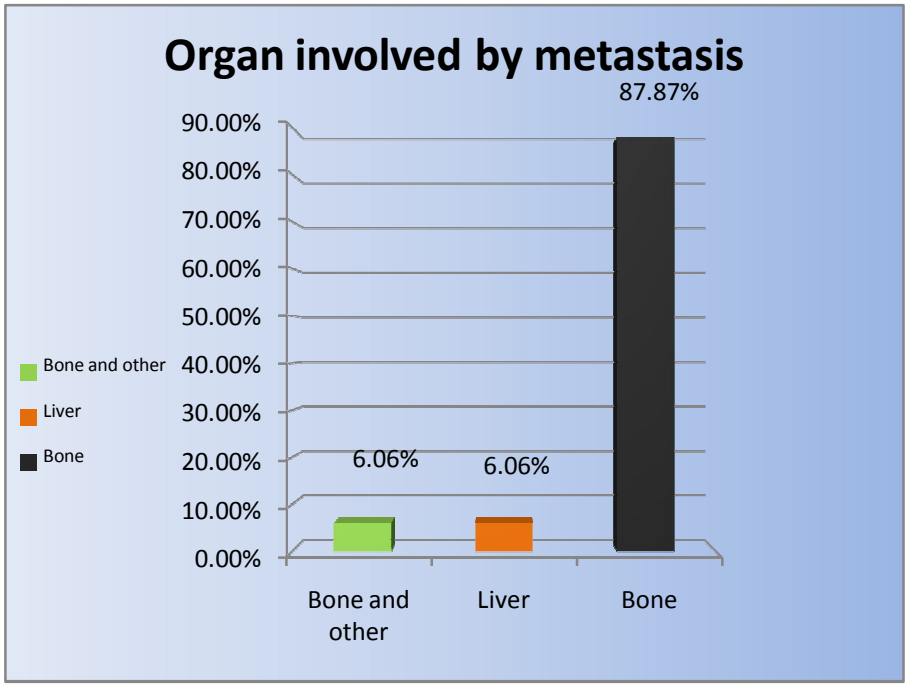


Regarding lymphovascular invasion, 92.5% of patients were free whereas, 7.5% have lymphovascular invasion.

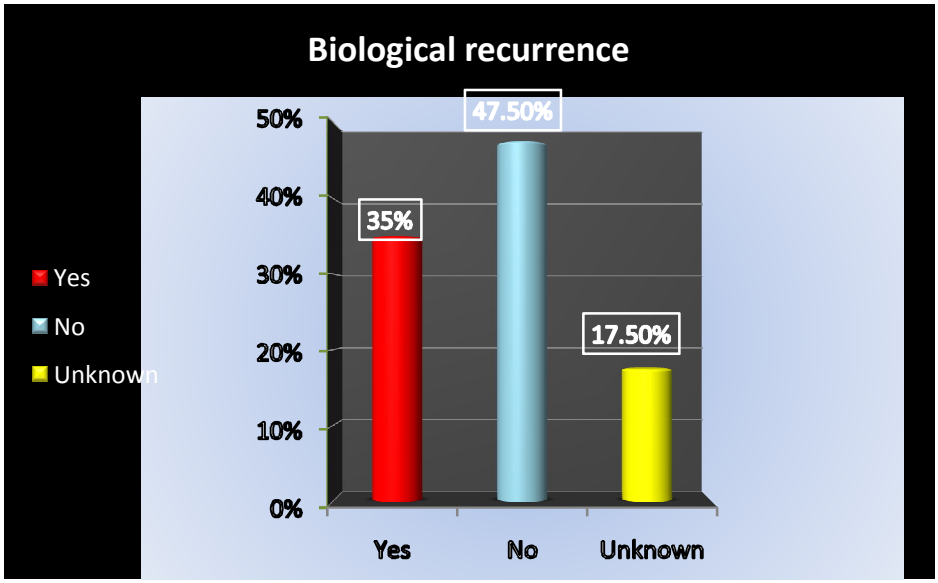


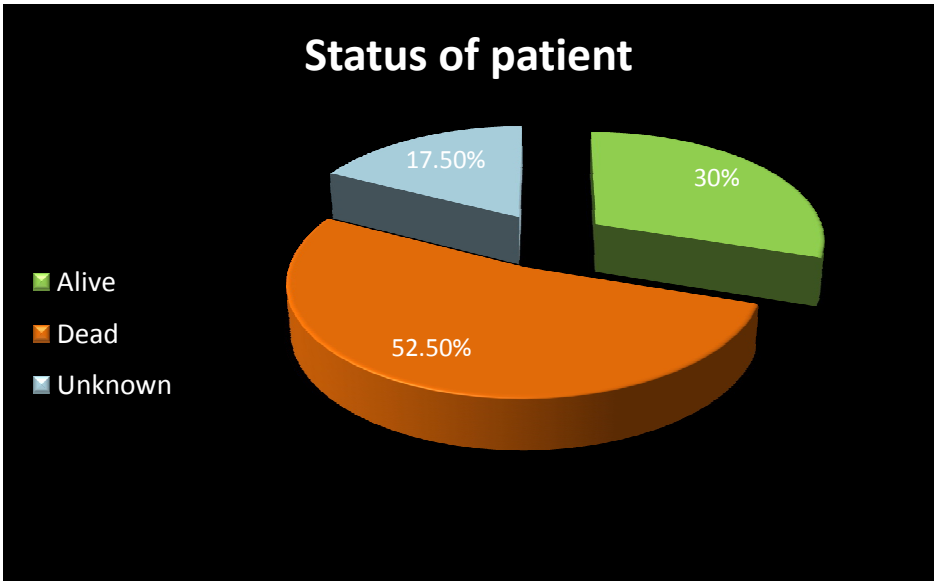
At time of diagnosis, a total of 33 patients (82.2%) had distant metastasis. The distant metastasis was distributed as following: 29 cases have bone metastasis, 2 cases have liver metastesis, one case has bone and pancrease metastasis, one case has bone, lung, liver.





The duration of follow-up was 6 years (72 months), biological recurrence were found in 14 patients (35%) whereas, 19 patients (47.5%) had no recurrence, 7 patients (17.5%) are missed. During this period more than half of patients (52.5%) had been documented as dead.





Immunohistochemical method

β-catenin immunostainig

Paraffin embedded blocks of PCa have been obtained from pathology department archive. Sections were cut serially at 5µm for immunohisto-chemical (IHC) staining. IHC analysis done using the automatic system (Bench- Mark XT, Ventana Medical System, Inc. Tucson, Arizona, USA). This fully automated processing for bar code labeled slides included baking of the slides, solvent free deparaffinization, antigen retrieval in a cell conditioning buffer CC1 (Mild: 36minutes conditioning, and standard: 60 minutes conditioning), incubation with Mouse monoclonal anti-β-catenin antibody (clone: 4, isotype: IgG1, Catalog no: 760-4242 Ventana Medical Systems), for 32 min, at 37_C. Application of ultra view TM universal DAB. Ultra view DAB includes: ultra view universal HRP, ultra view universal DAB inhibitor, ultra view universal DAB chromogen, ultra view universal DAB H2O2, and ultra view universal DAB copper. Counterstaining with haematoxylin (2021) for 4 minutes, and post-counterstaining with bluing reagent (2037) for 4 minutes. After staining, the sections will be dehydrated in ethanol, cleared in xylene, and covered with Mountex and cover slips.

Evaluation of β-catenin staining

The evaluation of staining was performed with a light microscope at the magnification of x40, blinded by the information on tumor grade, stage or clinical outcome. Membranous and cytoplasmic staining were evaluated separately. For cell membrance staining, four categories were used, (+++, ++, +, 0), 0) no expression, no detectable staining in < 10% of the membrance 1) weak but detectable discontinuous staining present in 10-39% of the membrance 2) moderate, clearly positive discontinuous staining present in 40-90% of the membrance and 3) intense continuous staining of the membrance create a honeycomb pattern (Elzagheid et al, 2002; Elzagheid et al, 2006). The cytoplasmic staining was also graded into four categories: (0) Negative, no detectable staining, (1) Weak, but detectable still staining, (2) Modereate, clearly positive but still weak, (3) Heavy staining, intense (Elzagheid et al, 2006). The membrance index (MI) was calculated with both the intensity of staining and fraction of positively-stained cells taken into account using the following formula:

$$\text{ı} = 0 * f0 + 1 * f1 + 2 * f2 + 3 * f3$$

Where ı ; is the staining index, f0-f3 are the fractions of the cells showing a defined level of staining intensity (from 0 to 3). Theoretically, the index could vary between 0 and 3 (Lipponen & Collan, 1992;. Buhmeida et al, 2008). The reproducibility of evaluation of β-catenin staining indices was tested by employing intra-observer reproducibility (90%).

Statistical analysis

Statistical analyses were performed using the SPSS® (SPSS, Inc., Chicago, USA) and STATA (Stata Corp., TX, USA) software packages (SPSS for Windows, version 18.0.3 and STATA/SE 11.1). Frequency tables were analyzed using the Chi-square test, with the likelihood ratio (LR) or Fisher's exact test being used to assess the significance of the correlation between the categorical variables. Differences in the means of continuous variables were analyzed using non-parametric tests (Mann-Whitney) or Kruskall-Wallis for 2- and K-independent samples respectively.

Analysis of variance was only used to derive the mean values (and 95% CI) of each individual stratum. Univariate survival analysis for the outcome measure [disease-specific survival (DSS) and disease-free survival (DFS)] was based on the Kaplan-Meier method, with log-rank (Mantel-Cox) comparison test. In all tests, $\mathbf{p < 0.05}$ was regarded as statistically significant.

CHAPTER IV

RESULTS

4. Results

Expression patterns of β-catenin

The expression pattern of β-catenin was premominantly membranous, in normal prostatic glands, hyperplastic prostatic glands and in tumor area as well. The expression patterns of β-catenin in PCa lesions are illustrated in the following figures respectively (Figure 4.1, 4.2, 4.3, 4.4, 4.5, 4.6). The mean value of β-catenin staining indice (MI) was **(2.5).**

Correlation of β-catenin expression with clinicopathlogical characteristics

The distribution of β-catenin expression in tumour sample in relation to clinicopathological characteristics is present in (Table 4.1, 4.2).

Using different cut-off points (mean, median, 4 tier score (0, 1, 2, 3), 2 vs 3). An interesting finding in our immunohistochemical study, β-catenin overexpresion (membranous) show a significant correlation with the age ($P< 0.024$) in that tumours of old patients (≥74 years) (13/18) overexpress β-catenin more than tumours of young patients (<74 years) (8/22).

Moreover, overexpression of β-catenin (membranous) were also significantly ($P<0.014$) associated with the higher grade (Gleason >7) in that Gleason grade >7 cancers (17/26) showed higher expression of β-catenin compared to Gleason grade (5-7) PCa (4/14).

There was no statistically significant difference in β-catenin immunoexpression as regards histopathological diagnosis, type of biopsy (core, TURP, radical prostatectomy), perineural invasion, lymphovascular invasion, tumor stage, recurrence.

Table 4.1. Expression of β-catenin in Libyan PCa patients as related to clinicopathological data and disease outcome

Features	Mean (2.5)	1,2,3	1,2 vs 3
Age	**0.024**	0.189	0.115
Type of biobsy	0.551	0.366	0.904
Diagnosis	0.335	0.897	0.666
Stage	0.574	0.893	0.929
Histological grade	**0.014**	**0.068**	**0.06**
Perineural invasion	0.970	-	-
Lymphovascular invasion	0.609	0.709	0.442
Metastasis	0.574	0.89	0.929
Biological recurrence	0.719	0.222	0.217
Status at the end point	0.895	0.580	0.483

Table 4.2. Expression of β-catenin in Libyan PCa patients as related to clinicopathological data and disease outcome

Features		Number (%)	B-catenin expression		P-Value
			Below mean	Above mean	
Age	≥74	18(45%)	5/19(26.3%)	13/23(61.9%)	0.024
	<74	22(55%)	14/19(73.7%)	8/21(38.1%)	
Type of biobsy	TURP	31(77.5%)	14/19(73.7%)	17/21(81%)	0.55
	Core biopsy	8(20%)	4/19(21.1%)	4/21(19%)	
	Radical prostatectomy	1(2.5%)	1/19(2.5%)	0	
Diagnosis	Acinar adenocarcinoma	39(97.5%)	19/19(100%)	20/21(95.2%)	0.525
	Ductal adenocarcinoma	1(2.5%)	0	1/21(4.8%)	
Histological grade	Gr II	14(35%)	10/19(52.6%)	4/21(19%)	0.014
	Gr III	26(65%)	9/19(47.4%)	17/21(81%)	
Perineural invasion	Yes	9(22.5%)	4/18(22.2%)	5/22(22.7%)	0.970
	No	31(77.5%)	14/18(77.8%)	17/22(77.3%)	
Lymphovascular invasion	Yes	37(92.5%)	1/19(5.3%)	2/21(9.5%)	0.609
	No	3(7.5%)	18/19(94.7%)	19/21(90.5%)	
Metastasis	Yes	33(82.5%)	15/19(78.9%)	18/21(85.7%)	0.574
	No	7(17.5%)	4/19(21.1%)	3/21(14.3%)	
Biological recurrence	Yes	13(40.6%)	7/16(43.75%)	10/16(62.5%)	0.719
	No	19(59.4%)	9/16(56.25%)	6/16(37.5%)	
Status at the end point	Alive	12(36.4%)	6/16(37.5%)	6/17(35.3%)	0.895
	Death	21(63.6%)	10/16(62.5%)	11/17(64.7%)	

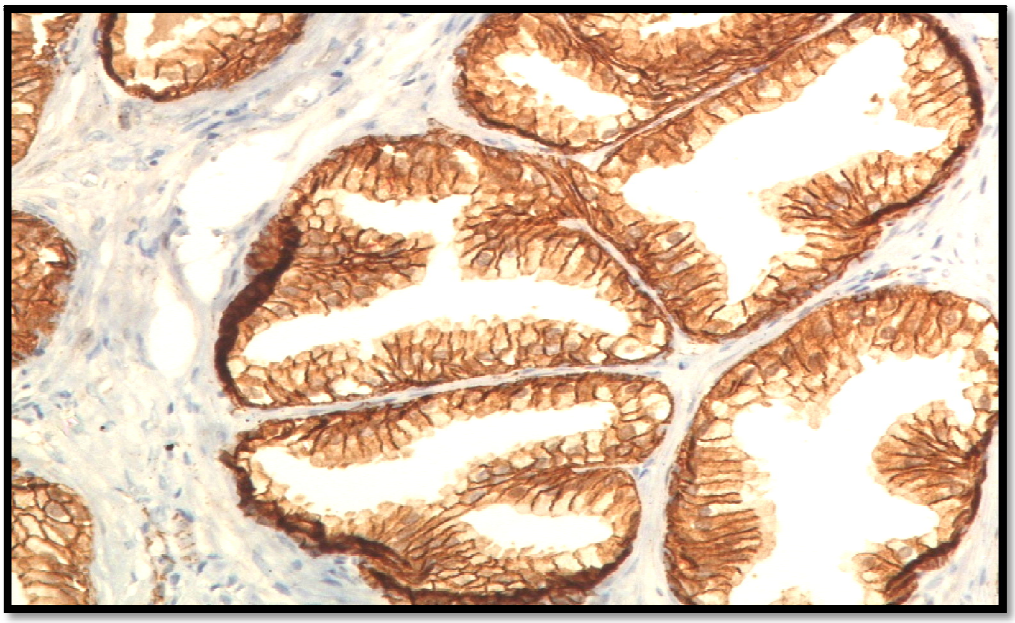


Figure. 4.1. (A)-Immunohistochemical staining of β-catenin. Membranous expression of β-catenin in being prostatic hyperplasia (IHCX20) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

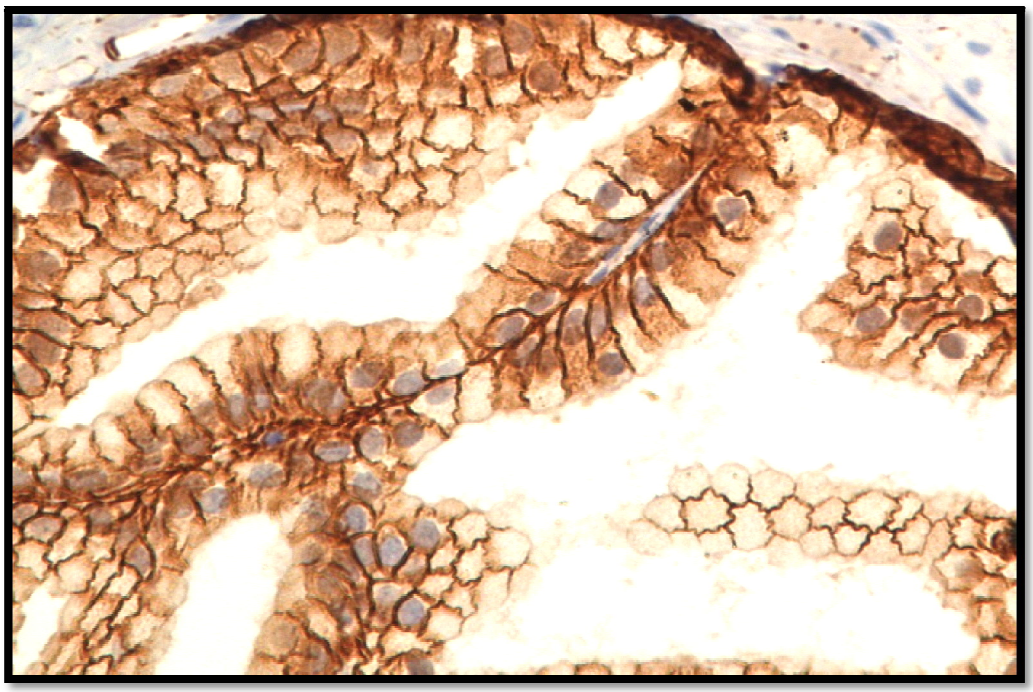


Figure. 4.1. (B). Immunohistochemical staining of β-catenin. Membranous expression of β-catenin in being prostatic hyperplasia (IHCX40) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

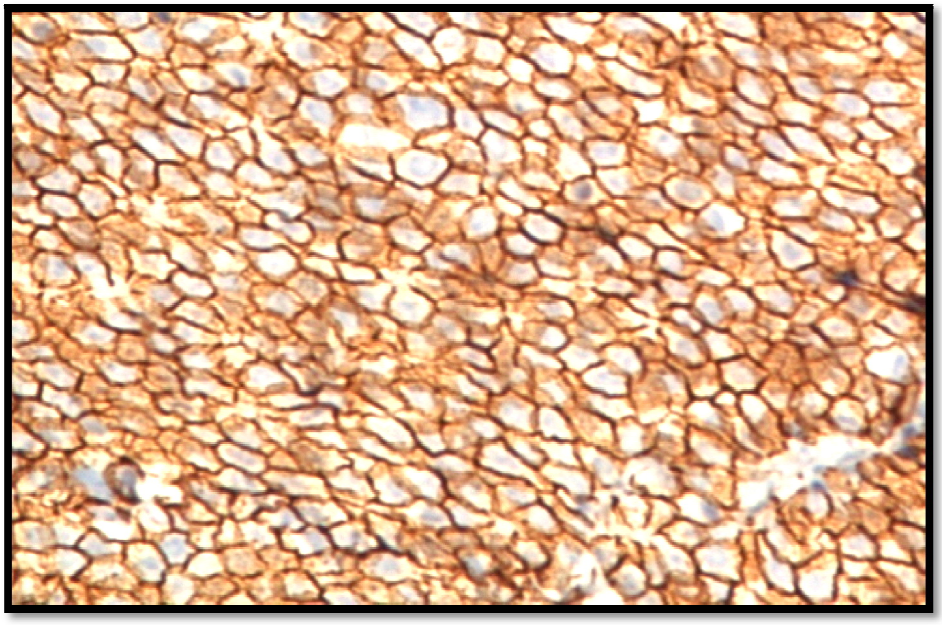


Figure 4.2. Strong membranous β-catenin expression in pattern five prostatic carcinoma (IHCX20) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

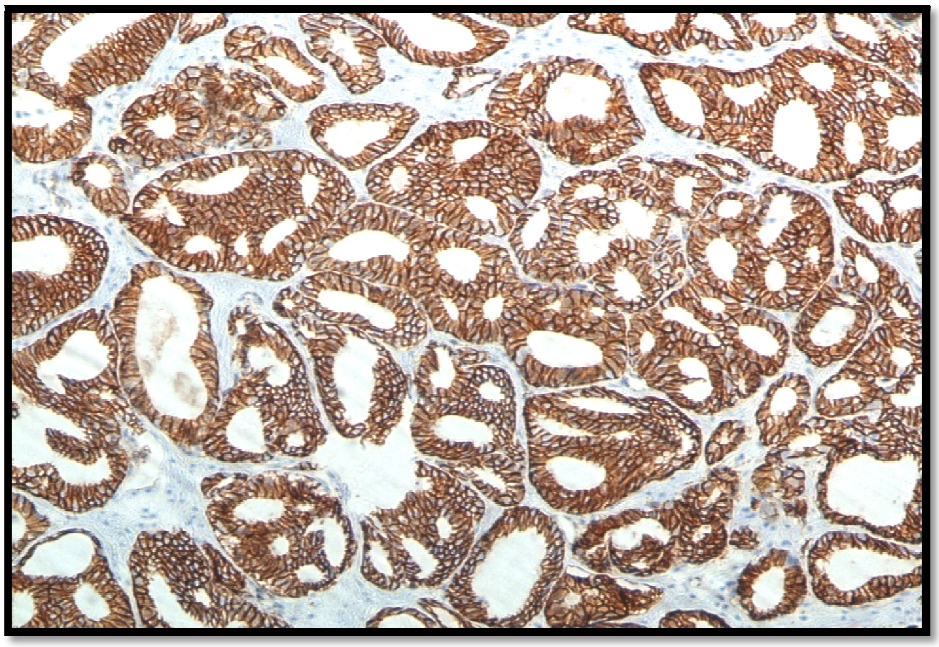


Figure 4.3 . Strong membranous β-catenin expression in pattern three prostatic carcinoma (IHCX20) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

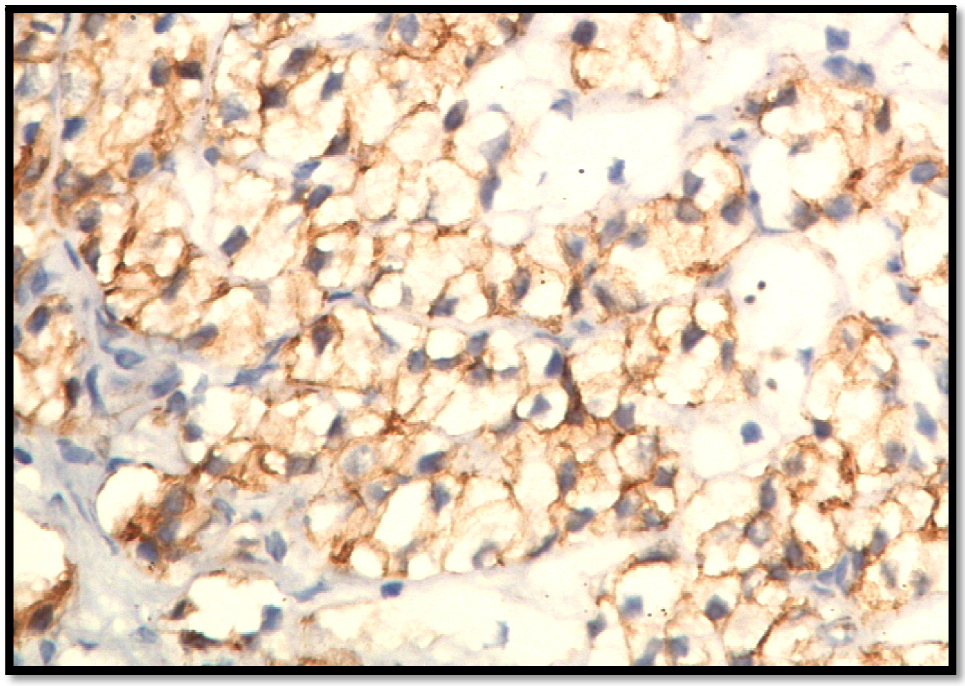


Figure 4.4. Moderate membranous β-catenin expression with weak membranous expression in some area of prostatic carcinoma (IHCX40) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

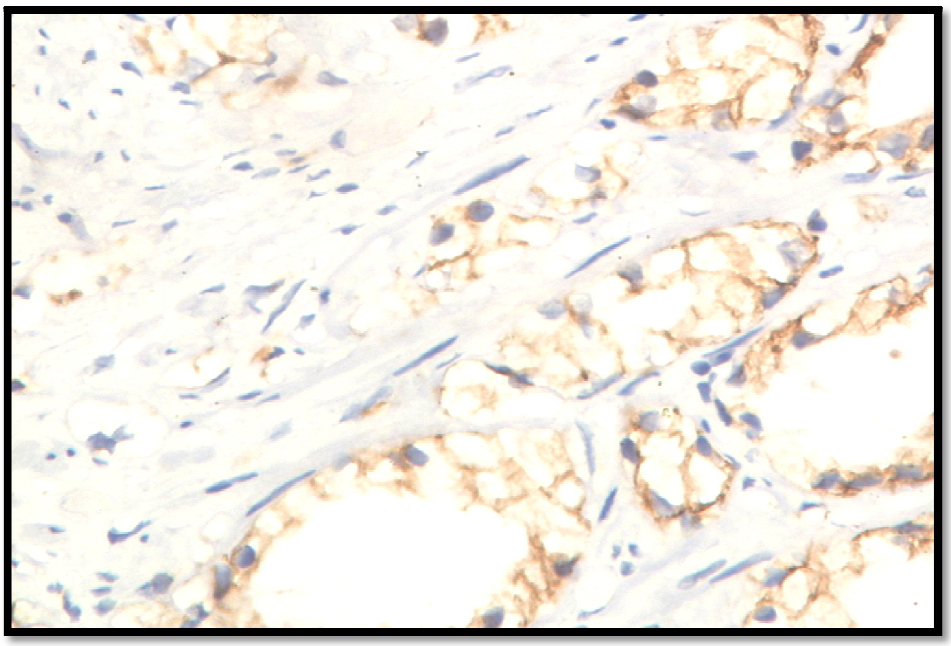


Figure 4.5. Weak membranous β-catenin expression of prostatic carcinoma (IHCX 40) (Department of Pathology, Faculty of Medicine, Benghazi University**)**.

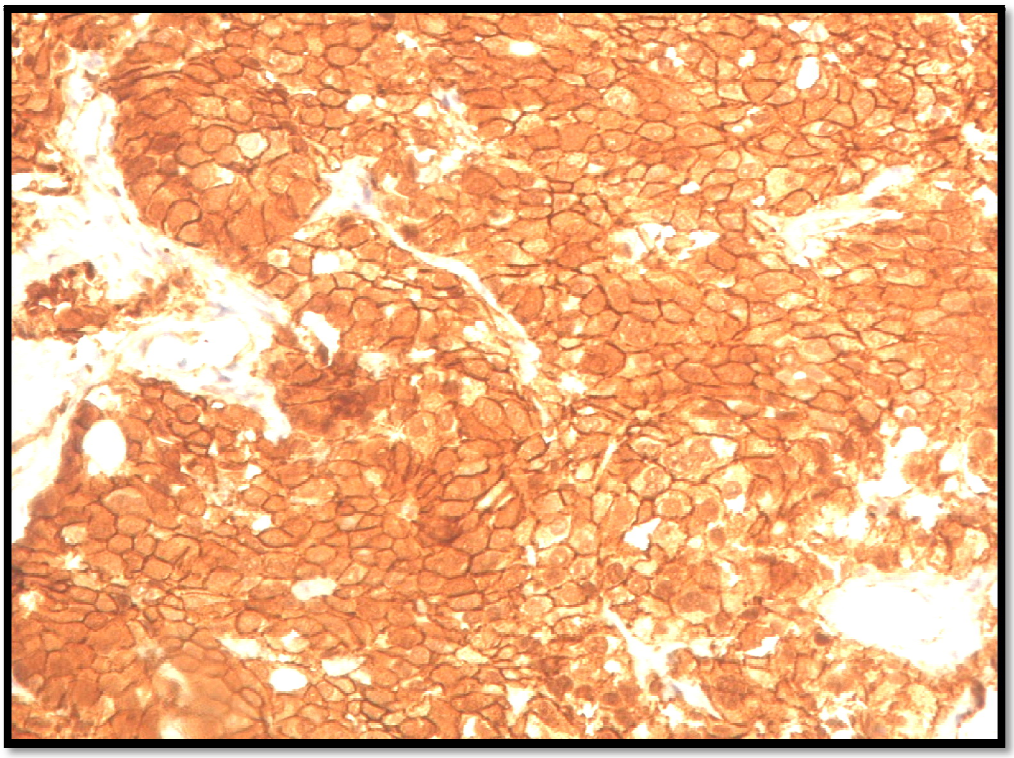


Figure 4.6. Strong membranous and moderate cytoplasmic β-catenin expression in prostate carcinoma (IHCX20) (Department of Pathology, Faculty of Medicine, Benghazi University).

CHAPTER V

DISCUSION

5. DISCUSION

PCa is a chronic and progressive disease frequently accompanied by irreversible and lethal metastasis. Defining the etiology, so as to provide measures of prediction and prevention, is the utmost priority of PCa research (Gunderson et al, 2011). NCI estimates that about ~240 890 American men will be diagnosed with PCa in 2011 and approximately ~33 720 will die of the disease. It is the most prevalent tumor in men and despite increasing efforts at early detection, 10–20% of the cases present bone metastasis at diagnosis. Most men diagnosed with PCa can survive the primary localized tumor; however, because of the widespread metastasis that are resistant to conventional treatment including improved surgical techniques, mortality rates remain extremely high. Development of PCa is prevalently asymptomatic, and once symptoms are noticed, it usually implies an advanced disease stage. Metastatic dissemination of cancer cells consists of series of sequential interrelated steps that lead to spread of the disease to distant organs such as bone, lymph nodes, rectum, urinary bladder, and brain, which ultimately leads to death. So, it is critical to understand the mechanisms that drive PCa to become metastatic. Moreover, it is also important to diagnose the disease at an early stage so that proper therapy can be administered, for which we need a predictable biomarker. Thus, by understanding the molecular events in the pathogenesis of PCa and detecting a reliable biomarker will offer improved diagnosis, prognosis, and therapy of the disease that will ultimately help us to eliminate PCa (Dasgupta et al, 2012).

The protein β-catenin has at least 2 functions of interest in PCa: it participates in cadherin-mediated adhesion, and it is the "molecular node" of the Wnt canonical signaling pathway (Wan et al, 2011). Previous studies implicated β-catenin in the pathogenesis of PCa because it localizes in tumor-cell nuclei in 20% to 40% of CRPC specimens (Chesire et al, 2002, de la Taille et al, 2003, Yardy et al, 2005). More recently, another group reported that activation of Wnt/β-catenin signaling is involved in PCa initiation and progression in a mouse model (Yu et al, 2009, Yu et al, 2011).

Together, these findings imply that the Wnt canonical pathway is involved in the pathogenesis of a subgroup of advanced PCa.

The present cohort of PCa patients enrolled at Benghazi University (Libya) is unique in that follow-up of patients covers 6 years. Accordingly, we could evaluate different clinicopathological features and outcome of PCa in Libyan patients. The aim of present study was to elucidate the biology of PCa in Libyan patients and cast further light on the issues related to prognostication of PCa while assessing the value of β-catenin expression profiles as predictive and prognostic factor. In addition, we used different approaches to analyze the expression of β-catenin.

According to the latest cancer incidence report from BCR, 2004, PCa was the fourth most cancer of men after Lung (19%), Colon and Rectum (11%), and Bladder (9%), there were 42 new PCa, accounting for 7% of all cancers in males. Age distribution shows that PCa mainly occurs in the elderly (85% of all cases occur in men aged 60 years or more) (El Mistiri et al, 2010). Worldwide comparisons are strongly influenced by the prevalence of PSA screenings. Therefore, with the highest rates recorded primarily in the developed countries of Oceania, Europe, and North America, largely because of the wide utilization of PSA testing that detects clinically important tumors as well as other slow-growing cancers that might otherwise escape diagnosis. In contrast, males of African descent in the Caribbean region (Uganda and Zimbabwe) have the highest PCa mortality rates in the world, which is thought to reflect partly difference in genetic, racial and ethnic factors (Jemal et al, 2011).

As shown in the current study, mean age of patients with PCa were 73 years, and 57.5% of our patient were 73 years age or older, which was almost similar to that of other investigators in Libya (El Mistiri et al, 2010) and neighboring countries e.g. Egypt (NCI), Egypt (Gharbiah), Suadi Arabia, Jordan (Elattar, 2005).

Eighty-nine percent of our PCa cases were Acinar adenocarcinomas, which is comparable to previous Libyan (El Hashmi et al, 2006; El Mistiri et al, 2010) and other findings (Chang et al, 2008).

Gleason score is the most used prognostic factor for PCa, with high scores particularly from 7 to 10 presenting a higher risk of death from PCa than low Gleason score

(Gleason score <4) cancers when patients aged 74 were treated conservatively (Albertsen et al, 1998). However, patients aged from 55 to 74 with Gleason score between 5 and 6 subjected to treatments are likely die from competing medical conditions and patients with Gleason score greater than 6 are likely to die from PCa despite treatment (Albertsen et al, 2005). After age 75 years average life expectancy in men is less than 10 years and there is general agreement that men older than 75 years are unlikely to benefit from PCa screening (Fowler et al, 2012). The distribution of Gleason grades has shifted over time, and in the era of PSA screening, most men are now diagnosed with Gleason 6 or 7 tumors (Martin et al, 2011). In our study, we found that (65%) of PCa cases were poorly differentiated adenocarcinomas (Gleasone 8-9), (35%) were moderately differentiated adenocarcinoma(Gleasone 5-7). Moreover, we found that (82.5%) of PCa cases were presented with distant metastasis at time of diagnosis. A similar observation has been previously reported by Rebbeck et al. who observed that a significantly greater proportion of tumors in Africa had a high Gleason score or high tumor stage compared with those in the USA or UK. In the UK and USA, the most common Gleason scores are 6 and 7, whereas the distribution in other regions is more uniform, with lower (≤5) and higher (≥8) scores representing a greater proportion of tumors than in the USA (Rebbeck et al, 2013). Delays in diagnosis could be an important factor in Libyan patients having high PSA level, high Gleason score, high tumor stage.

There have been a number of contradictory IHC studies of β-catenin expression in PCa (Table 2.11). It has been observed that β-Catenin expression and localization change during human prostate cancer progression, however, results are inconsistent. Several studies have seen an increase in β-Catenin expression and nuclear localization in late stage cancer samples this is particularly true of advanced, metastatic and hormone refractory prostate cancer, while others have reported a loss in nuclear expression in advanced tumours (Francis et al, 2013).

In this study, we examined the expression and localization of β-catenin protein in a subset of PCa and a number of adjacent histologically normal and hyperplastic mucosa. The results showed that a membranous staining pattern was well preserved in prostatic adenocarcinomas without detectable nuclear immunoreactivity. These results are in general agreement with those of previous studies in which no nuclear β-catenin

immunostaing was observed in prostatic adenocarcinomas (Morita et al, 1999, Saha et al, 2008).

An interesting finding in our immunohistochemical studies is that the membranous overexppresion of β-catenin staining occurs mainly in cases with higher Gleason scores (>7) prostatic adenocarcinomas. This finding is consistent with finding of Whitaker et al. who observed that increased β-catenin expression in only high Gleason score (>7) prostate cancer and a gradual loss in nuclear distribution with increasing Gleason grade (Whitaker et al, 2008). The same observation was demonstrated by Chen et al. who observated the High levels of Wnt-1 and β-catenin expression were associated with advanced, metastatic, hormone-refractory prostate carcinoma, in which they can serve as markers of disease progression (Chen et al, 2004). In distinct contrast to our results, however, Bismar et al. reported that the loss of β-catenin membranous staining noted in a small fraction of prostatic adenocarcinomas occurs mainly in cases with higher Gleason scores (Bismar et al, 2004). The studies by Kallakury et al. further showed that loss of β-catenin expression, which occurred in 4% to 5% of the prostatic adenocarcinomas in their series, was associated with high tumor grade (Kallakury et al, 2001). Although, (Szász et al, 2008; Lazari et al, 2013) reported that there were no signification association between β-catenin expression and Gleason score.

However, some studies have demonstrated that the membranous overexpression of β-catenin is significantly associated with the metastatic prostate cancer cells in the bone and that the high frequency of expression suggests its involvement in the intercellular adhesion of the metastatic cells in the bone (van Oort et al, 2007, Saha et al, 2008).

The discrepancy between our results and other may be attributed to significant differences in the methodologies employed for samples collection, fixation and the protocols used for immunohistochemical staining.

In the current study, we did not find any significant correlation between β-catenin expression and lymphovascular invasion. A similar finding has been reported by (Morita et al, 1999).

CHAPTER VI

CONCLUSIONS & RECOMMENDATIONS

6.1. Summary and conclusions

- PCa is the second most common cause of cancer and the sixth leading cause of cancer death among men worldwide.
- In Libya, PCa was the fourth most cancer of men after Lung , Colon and Rectum , and Bladder.
- PSA testing has revolutionized the diagnosis of PCa, since it is now possible to detect most prostate tumors at early stages, unlike other cancers that lack a straightforward method for early detection.
- Libyan men represented a particularly high risk group for the development of PCa, (82.5%) have pretreatment PSA level greater than 20ng/ml, (82.5%) stage VI with (94%) have bone metastesis, (65%) high grade cancer (Gleason score8-10). These are at a high risk of biochemical progression (that is, further cancer growth), regardless of whether they received surgery or radiation.
- β-catenin immunostaning results show overexpression of β-catenin in PCa Libyan patients.
- β-catenin overexpression showed significant correlation with age ($p<0.024$), Gleasone score ($p<0.014$).
- We concluded that changes in expression and cell distribution of β-catenin correlated with the progression degree of prostate adenocarcinoma, suggesting a role of this molecule as marker of progression and prognosis.

6.2.Recommendations

- Establishment of electronic archives to facilitate collection of data for further studies in the future.
- Provision of the National cancer registry of Libya, to give more precise information.
- Provision of the National Guidline in histopathology reporting.
- The members of Oncology, Pathology, Surgon and Radiology must work as one team for more productive result.
- PSA–based screening Programs should start in men aged 50 to 74 years, along with other screening methods, such as digital rectal DRE or transrectal ultrasonography.
- Raising the awareness of PCa in Libyan community and implementing the National Awareness and Early Diagnosis Initiative, led by Department of Health will provide a vehicle for earlier diagnosis and better outcome particularly for those at higher risk of PCa.
- For initial biopsy should be using extended biopsy schemes (10–13 cores) including laterally directed biopsies.
- The anatomic location or locations of carcinoma within total prostatectomy specimens should be specified in the pathology report whenever possible.
- We recommend continuous monitoring of the newly diagnosed cases, measuring of the morbidity caused by this disease and keeping an eye on its mortality rates.
- Further studies with larger numbers of patients to prove the significant effect of β-catenin as markers of progression and prognosis among Libyan patients.
- Findings merit follow-up in additional studies with larger sample size to confirm the association and to investigate the underpinning of the genetic association (PTEN, AR).

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قدمت هذه الدراسة لقسم علم الأمراض استكمالا لنيل الإجازة العليا

إعداد

وردة محمد مصباح سعيد

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الملخص العربي

ملخص البحث:

الهدف:

هدف هذه الرسالة البحثية هي تسليط الضوء على عوامل الالتصاق في سرطان البروستاتا بواسطة الكشف على الجزئيات اللاصقة (بيتى كاتينين) وتقييم دورها في حالات مرضى سرطان البروستاتا في ليبيا وتقييم علاقة هذه الجزئيات بعوامل التنبؤ التقليدية وأثرها على حياة المرضى.

المرضى وطرق الدراسة:

تم اخذ عينات 40 مريض من مرضى سرطان البروستاتا في شرق ليبيا من واقع سجلات قسم علم الأمراض بجامعة بنغازي ومن ثم متابعة هذه الحالات في قسم الأورام بمركز بنغازي الطبي. وقد تم صبغ هذه العينات بعامل الالتصاق المذكور أعلاه واختبار هذه العينات إحصائيا.

النتائج:

بعد صبغ العينات بعامل التنبؤ وجد أن هناك زيادة في الجزئيات اللاصقة في حالات سرطان البروستاتا لدى المرضى الليبيين، مع وجود علاقة بين هذه الزيادة في الجزئيات اللاصقة وتقدم عمر المريض وزيادة تصنيف قليسون.

المقدمة:

طبقا لتقرير المعهد القومي للسرطان، يأتي سرطان البروستاتا في المرتبة الثانية في اكتر أنواع السرطانات شيوعا بين الرجال في الولايات المتحدة الأمريكية وفى واقع الأمر، فان رجلا من بين ستة رجال يتم تشخيص إصابته بسرطان البروستاتا. كما إن الرجال الذين يخضعون لعوامل خطر محددة هم اكتر عرضة من غيرهم للإصابة بالمرض.

في شرق ليبيا سرطانات البروستاتا هي رابع أكثر الأورام شيوعا بين الذكور، عام 2004 تم تسجيل 42 حالة جديدة، وهى تشكل 7% من مجموع الحالات.

سرطان البروستاتا وعوامل الخطر:

العمر: يمثل العمر عامل الخطر الرئيسي في سرطان البروستاتا، فالإصابة بالمرض نادرة في الرجال تحت سن الخامسة والأربعين، وتزداد فرصة الإصابة بسرطان البروستاتا بشدة كلما تقدم العمر. ومعظم الرجال المصابين بسرطان البروستاتا في الولايات المتحدة الأمريكية فوق الخامسة والستين.

التاريخ العائلي: يزداد خطر إصابة الرجل بسرطان البروستاتا إذا كان والده أو أخوه مصاباً بالمرض.

العرق: سرطان البروستاتا أكثر شيوعاً في الأمريكيين من أصل أفريقي عن الرجال البيض، بمن فيهم البيض من أصل أسباني. كما أنه أقل شيوعاً في الأمريكيين من أصل أسيوي والأمريكيين الأصليين.

النظام الغذائي: تشير بعض الدراسات إلى أن الرجال الذين يعتمدون في نظامهم الغذائي على أطعمة غنية بالدهن الحيواني أو اللحوم يزيد لديهم خطر الإصابة بسرطان البروستاتا. الرجال الذين يعتمدون على الأنظمة الغذائية الغنية بالفواكه والخضروات يقل لديهم خطر الإصابة بالمرض.

تشخيص سرطان البروستاتا:

الاختبارات والإجراءات التالية التي يمكن استخدامها:

دلالات الأورام: وهو الاختبار الذي يقيس مستوى (PSA) في الدم ، ارتفاع مستواه فى الدم عن المعدلات الطبيعية في المرضى الذين يعانون من عدوى أو التهاب في البروستاتا، أورام البروستاتا.

الموجات فوق الصوتية من خلال المستقيم : إجراء يتم فيه فحص البروستاتا بالموجات الفوق صوتيه من خلال وضع الالمسبار (جهاز الفحص) داخل الشرج. يمكن أيضا من خلال الفحص بهذه الطريقة اخذ عينه من البروستاتا او اى تورمات تظهر داخلها.

اخذ عينه من الورم الموجود بالبروستاتا يمكن من فحص انسجتها تحت الميكروسكوب و تحديد درجة (gleason score) وتتراوح درجة غليسون من 2-10.

مراحل سرطان البروستاتا :

عندما تم تشخيص المريض مع سرطان البروستاتا ، من المهم تحديد تطور المرض وإمكانية وصف العلاجات المناسبة لذلك. العملية المستخدمة لمعرفة تقدم السرطان، وكيفية انتشاره تسمى التدريج. سيقوم الطبيب بحاجة إلى معرفة إذا تم احتواء المرض في البروستاتا أو انتشاره إلى مناطق أخرى من الجسم. وتستخدم هذه العملية انطلاق لتحديد مرحلة المرض.

مع سرطان البروستاتا هناك أربع مراحل للمرض. كما ينتقل سرطان البروستاتا من المرحلة الأولى إلى المرحلة الرابعة، فإن الخلايا السرطانية تقوم بالانتقال من داخل البروستاتا إلى خارجها إلى الأنسجة المجاورة والغدد الليمفاوية وغيرها من الأجهزة في مرحلة متقدمة من المرض.

عندما يتم فحص أنسجة البروستاتا تحت المجهر، فإن التصنيف المستخدم لذلك يسمى قليسون والدرجة المحتملة تتراوح ما بين (2-10) وبالتالي (2) تعتبر درجة منخفضة وتتوافق مع أدنى فرصة إلى تتطور السرطان وانتشاره ويشير(10) إلى ارتفاع فرصة تتطور السرطان وانتشاره داخل الجسم.

لتصنيف مراحل سرطان البروستاتا تي ان ام حدد النظام الأساسى لذلك.
تي -- يصف حجم المنطقة الرئيسية لسرطان البروستاتا.
إن -- يصف ما إذا انتشر سرطان البروستاتا إلى أي الغدد الليمفاوية وإلى أي مدى.
إم (الإنبثاث) – يعني الإنتشار البعيد لسرطان البروستاتا ، على سبيل المثال ، إلى العظام أو الكبد.

المرحلة الأولى:
في المرحلة الأولى، خلايا السرطان توجد في البروستاتا فقط. ولايمكن الشعور بها فى الفحص الدورى للمستقيم وغير مرئية من قبل التصوير (الإشعاعات). وهى توجد عادة عن طريق الصدفة أثناء عملية جراحية لأسباب أخرى، مثل تضخم البروستاتا الحميد. وتكون درجة قليسون منخفضة.
المرحلة الاولى من سرطان البروستاتا تسمى ايضا المرحلة (أ1).

المرحلة الثانية:

في المرحلة الثانية ، السرطان هو أكثر تقدما مما كانت عليه في المرحلة الأولى ، ولكن لم ينتشر خارج البروستاتا. وتكون درجة (gleason score تتراوح بين 2-10. المرحلة الثانية من سرطان البروستاتا تسمى أيضا المرحلة (أ2)، (ب1)، (ب2).

المرحلة الثالثة:

في المرحلة الثالثة ، خلايا السرطان انتشرت خارج الطبقة الخارجية من البروستاتا إلى الأنسجة المجاورة و انتشرت في الحويصلات المنوية. ويكون (gleason score) يتراوح بين 2-10. المرحلة الثالثة من سرطان البروستاتا قد تسمى ايضا المرحلة (ج) لسرطان البروستاتا.

المرحلة الرابعة:

في المرحلة الرابعة ، ينتشر السرطان فى الجسد إلى الغدد الليمفاوية القريبة أو البعيدة من البروستاتا أو إلى أجزاء أخرى من الجسم ، مثل المثانة ، المستقيم والعظام والكبد ، أو الرئتين. سرطان البروستاتا المنتشر غالبا ما ينتشر الى العظام. المرحلة الرابعة من سرطان البروستاتا تسمى ايضا المرحل (د1)أو (د2) لسرطان البروستاتا.