CHAPTER I

INTRODUCTION

Prostate Structure and Biology

The prostate is a male accessory reproductive organ, which is found in all orders of mammals, including monotremes (1). McNeal described three anatomically distinct zones of the human prostate gland: the *peripheral zone* representing 70-75%, the *central zone* representing 20-25% and the *transition zone* accounting for 5-10% of the prostate gland (2). In the *peripheral zone,* ducts radiate laterally from the distal prostatic urethra and coincide with the ejaculatory duct axis (3). The wedge-shaped *central zone* surrounds

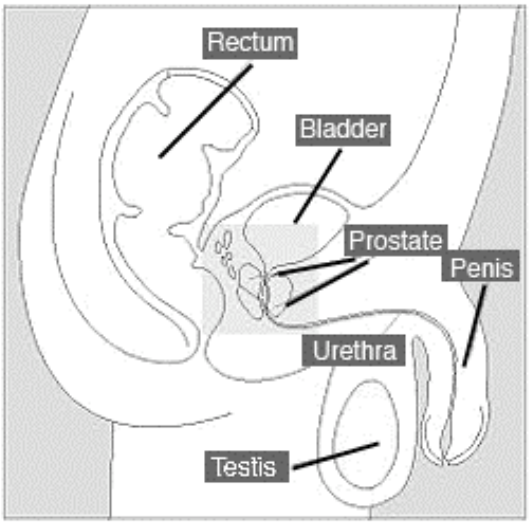

Figure 1. The Adult Prostate and Surrounding Structures. The prostate is located just beneath the bladder, in front of the rectum. The urethra that carries urine from the bladder out through the penis, bisects the prostate.

the ejaculatory ducts and has typically larger acini and more complex ductal branching than the peripheral zone. *Transition zone* is made of ducts arising from the urethra and is separated from the surrounding periurethral glands by the preprostatic sphincter. The *anterior fibromuscular stroma,* representing the most anterior part of the prostate gland, is composed of preprostatic sphincter, anterior detrusor muscle, internal sphincter, and a portion of the striated urethral sphincter (4).

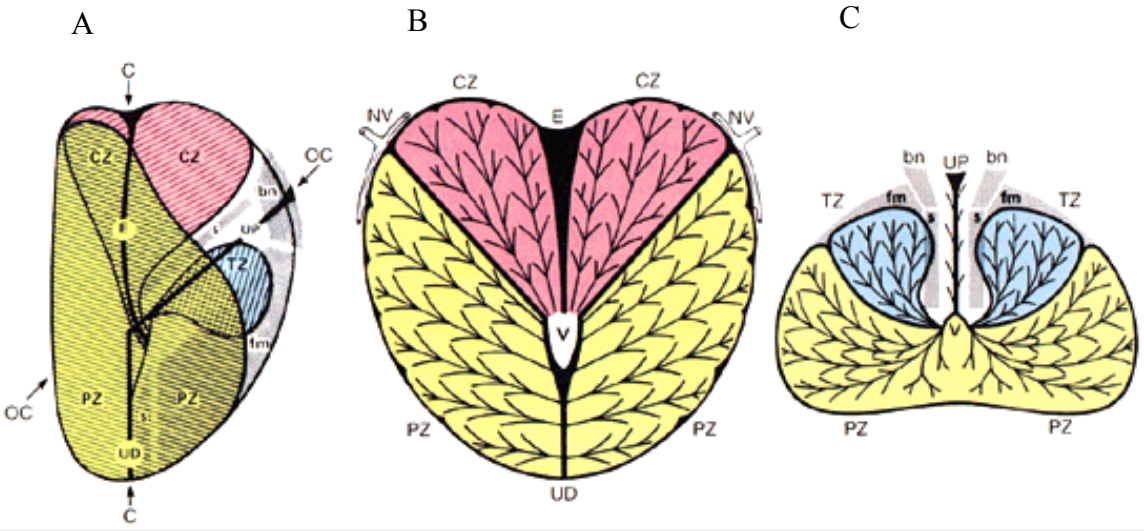

Figure 2. Zonal architecture of the Human Prostate. ***A:*** Sagittal, ***B:*** Coronal and ***C:*** Oblique sections of the prostate. Ducts radiate from verumontanum (V). bn, bladder neck; C, coronal plane; CZ, central zone; E, ejaculatory duct; fm, fibromuscular stroma; NV, neurovascular tissue; OC, oblique coronal plane; PZ, peripheral zone; s, sphincter; TZ, transition zone; UD, distal prostatic urethra; UP, proximal prostatic urethra. (From McNeal JE. The prostate gland: morphology and pathobiology. *Monogr Urol* 1988;9:36–54)

Ductal Branching Morphogenesis

Development of the prostate gland is initiated during fetal life and is completed at sexual maturity. Prostate development is a culmination of numerous coordinated cellular

processes including ductal branching morphogenesis and canalization, epithelial and mesenchymal differentiation, and proliferation. Prostatic buds emerge from the urogenital sinus on day 17 in embryonic mice, on day 19 in embryonic rats and during the tenth week in human fetuses (5). The spatial pattern of the emerging prostatic buds provides the foundation of the subsequent development of the rodent prostate into four distinct lobes – *dorsal, ventral, lateral* and *anterior.* In contrast, the human prostate is organized into zones as described earlier (6).

In mouse prostate, 80% of the branching morphogenesis occurs in the first 15 days of postnatal life. During this time, individual prostatic buds emerging from urethra elongate and branch (7) giving rise to a branching pattern that is characteristic of each lobe of the rodent prostate. The *ventral* prostatic buds, for example, branch dichotomously at regular intervals. In contrast, the *dorsal* prostatic ducts elongate considerably before branching to numerous tight spaced ducts. In the *lateral lobe,* the ducts emerging from the urogenital sinus close to the dorsal ducts, wrap around the periphery of the prostate with a dichotomous branching pattern similar to the *ventral lobe* (7).

Mesenchymal and Epithelial Differentiation

In addition to ductal branching morphogenesis, the first 15 days of postnatal life in rodents also witnesses epithelial and mesenchymal (stromal) differentiation in the prostate. In human prostate, the action of androgen on prostatic mesenchyme during fetal and prepubertal development induces ductal budding and epithelial proliferation

accompanied by differentiation into luminal and basal epithelial cells (8, 9). Basal epithelial cells characterized by the expression of cytokeratin 5 and 14 as well as p63, localizes along the basement membrane to form a continuous layer. Tall columnar luminal epithelial cells, chartacterized by the expression of cytokeratins 8 and 18 line the ductal lumina and produce the prostatic secretions (8, 10).

Androgen receptor (AR) is another differentiation marker for prostatic epithelium. Although AR is not expressed in the epithelia in the embryonic prostatic buds, the mesenchyme of the surrounding urogenital sinus exhibits high levels of receptor expression. During neonatal period, the differentiating epithelia begin to express low levels of AR (11). In rodent prostate, AR expression in the epithelial compartment can be detected at approximately 2 to 6 days postnatally (8, 12), and by day 15 all the luminal cells stain positive for AR (13). In human prostate, AR can be detected in the urogenital sinus mesenchyme before prostatic budding is initiated (12). With differentiation of the mesenchyme, the interductal fibroblasts lose AR expression, even as the smooth muscle cells retain the receptor expression (12). The adult prostate tissue both in human and rodents is largely growth quiescent, in spite of high levels of androgen. This raises the hypothesis that, in normal adult prostate, the principal function of AR is not to induce proliferation but to maintain the cytodifferentiation of the epithelia and the surrounding mesenchyme.

Epithelial-Mesenchymal-Transition (EMT)

During recent years, the EMT phenotype has emerged as a central process during embryonic development, chronic inflammation and fibrosis, as well as cancer progression. The EMT phenotype involves the transition of polarized epithelial cells to a highly motile fibroblastoid or mesenchymal phenotype. The role of EMT in normal prostate development and growth is not properly understood. The potential role of EMT

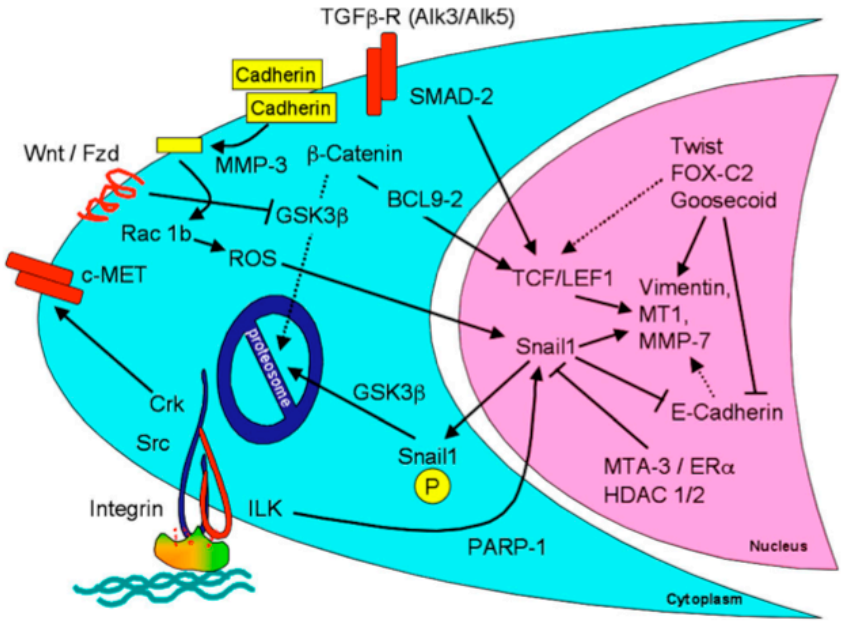

Figure 3. TGFβ-Mediated EMT Mechanism. The major signaling events reported in EMT are summarized. The TGFβ axis interacts with other signaling molecules such as the Wnt, TCF-LEF1, BCL9-2 to repress E-cadherin expression. This is accompanied by a concomitant increase in Vimentin expression. The c-Met receptor tyrosine kinase also stimuates EMT. (From Thomson et al. Journal of Cell Biology, 2006;172:973-981)

in the progression of PCa to a more invasive phenotype is examined in greater details in Chapters V and VI. In the context of tumorigenesis, EMT has been studied in various

tissue culture models of epithelial cells and transgenic mouse tumor models. In a vast majority of these models, TGFβ signaling cooperates with oncogenic Ras or Receptor Tyrosine Kinases (RTKs) to cause EMT and metastasis. Several other signal transduction pathways, such as the Wnt/β-catenin, MAPK, Notch, Sonic Hedgehog pathways, have also emerged as critical modulators for EMT, often correlating with tumor progression and metastasis *in vivo.* As depicted in Figure 3, these pathways can be stimulated by specific signals but also involves a great deal of cross talk between each other as well as with the TGFβ signaling axis to induce EMT.

Diseases of the Prostate

The human prostate can be diagnosed with both benign and malignant conditions. The presence of Lower Urinary Tract Symptoms or LUTS characterized by urgent need to urinate, frequent urination and nocturia, can be indicative of a benign or malignant condition. Prostatitis and Benign Prostatic Hyperplasia are the two most commonly diagnosed benign conditions and adenocarcinoma is the most frequently diagnosed malignant condition encountered in the clinic.

Prostatitis

Two-ten percent of men worldwide are diagnosed with prostatitis in their lifetime. According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), prostatitis can be classified into the following four categories:

Category I: **Acute Bacterial Prostatitis:** This category is relatively rare and occur in about 2-5% of patients presenting with prostatitis conditions. It is typically caused by infection by uropathogenic bacteria and patients manifest an acute onset of local (perineal prostatic pain, dysuria and obstructive urinary symptoms) and systemic symptoms (sepsis, fevers, chills and malaise). These patients respond well to antomicrobial treatment.

Category II: **Chronic Bacterial Prostatitis:** This form of prostatitis is also relatively rare accounting for 2-5% of the cases. Chronic infection of the prostate gland is caused by uropathogens and causes intermittent local symptoms only. Chronic prostatitis can also be treated successfully with antimicrobial agents.

Category III: **Chronic Nonbacterial Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS):** This is the most common form of prostatitis with 90-95% of the cases falling under this category. CP/CPPS is characterized by local symptoms such as pelvic pain, urinary symptoms and ejaculatory symptoms. No uropathogen has been associated with this disease and as such they are unresponsive to antimicrobial treatment. Depending on the presence or absence of leukocytes in the prostatic fluid, this form of prostatitis has been further classified as **inflammatory (IIIA)** or **non-inflammatory (IIIB)**.

Category IV: **Asymptomatic Inflammatory Prostatitis:** No pathogens have been implicated in this form of prostatitis and is frequently diagnosed as a result of incidental observation of leukocytes in prostatic secretions or the prostate tissue during evaluation of other disorders such as prostate biopsies following elevated PSA. Usually, no treatment is recommended for asymptomatic inflammatory prostatitis.

Although prostatitis is a non-life-threatening disorder of the prostate gland, recent studies have suggested a correlation between chronic inflammation and increased cancer risk. However, other studies have found no statistically significant correlation between prostatitis and occurrence of prostate cancer. Hence, further investigation must assess whether prostatitis is a precursor to prostate cancer.

Benign Prostatic Hyperplasia (BPH)

The incidence of BPH, another common disease of the prostate, increases with age: 50% of the male population over the age 50 is diagnosed with this disease and it is clinically evident in 80% of males by age 80 (14, 15). BPH occurs in the transitional zone of the prostate and is associated with excessive cell proliferation resulting in an enlarged prostate gland. A majority of patients diagnosed with BPH also present symptoms of moderate to severe LUTS.

BPH is diagnosed by Digital Rectal Examination (DRE), Uroflowmetry (which measures the time in which a given volume of urine is voided) and by measuring Prostate Specific Antigen (PSA) in the serum. If the PSA levels are below 4ng/ml, BPH is the more likely cause of LUTS in patients. If the PSA is greater than 4ng/ml, patients are subjected to further tests such as a prostate biopsy to rule out prostate cancer.

Prostate Cancer (PCa)

Multiple molecular events control PCa initiation, growth, invasion and metastasis. In spite of the prevalence of the disease, our knowledge of the genetic alterations occurring during this process is limited. While BPH arise mostly in the transitional zone, PCa occurs primarily in the peripheral zone of the prostate gland. PCa is the second leading cause of cancer related death in the male population of the United States, and accounts for a third of all cancers diagnosed. The PCa incidence increased dramatically in 1990s. This increase can be mainly attributed to the discovery of PSA screening which led to better diagnosis of PCa. Before PSA assays became routine, many patients were diagnosed with PCa by clinical syndromes or Digital Rectal Examination (DRE). Such diagnostic methods were clearly ineffective to a large extent because in majority of cases, the tumor had already reached an advanced stage and had extended beyond the organ capsule or metastasized. Typically in such cases, the serum PSA levels are higher than 10ng/ml. In contrast, many cases today are detected by PSA levels in the 2.5-10ng/ml range. Thus, PSA screening has revolutionized the clinical management of PCa and improved the chances of curative treatment. However, PSA screening for early detection of PCa has its limitations also. Since PSA is organ specific rather than cancer specific, elevated PSA levels can also be observed in prostatitis, BPH, Prostatic Intraepithelial Neoplasia (PIN), and other non-malignant disease of the prostate. Hence serum PSA levels alone cannot distinguish between adenocarconima and other benign diseases of the prostate. Indeed, only about 25-30% of prostate biopsies obtained from patients with a serum PSA level of 4-10ng/ml actually have foci of adenocarcinoma.

High grade PIN, characterized by thickening of the epithelial layer, is the earliest detectable precursor lesion of PCa (16). The basal cell layer remains intact in low grade and high grade PIN but is lost in adenocarcinoma. Prostate tumor is multifocal and several distinct foci of adenocarcinoma and PIN, varying in the degree of cellular dysplasia can be detected in the same histological section (17, 18). Consistent with this heterogeneous characteristic of PCa, tissue disorganization and genetic alterarions vary significantly between different foci and even within the same contiguous carcinoma. Hence, to better characterize malignant lesions of the prostate, histological grading (G1-G3) has been replaced by Gleason grading. This form of grading takes into consideration the degree of tissue disorganization in the two most prominent foci of the carcinoma (19, 20).

The heterogenous nature of prostate carcinoma is further highlighted by the fact that while a considerable majority retains an indolent growth pattern, in about 30% of the patients the lesion becomes locally invasive or metastasizes to distant organs such as bones, liver and lung (21). Throughout the initiation and progression of PCa, androgen receptor (AR) is expressed. Androgen depletion remains the gold standard for treating PCa. Although the tumor initially regresses, the tumor inevitably becomes hormone refractory and continues to grow in hormone-depleted conditions. The molecular mechanisms by which prostate carcinoma progresses, from an androgen-dependent to an androgen-independent state, is still not fully understood.

Although the etiology of PCa remains far from totally unraveled, some of the established non-modifiable risk factors include age, a family history of PCa and race. African-Americans are more susceptible to this disease compared to Caucasians and Asians. After standardizing to a common age standard, the rate of incidence of PCa varies widely from 1 in 100,000 men annually in China compared to 62 in 100,000 white men and 82 in 100,000 African-American men in the United states in the 1980s. (22).

Among modifiable risk factors, nutritional and environmental factors are thought to have a profound influence on the occurrence of PCa. Some studies have reported a statistically significant correlation between *per capita* consumption of fat, animal fat, red meat, and dairy products and a higher risk of PCa (23). It can be argued that it is not possible to draw firm conclusions from such correlational data, because other factors that vary with dietary habbits could account for this association. Nonetheless, the wide variability in rates of PCa occurance among different countries and races, taken together with strong correlations between nutritional habbits and PCa incidence strongly suggest that some aspects of diet and lifestyle may influence risk of PCa.

Many genes have been implicated in the development and progression of PCa. A few of the most studied are listed and discussed briefly below.

Molecular Genetics of Progression of Prostate Cancer

The molecular genetics of progression of PCa is still far from fully understood.

It is widely accepted that, similar to other cancers, development and progression of PCa is the culmination of sequential genetic events. Each of these somatic genetic changes confers upon the cells harboring such modifications, a selective growth advantage over their neighboring normal calls.

Loss of Heterozygosity

Loss of Heterozygosity (LOH) refers to large part or whole chromosomes being deleted from cells and usually is indicative of the possible location of a Tumor Supressor Gene. Cell clones with LOH are bestowed a selective growth advantage due to the fact that one allele only fails to suppress cell growth. LOH has been identified in a high percentage of prostate tumor patients in chromosomes 8p, 10q and 16q (24,25) indicating that prostate tumor suppressor genes may be located in these regions. LOH in 18q (26) and 17q (27) have a lower rate of penetrance in PCa.

Based on gene functions, several candidate genes have been investigated for their role in PCa progression, primarily by screening for somatic mutations. Such approaches led to the identification of gene mutations in Mxi-1 (28), KAI-1 (29), p53 (30), RB (31), etc. The inherent heterogeneity of prostate tumors combined with a rareness of mutations in common oncogenes and tumor suppressor genes, makes it difficult to identify molecular or genetic signatures of PCa. Fortunately, the emergence of advanced technologies such as tissue microarrays, laser capture microdissection etc. combined with the availability of databases such as the human genome has made possible the

development of genome wide analytical approaches to identify novel genetic pathways in the initiation and progression of PCa.

Androgen Receptor (AR) in Prostate Cancer

Both normal prostate development and prostate tumor depends on androgen stimulation for growth. AR is a member of the nuclear receptor superfamily of ligand-activated transcription factors. Two biologically active androgen binds to AR to mediate its transcriptional activity. Testosterone (T), the major circulating androgen, is secreted by the testis. Dihydrotestosterone (DHT), a more potent androgen, is a 5α-reduced metabolite of T. DHT is required for male reproductive tract development, whereas T is the active androgen in muscle (32).

The AR molecule is structured into several modular domains. It contains an amino-terminal transactivation domain, a central DNA binding domain (DBD), a linker hinge region which leads into the carboxy-terminal ligand binding domain (LBD) (33). AR function is regulated by two major activation domains. Androgen-mediated transcriptional activity is modulated by Activation Function 1 (AF1) in the amino-terminal region (33). Activation Function 2 (AF2) in the LBD maintains the structural integrity of the receptor and is dependent upon androgen binding to AR (34).

The AR gene is X-linked implying only a single copy is present. Hence, LOH is not applicable to AR gene in men. Recent works have identified genetic alterations resulting in an abnormal gain of function of AR in at least a subset of advanced PCa. AR

continues to be expressed in high levels in all advanced stage of the disease, indicating a role for AR in PCa progression (35-38). Somatic mutations resulting in increased AR activity as well as mutations that broaden ligand specificity of AR has been identified both in primary prostate tumors and cell lines derived from them (39). For instance, in the LNCaP cells, derived from a lymph node metastasis of PCa patient, a threonine to alanine mutation (T877A) in the hinge region of AR causes the androgen antagonist, hydroxyflutmide, to act as an agonist and activate AR mediated transcription (40-42).

Thus, the AR is essential both for normal development and differentiation of the prostate. During tumor initiation and progression, AR can acquire gain of function mutations and/or gene amplification to confer a selective growth advantage on tumor cells which now proliferate under conditions where normal prostate cells would have been growth quiescent.

Ras Mutations in Prostate Cancer

The Ras family of proteins includes small enzymatic molecules that hydrolyze guanine nucleotide triphosphatase (GTPase). There are three genes included in this family: K-, N-and H-Ras. Ras bound to GTP activates the Raf-MEK-MAPK pathway and also couples the activation of growth factor receptor to downstream signals. H-Ras of the Ras family of genes was the first identified human oncogene (43). Activating mutations in the Ras genes have been identified in a number of human malignancies including those of lung, pancreas and colon.

Mutations leading to constitutively active H-Ras are relatively rare in PCa and was first identified in 1987 (44). Interestingly, the rate of Ras mutations in PCa is much higher in Asian men compared to American men. Primary PCa samples obtained from American men exhibit a Ras mutation rate of 0-5% (45, 46). In contrast, Ras mutations in Asian men with PCa are significantly higher and range from 13-27% (47, 48). This data indicates that, in the context of prostate tumor, the etiology and disease progression is considerably different among various ethnic backgrounds.

Insulinlike Growth Factor, Phosphoinositide 3-Kinase and Phosphatase and Tensin Homologue Pathway in Prostate Cancer

Insulin-like Growth Factor -1 (IGF-1) binds to its receptor, IGF-1R, to activate and recruit the PI3-K complex to the plasma membrane (49). This leads to the activation of another kinase, AKT, which, in turn phosphorylates a number of downstream targets. The activation of AKT results in decreased apoptosis and increased proliferation. Moreover, activated AKT has also been implicated in regulating cell adhesion and cell motility (50).

PTEN, a tumor suppressor gene, inactivates the PI3-K pathway by dephosphorylating the phosphorylated lipids produced by PI3-K (51). Thus a loss of PTEN in tumor cells results in constitutively active AKT culminating in impaired apoptosis and enhanced proliferation. A number of studies have demonstrated that increasing plasma levels of IGF-1 resulting in activation of PI3-K and its downstream target AKT, correlates with an increased risk of developing PCa (52). Similarly loss of PTEN resulting in constitutive activation of AKT has also been implicated in disease

progression. LOH associated with PTEN loss involves deletion of chromosome 10q23 and has been reported in 20-60% of prostate tumors (53, 54). Thus, loss of PTEN expression is indicative of poor prognosis and has been reported with higher frequency in higher-grade and higher-stage tumors.

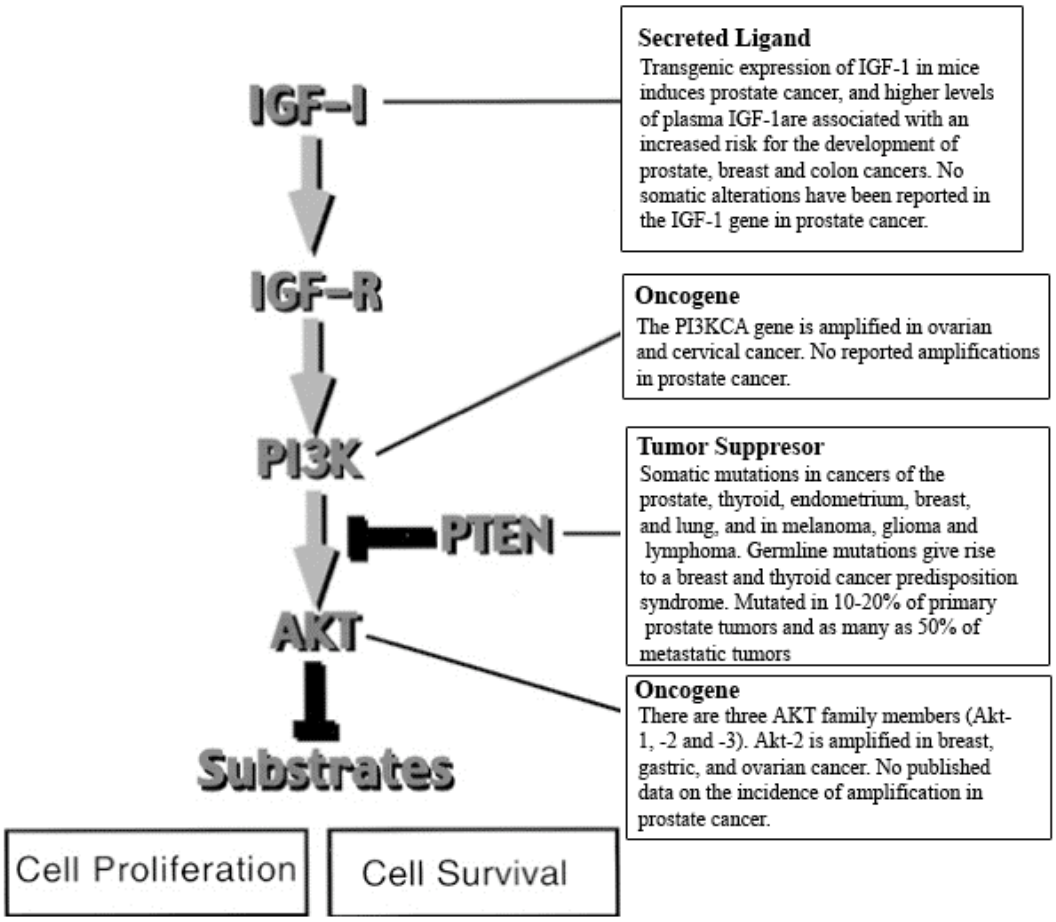

Figure 4. Insulin-like Growth Factor, phosphoinositide-3 kinase (PI3K) and PTEN Pathway. Ligand binding to IGF-R leads to the recruitment and activation of kinases such as Akt. Akt activation results in phosphorylation of downstream substrates that regulate cell survival and proliferation. The tumor suppressor PTEN inhibits phosphorylation of Akt, and a loss of PTEN results in impaired apoptosis and enhanced proliferation. (From Prostate Cancer: Principles and Practice Section I;page 56:Lippincott Williams and Wilkins Publications.)

Wnt Signaling in Prostate Cancer

The Wnt family of proteins, comprising of 19 members, are cystein rich glycoproteins, which functions mainly to modulate branching morphogenesis and to control body axis symmetry during development (55). Canonical Wnt signaling functions through the Adenomatous Polyposis Coli (APC)/β-catenin pathway (56, 57). In the absence of Wnt signaling, β-catenin is sequestered in the cytoplasm where it is targeted

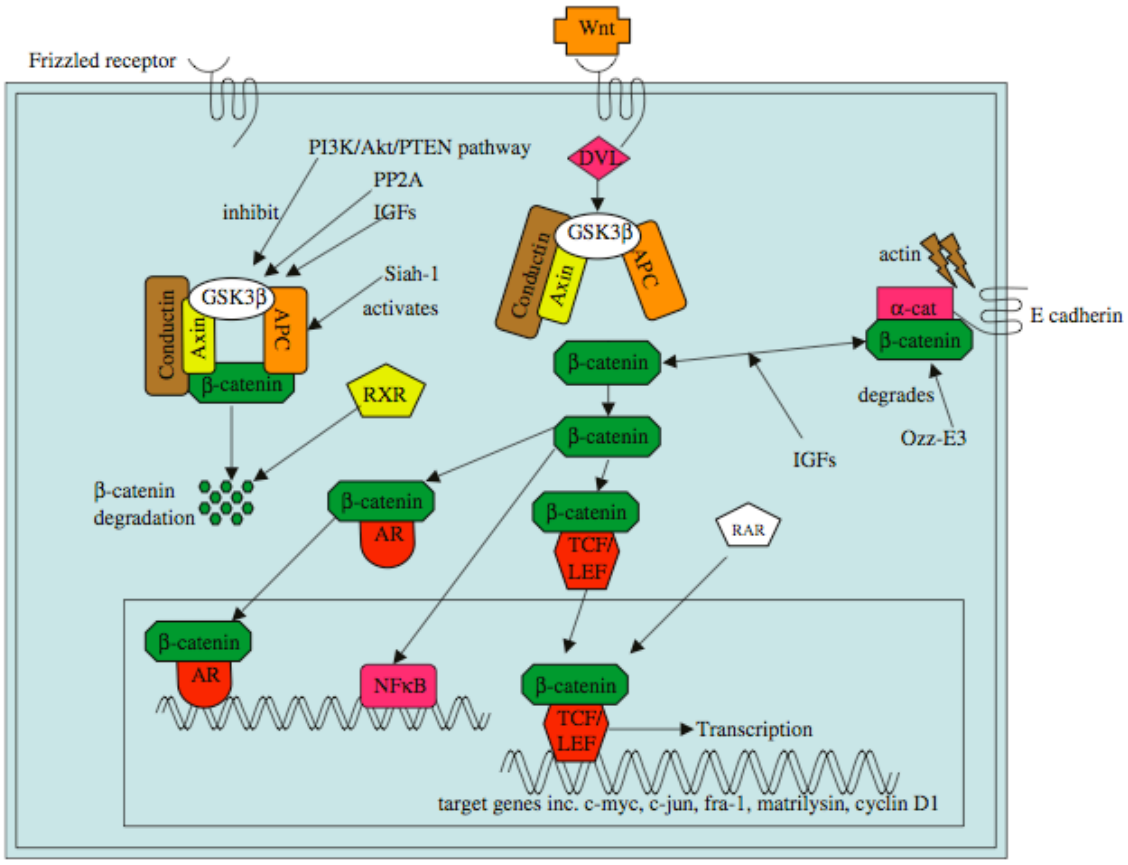

Figure 5. Wnt Signaling in Prostate Cancer. Cross-talk between Wnt signaling and other pathways such as protein phosphatase2A (PP2A), IGF, AR, Retinoic acid receptor A(RAR), and NFκB. (From Brewster et al. Prostate Cancer and Prostatic Diseases, 2005;8:119-126.)

for degradation by a protein complex consisting of APC, axin and GSK-3β. Active Wnt signaling inhibits the APC/axin/GSK-3β complex resulting in the stabilization and nuclear accumulation of β-catenin (56, 57). Once inside the nucleus, the β-catenin recruits transcription factors such as Lymphoid Enhancer Factor 1 (LEF), T-cell Transcription Factor 1 (TCF), and binds to DNA to activate target gene transcription. Mutations in APC and β-catenin can result in inappropriate stabilization and nuclear accumulation of β-catenin culminating in the misexpression of TCF-regulated genes, such as c-Myc, in the tumor cells.

Canonical Wnt signaling may promote "osteomimicry" in metastatic PCa cells in addition to enhancing proliferation and survival (58). Osteomimicry in PCa occurs when the tumor cells acquires properties of osteoblasts. Several studies have reported that metastatic PCa cells express the bone matrix protein osteopontin (OPN), the OPN receptor CD44 and the bone-specific transcription factor RUNX2 (58). It has also been demonstrated that a bone metastatic PCa cell line, C4-2B cells, produces mineralized matrix *in vitro* (59). Thus, osteomimicry is an autocrine function of the canonical Wnt signaling pathway that contributes to the osteoblastic nature of PCa bone metastatic lesions.

Transforming Growth Factor Beta (TGFβ) Pathway in Prostate Cancer

The TGFβ pathway has a multifaceted role in the prostate and is involved in regulating proliferation, growth arrest, differentiation and apoptosis of prostatic stromal and epithelial cells. It has also been implicated in the osteoblastic metastasis of PCa (60).

One of the salient features of the TGFβ signaling pathway is that while it is growth inhibitory in normal prostate cells, it can also enhance prostate tumor growth and metastasis. During carcinogenesis, prostate epithelial cells become resistant to growth suppression and induction of apoptosis by TGFβ (61). However, the mechanisms by which TGFβ acts as a tumor suppressor at one end of the spectrum and a tumor promoter at the other end remains poorly understood, mainly due to the complexity and diversity in TGFβ signaling mechanisms among various tissues and cell types.

As has been discussed earlier, androgens, signaling through the AR, are crucial for both normal growth and development of the prostate gland as well as during tumorigenesis. In hormone-depleted condition, such as following castration, the prostate gland regresses accompanied by a rapid upregulation of TGFβ ligands and receptors (62-64). Androgens (DHT, R1881), on the other hand, suppress TGFβ expression in prostatic cells in culture (65, 66). Physiological levels of TGFβ is sufficient to induce apoptosis in normal prostatic epithelial cells in culture (67). Moreover, when dominant negative TβRII is targeted to the prostate of transgenic mice, apoptosis was inhibited with a concomitant increase in cellular proliferation (68). All these studies indicate that TGFβ exerts a growth inhibitory effect in normal prostatic epithelial homeostasis. Loss of TβRI and TβRII expression accompanies acquisition of resistance to TGFβ-induced apoptosis during prostate carcinogenesis in humans (69-71). Dominant-negative TβRII blocks the growth inhibitory effects of TGFβ on DP-153 cells and promotes their malignant transformation, inducing carcinomas as early as 4 weeks in athymic mice (72). DP-153 is

a spontaneously immortalized non-tumorigenic rat prostatic epithelial cell line developed from the dorsal prostate of a Lobund –Wistar rat (73).

A growing body of literature suggests that stromal cells are critical in promoting and maintaining malignant transformation of prostatic epithelial cells. However, the mechanism by which they promote tumorigenesis is poorly understood. Recent reports have suggested that TGFβ may have crucial roles in prostate stromal cell function and can modulate stromal-epithelial interaction. Fibroblast cells in the normal prostate stroma have high levels of TGFβ-receptors and can trandifferentiate into smooth muscle cells following TGFβ stimulation (74). Bhowmick et al. have demonstrated that knocking out TGFβ signaling in the stromal compartment led to malignant transformation of the prostatic epithelium (75). In this study, *cre-lox* targeted with a fibroblast specific promoter was used to selectively knock out TβRII in the fibroblast (75). This resulted in PIN thus providing evidence that TGFβ can affect prostatic epithelium growth by modulating stromal-epithelium interaction. It has been suggested that TGFβ exerts its influence on the stroma by suppressing the production of growth factors, such as hepatocyte growth factor (75), which are induced by androgens acting through AR in these fibroblasts. Indeed, it has been reported that TGFβ can block AR signaling in the prostate fibroblast, ostensibly by promoting the translocation of AR from the nucleus to the cytosol (76). Thus, a disruption in TGFβ induced stromal-epithelial interactions can result in uncontrolled proliferation of the epithelium leading to malignant transformation.

TGFβ Signaling Through Smad Molecules

TGFβ signals through two transmembrane serine/threonine kinase receptors, TβRI and TβRII. The Smad family of proteins represents the most widely studied intracellular mediators of the TGFβ signaling pathway. This family consists of activators (Smad2 and 3), mediator (Smad4) and inhibitor (Smad7) of TGFβ responses. The Smad proteins are made up of three distinct domains - the highly conserved N-terminal MH1

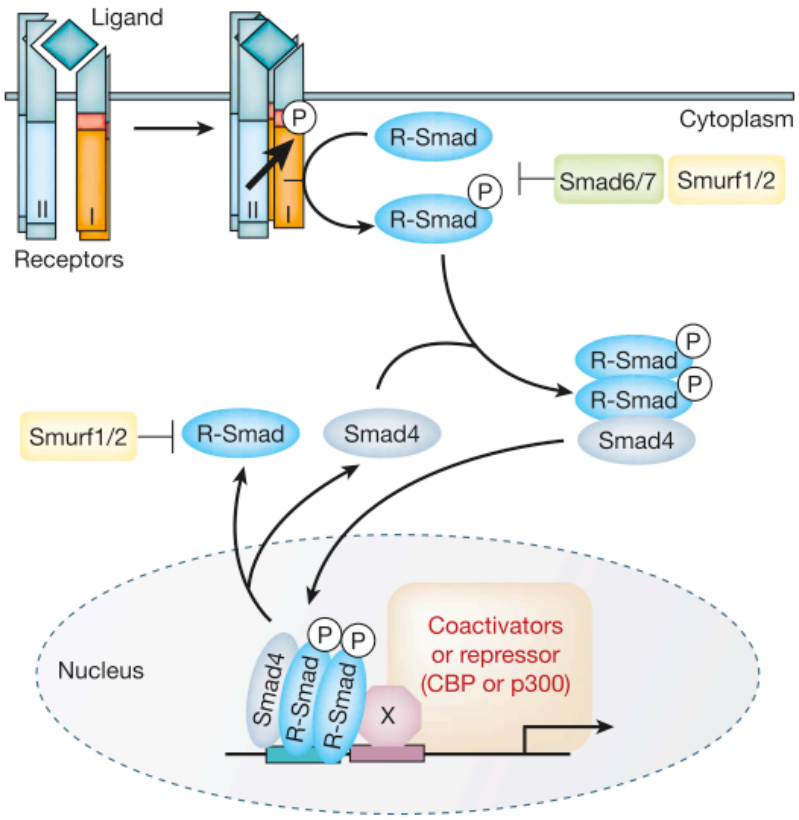

Figure 6. Smad-dependent TGFβ Signaling. Ligand binding to receptors activates Smads (R-Smads) which forms a complex with smad4. Activated Smad complexes translocated to the nucleus to regulate transcription of target genes. (From Zhang et al. Nature 2003;425:577-584).

and C-terminal MH2 domain and a poorly conserved middle linker region (77). The MH1 domain is involved in DNA binding and the MH2 domian is crucial for protein-protein interaction (77). In the cytoplasm, Smad2 and Smad3 remain bound to microtubules (78) and in this conformation they remain inhibited through the association of N-terminal and C-terminal domains (79). Stimulation in the form of phosphorylation of C-terminal serines by an activated TβRI, relieves the Smads from this inhibition which then translocate to the nucleus (78). In the nucleus, the Smad molecules can activate transcription of its target genes by binding to promoter regions through the MH1 domain and associating with other proteins through the MH2 domain.

TGFβ Signaling Through Non-Smad Molecules

Although the Smad-dependent TGFβ pathway has been well studied, Smad-independent TGFβ activation remains largely unexplored. Recent reports suggest a cross-talk between the TGFβ and the Mitogen Activated Protein Kinase (MAPK) pathway (73). It has also been demonstrated that the immunophilin FKBP12 binds to TβRI (80, 81) to prevent ligand-independent phospho-activation of TβRI by TβRII (82, 83) thereby modulating the TGFβ signaling axis. The cytoplasmic domains of TGFβ receptors interact with a number of proteins, e.g., the Bα subunit of Protein Phosphatase 2A (PP2A) associates with the cytoplasmic domain of TβRI (84) and clusterin interacts with the cytoplasmic domain of both TβRI and TβRII (85). Interestingly, the expression of Clusterin, also known as ApoJ or TRPM2 (85), is induced following androgen withdrawal in rat prostates (86). Moreover, TGFβ stimulation also increases clusterin expression by inducing c-fos (87).

The TGFβ receptors can physically interact with other proteins also. TGFβ-receptor-interacting-protein-1 (TRIP-1) directly associates with TβRII to inhibit both TGFβ and Smad3-induced PAI-I promoter activity (88). Another protein DAXX interacts with TβRII to modulate TGFβ-induced cJun-N-Terminal kinase (JNK) activity and apoptosis (89). Homeodomain-Interacting Protein Kinase-2 (HIPK2) can mediate the TGFβ-induced activation of JNK, through interaction with DAXX (90).

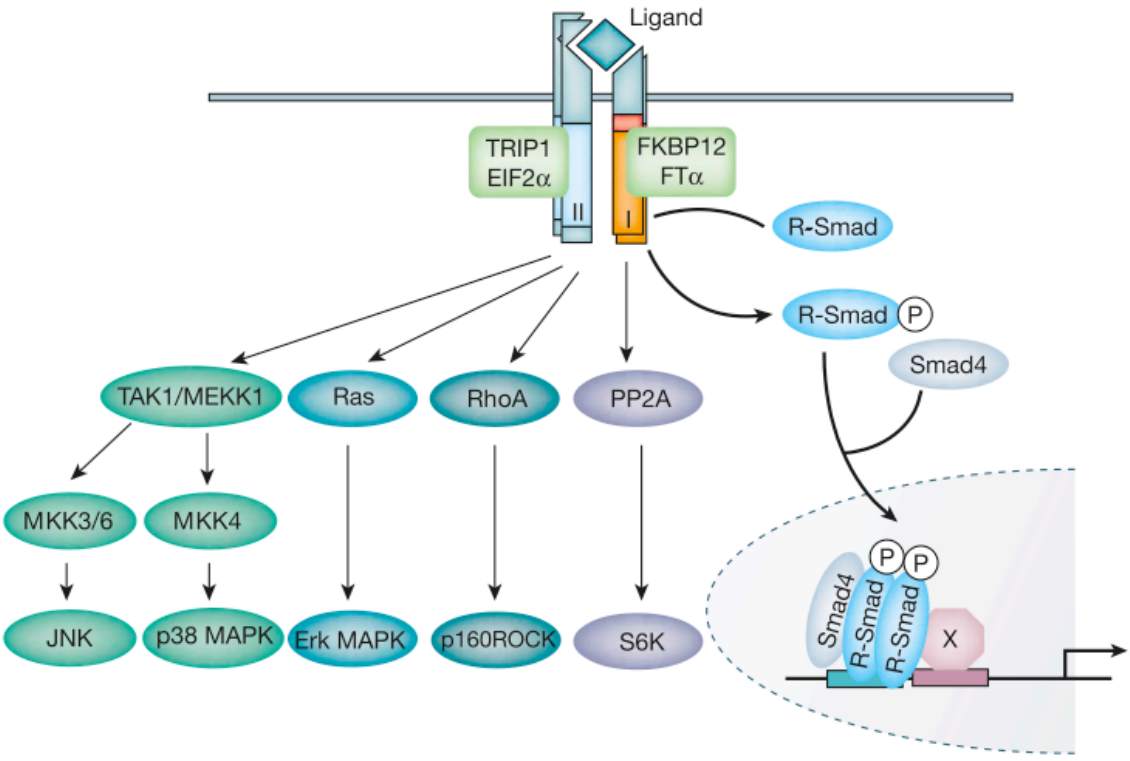

Figure 7. Smad-independent TGFβ Signaling. Apart from proteins that interact with receptors and smads, other proteins can associate with Type I and II receptors to regulate TGFβ signaling without an apparent direct effect on Smad activation. (From Zhang et al. Nature 2003;425:577-584).

In summary, the TGFβ signaling pathway has a wide plethora of responses both in the prostatic stroma and epithelium. TGFβ can elicit its activity either by a Smad-dependent or -independent mechanism. The complexity and the diversity of this signaling axis call for the identification of effector molecules through which TGFβ modulates individual responses in the prostate. This can potentially be of immense value in identifying molecular targets for rational drug design aimed at better clinical management of PCa.

Stathmin

Stathmin is a ubiquitous cytosolic phosphoprotein that possesses the capacity to bind tubulin and interfere with microtubule dynamics. The gene for human stathmin is located in 1p36.1-p35 (91) and encodes for an 18 kilo-Dalton protein. Stathmin has also been referred to as p19 (92), prosolin (93), Lap18 (91), metablastin (94) and Oncoprotein 18 or Op18 (95). Overexpression of the protein has been associated with leukemia (95), breast (96) and ovarian cancer (97). The functions of stathmin can be broadly classified as: a) regulation of microtubule dynamics (98), and b) non-microtubule functions which include i) regulation of prolactin, ii) hormonal regulation of various anterior pituitary cell types and iii) regulation of differentiation of muscle cells by growth factors, hormones and neurotransmitters (99).

Stathmin was initially studied either because of its complex pattern of phosphorylation at its N-terminal serine residues or because of is elevated expression in a variety of human malignancies. Subsequently, stathmin was identified as a microtubule

destabilizing protein that promotes microtubule catastrophe, which is a phenomenon by which microtubules transition from growing to shrinking states (100).

The microtubules are critical for diverse cellular functions such as maintaining cell shape, cellular transport, motility and cell division. The microtubules interact with a variety of proteins, referred to as microtubules-associated-proteins (MAPs). Interaction with MAPs regulates the distribution of microtubules in the cell. Of special interest is the interaction of microtubules with the actin cytoskeleton. The microtubule-actin interactions can be either structural or regulatory. Rodriguez et al. demonstrated that the physical association of microtubules with actin filaments facilitates the movement of the complex with the retrograde flow of actin. Attachment of microtubule ends to the actin cable provides another instance of structural interaction between microtubules and the actin cytoskeleton. Gunderson et al. showed that this interaction is critical for the cell to pull the mitotic spindle to the proper cortical location. These structural interactions can be modulated by a host of cross-linking and motor proteins. The interaction between microtubules and actin can also be modulated through signaling molecules, such as through the regulation of Rho-type GTPases. Such interactions have been implicated in the regulation of microtubule stability. The dynamic instability of microtubules is characterized by switching between alternate phases of growth and shrinkage. During interphase, the kinetics of such switching between the phases is slowed down and the microtubules are relatively stable. However, during mitosis the interphase microtubules undergo rapid depolymerization followed by repolymerization to constitute the mitotic spindles.

Stathmin binds free tubulin heterodimers to form a ternary tubulin-sequestering complex to exert its effect on microtubules (101). This complex consists of two tubulin heterodimers, arranged head to tail (102), with each of the two tandem helical repeats of stathmin binding along one heterodimer (103). *In vitro* tubulin binding assays have demonstrated that the N-terminal non-helical region of stathmin promotes microtubule catastrophe and the tandem helical repeats are required for tubulin-sequestering activity (104).

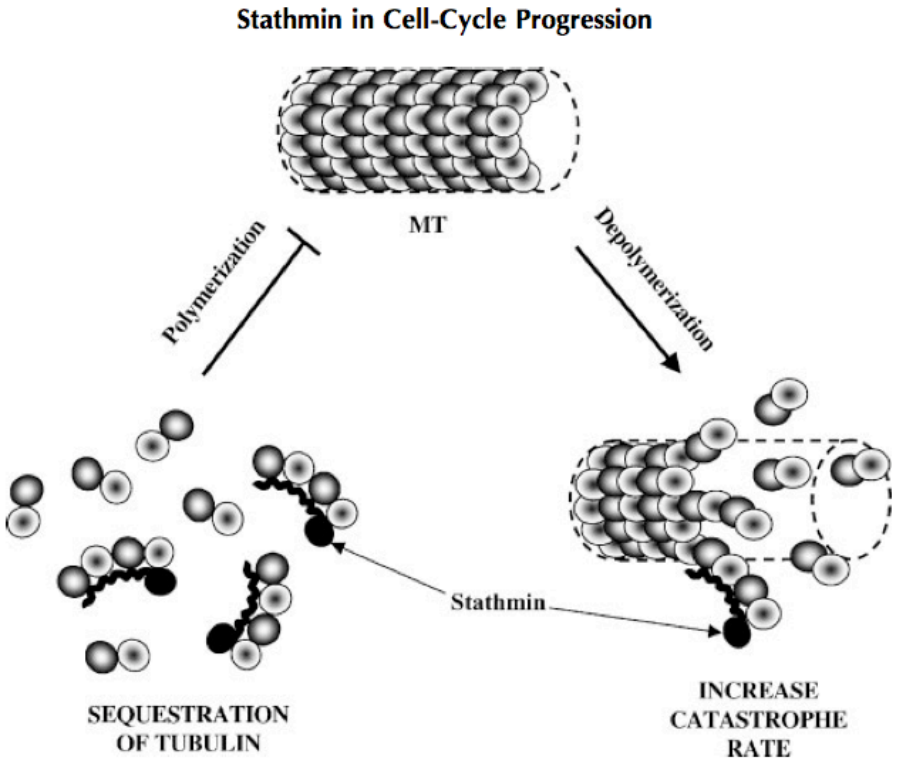

Figure 8. Role of stathmin in the regulation of microtubule (MT) dynamics. Stathmin sequesters unpolymerized tubulin by binding two α/β-tubulin heterodimers represented here by light and dark shaded circles respectively. Stathmin can also bind to the ends of polymerized MTs to increase the rate of catastrophe by inducing a conformational change that promotes MT depolymerization. (From Atweh et al. Journal of Cellular Biochemistry 2004;93(2):242-250).

Human stathmin is a substrate for both cell-cycle regulating and signal-transducing kinase systems. Phosphorylation of stahmin by various kinases has been

discussed in Chapter IV. During mitosis, the microstubule destabilizing activity of stathmin is inactivated by phosphorylation (105). However, stathmin exists in predominantly unphosphorylated state during interphase (106). In metaphase-blocked human cell lines, all four N-terminal serine residues are phosphorylated (107), implying that the microtubule destabilizing activity of stathmin is inactivated by multisite phosphorylation during spindle assembly.

Stathmin as a Potential Target for Anti-Cancer Therapy

Several studies have indicated that stathmin promotes cell growth and tumorigenesis. For example, loss of stathmin expression in K562 leukemic cells abrogates anchorage independent cell growth and causes growth arrest (108). *In vivo*, antisense inhibition of stathmin has been shown to result in inhibition of tumorigenecity of leukemic cells (108). It has recently been demonstrated that, the malignant phenotype of prostate cancer cells *in vitro* is inhibited by adenovirus-mediated gene transfer of anti-stathmin ribozyme (109). Efficient knockdown of stathmin expression using this system resulted in a dramatic growth inhibition and decreased clonogenic potential in LNCaP cells (109). Growth inhibition in LNCaP cells was accompanied by an accumulation of cells in the G2-M phase of cell cycle (109). Despite a growing body of literature implicating stathmin in various human cancers, no down-stream effectors of stathmin have been identified yet.

Anti-microtubule drugs, such as Taxotere that, like Taxol, inhibits microtubule assembly to block the cell cycle, have been used as chemotherapeutic agents in breast,

ovarian and PCa patients. Therefore, one strategy would be to use combinatorial therapy of anti-stathmin strategies with anti-microtubule drugs. This may be a potent anti-cancer strategy since both therapies target the same microtubule pathway. Indeed, stathmin antisense molecules have been reported to sensitize K562 cells to Taxol treatment (110), thereby inhibiting their proliferation and clonogenic potential. Similar observations in breast (111) and prostate cancer cell lines (112) suggest that stathmin represents an important molecular target for developing novel anti-cancer therapies.

A second strategy would be to target PAK1, which is activated by Epidermal Growth Factor (EGF) to phosphorylate stathmin at Serine 16 (113, 114). EGF binds to its receptor, EGFR, and activates PAK1 through the Rac/Cdc42 pathway. EGFR expression is elevated in androgen-independent PCa and has been correlated with poor prognosis. Since EGFR acts through PAK1 to regulate the microtubule destabilizing activities of stathmin, pharmaceuticals such as Erbitux that target EGFR may also inhibit stathmin activity. Thus, combinatorial treatment involving inhibition of stathmin with small molecule inhibitors and Erbitux may represent another potential treatment strategy for PCa patients. Erbitux has already been approved for treatment of colorectal cancer (115, 116) and non-small cell lung cancer (116).

Combinatorial strategies that target microtubule function to inhibit the cell cycle and prevent tumor growth have not yet been evaluated *in vivo* in PCa. Understanding the pathways, which activate stathmin expression, would facilitate in designing specific combinatorial therapeutic strategies for the treatment of PCa.