

Cancer Genetics, Cancer Biology, and Tumor Growth and Metastasis: The Interaction of Cancer and Its Host Environment

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Renal Cell Carcinoma

Premalignant Lesions

Unlike prostate cancer, precursor lesions for renal cell carcinoma (RCC) are not well understood. Renal intraepithelial neoplasia (RIN) and dysplastic changes have been described in the literature [1]. Some of these lesions have common genetic alterations with RCC, share spatial orientation, and have a premalignant appearance, which suggests an evolutionary relationship to carcinoma [2]. Given the sparse data and limited characterization, it is likely that this premalignant state is short lived or that the majority of RCCs occurs *de novo*. This further suggests that the time from genetic insult to overt carcinoma is rapid, emphasizing the need for early surgical intervention for curative intent.

Molecular Pathogenesis

Almost 100 years ago, Von Hippel and Lindau described a familial pattern of vascularized retinal growths, which was later recognized to be part of an autosomal dominant disorder. These patients were predisposed to develop hemangioblastomas, pheochromocytomas, and clear-cell RCC. In 1993, the *VHL* gene was discovered at 3p25.3, a region that is frequently deleted in RCC. Somatic mutations, promoter methylation, or loss of

heterozygosity of *VHL* is found in up to 90% of sporadic RCCs [3,4]. The VHL protein is best known for its role as the substrate recognition component of an E3 ligase and targeting of hypoxia inducible factors (HIF) for ubiquitination and degradation [5]. In hypoxic environments or in the absence/inactivation of VHL protein, the alpha subunit of HIF heterodimerizes with HIF β and translocates to the nucleus, and transcribes a number of genes including VEGF, PDGF- β , and TGF- α (Figure 1.1) [6]. The unregulated activation of this pathway is a main driver of angiogenesis, invasion, and metastasis in the majority of sporadic RCCs.

Targeting of the VEGF pathway has been mainstay of treatment for metastatic or unresectable RCC. Small molecule tyrosine kinase inhibitors (TKI) have been successful at disrupting VEGF signaling, resulting in improved patient survival in the metastatic setting. VEGF and PDGF β can stimulate the proliferation and migration of endothelial cells. The establishment of an enriched blood supply can facilitate the establishment of metastatic niches and lead to disseminated disease. As a result of this high metastatic potential, there is no currently approved neoadjuvant systemic approach for RCC using targeted therapies such as sunitinib or pazopanib. The use of these agents is also not approved in the adjuvant setting after

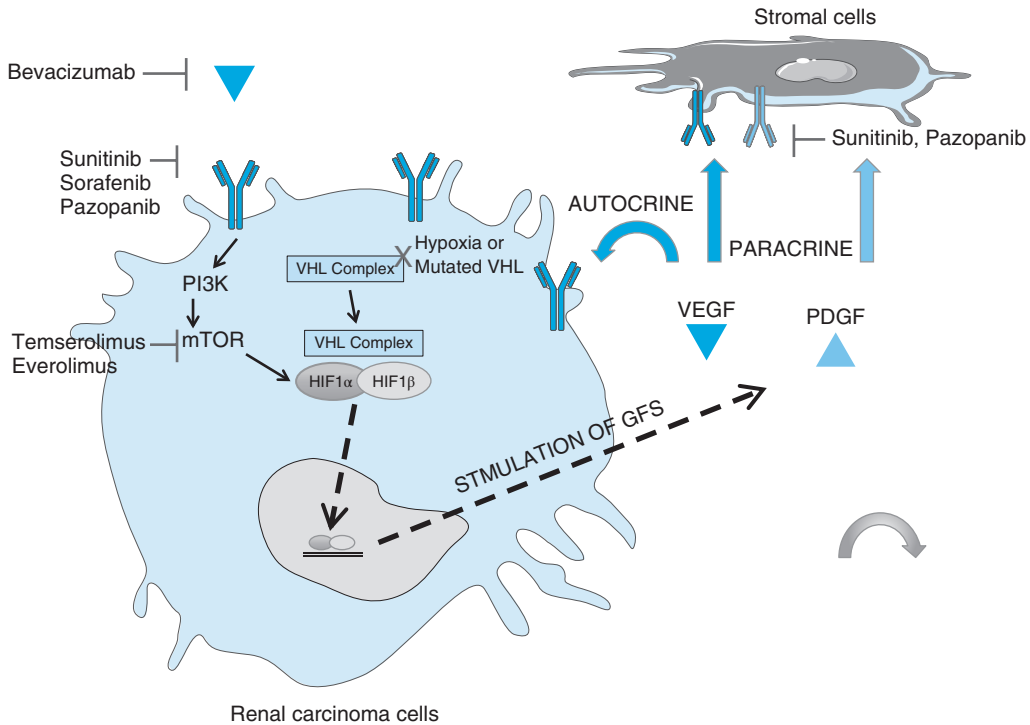


Figure 1.1 Molecular dysregulation of renal cell carcinoma. Under normal hypoxic conditions or in the presence of VHL mutations, HIF α and HIF β form a heterodimer, translocate to the nucleus and function as a transcription factor. Small molecule tyrosine kinase inhibitors (TKIs), Sunitinib and Pazopanib, or monoclonal antibodies (Bevacizumab) can abrogate VEGF signaling in RCC. TKIs can also attenuate the PI3K/mTOR and MAPK pathways shown. Temsirolimus and everolimus can directly antagonize mTOR signaling, inhibiting growth in certain RCCs. Adapted from Clin Cancer Res. December 15, 2006; 12(24):7215–7220.

nephrectomy. Multiple studies have failed to show a survival benefit of adjuvant TKI use or immunotherapy after definitive surgery underscoring the importance of early intervention with upfront surgery [7].

Loss of chromosome 3p is the most frequent genetic mutation in RCC. In addition to *VHL*, this region also contains the gene, *PBRM1* (3p21). *PBRM1* is a purported “gate-keeper” gene and plays a significant role in DNA repair, replication, and transcription. Somatic mutations have been found in 41% of clear-cell renal carcinomas but may be as high as 50% [8,9]. Loss of the *PBRM1* has been correlated with advanced stage, higher-grade disease, and worse patient outcomes [10]. Alterations of chromosome 3p may mark a key genetic event, either inherited or acquired, that drives early tumorigenesis.

Multiple genetic changes have been observed in RCC, including gain of 5q containing *TGFB1* and *CSF1R* and deletion of 14q harboring the tumor suppressor candidate, *NRXN3*.(11) Loss of 14q was associated with higher-grade disease and worse survival [11,12].

mTOR is a serine/threonine kinase that couples with adapter proteins forming two distinct complexes, mTORC1 and mTORC2. mTORC1 activation has been implicated in >50% of RCCs [13]. Interestingly, HIF-1 α has been shown to increase the expression of REDD1, a known inhibitor of mTORC1 [14]. Under hypoxic conditions, the stabilization of HIF-1 levels lead to the inhibition of mTOR signaling. This inhibition is dependent on the gene products of *TSC1* (tuberous sclerosis complex 1) and *TSC2* [14].

Mutations in TSC1 and PTEN may abrogate the effect of the HIF-1 signaling axis on mTOR inhibition, resulting in a second and distinct mechanism of carcinogenesis [15].

Everolimus binds to FKBP-12 and inhibits the activity of mTORC1. A Phase III trial that examined the effect of everolimus in patients with metastatic RCC who had progressed on TKI therapy was stopped early when 37% of the total progression events were shown in the everolimus group compared to 65% in the placebo arm [16]. In 2010, the final results of the trial showed a 3-month progression-free survival advantage following treatment with everolimus [17]. Temsirolimus, an intravenous inhibitor of mTORC1, increased overall survival in untreated patients with metastatic RCC and poor prognostic features [18]. Similar to TKI therapy, there is no role for mTOR inhibitors in the treatment of localized RCC.

The discovery of TKIs has revolutionized the treatment of metastatic disease and improved overall survival. Surgery remains the main treatment for localized disease. With the development of next-generation TKIs, targeted therapy may complement a surgical approach for early-stage disease.

Bladder Cancer

Bladder cancer is the fourth-most common neoplasm in males, consisting predominantly of urothelial carcinoma. The pathological stage of the tumor distinguishes between nonmuscle-invasive disease and muscle-invasive disease. Use of “molecular grading” may also aid conventional staging parameters and further define muscle- versus nonmuscle-invasive disease. Common alterations in cell-cycle regulation and growth pathways of bladder cancers are described next.

Cell-Cycle Regulation

Alterations in cell-cycle regulation pathways were found in approximately 90% of all muscle-invasive bladder cancers [19]. In this study,

the Cancer Genome Atlas Research Network (CGARN) found that *TP53* mutations were found in 49% of cancers. Other studies have found that mutations in *TP53* were associated with recurrence of nonmuscle-invasive bladder cancer as well as disease progression and poor prognosis [20,21]. There are conflicting data regarding the utility of p53 alteration when used to direct the administration of neoadjuvant therapy [22–24]. Although a common event in carcinogenesis, using p53 alterations as a sole biomarker to dictate treatment is of unclear clinical significance.

Studies have also incorporated other cell-cycle regulators in conjunction with p53 to better risk stratify patients. Garcia del Muro *et al.* examined the relationship of p53 and p21 overexpression to survival [25]. P53 regulates p21 expression, a cyclin-dependent kinase inhibitor, which can arrest cell growth by inhibiting Rb phosphorylation. Patients with T2–T4a, N0 disease received neoadjuvant chemotherapy followed by either radiation or surgery, depending on residual disease status. Patients harboring tumors that overexpressed p53 and p21 had a worse overall survival compared to patients with normal expression levels. A retrospective study showed that patients with pT1 disease treated with radical cystectomy were 24 and 27 times more likely to have disease relapse and cancer-specific death if alterations were found in p53, p27, and Ki-67 expression [26]. The combination of increased p53 and pRB expression with alterations in p21 levels resulted in an 8% five-year survival rate after cystectomy in another study [27].

Other genes and proteins involved in mediating p53 signaling have also been implicated in promoting bladder carcinogenesis. Loss of chromosome 9 is thought to be an early event occurring in more than 50% of all cases [28]. *CDK2NA/ARF* maps to 9p21, a region commonly lost in bladder cancer. This region encodes p16^{ink4A} and p14^{ARF}, respectively [29]. Cycle D1 can complex with CDK4, which results in the phosphorylation of Rb and release of E2F, allowing for progression

of the cycle. In the absence of mutation, p16 can form a binary complex with CDK4 antagonizing the effect of cyclin D1 and preventing the cell from progressing into S phase [30]. Frequent deletion of p16^{ink4A} and the resulting loss of p16 in bladder cancer allow the function of Cyclin D1 to go unchecked. Loss of p14 allows MDM2, the E3 ubiquitin ligase, to downregulate p53 protein levels further destabilizing cell-cycle regulation [31]. In one study, homozygous and heterozygous deletions in *CDK2NA/ARF* have been reported to occur in 14% and 12% of bladder cancers, respectively [32]. More recently, recurrent focal deletions in *CDK2NA/ARF* were found in 47% of tumors [19].

FGFR3 and Receptor Tyrosine Kinases

Fibroblast growth factor receptor 3 is part of a family of receptor tyrosine kinases that have been implicated in angiogenesis, apoptosis, and chemotaxis [33]. Inherited mutations in *FGFR3* have been well studied because of resulting achondroplasia and skeletal dysplasia [34]. Acquired mutation of *FGFR3* is a common event in low-grade and nonmuscle-invasive bladders cancers [35,36]. *FGFR3* mutation has also been associated with a low recurrence rate in nonmuscle-invasive bladder cancers treated with transurethral resection of bladder tumor (TURBT) and, in conjunction of normal MIB-1 expression, may be a better predictor of outcome than pathological staging [37,38]. The role of *FGFR3* mutation in muscle-invasive disease is less well established.

FGFR3 can activate multiple downstream pathways including the Ras/Raf/MEK/ERK and PI3K/AKT signaling axis (Figure 1.2). *HRAS* has been shown to be frequently mutated in bladder cancer and is known to be activated by *FGFR3* through adaptor proteins [39]. Activation of the resulting MAPK pathway may serve as a potential target for therapy. Small molecular inhibitors of *FGFR3* have shown promise in preclinical studies [40]. However, mutation in downstream mediators may convey early resistance and

limit therapeutic benefit. Targeting the c-RAF/MEK/ERK pathway could complement *FGFR* inhibition or be considered as monotherapy with growth inhibition shown in xenograft models [41].

The PI3K/AKT pathway is also known to be active in bladder cancer with activating mutations found in 17% of cases [19]. In this study, AKT was overexpressed in 12% of patients, *TSC1* truncated in 6%, and *PTEN* mutated in 2% of specimens. PI3K and AKT have known small molecular inhibitors currently being used in clinical trials, but no data is available with respect to bladder cancer. mTOR inhibitors, such as everolimus, have shown modest clinical benefit in metastatic transitional cell carcinoma [42].

EGFR, ERBB2 (HER2), ERBB3, and ERBB4 have also been shown to be overexpressed in bladder cancer [43–45]. A Phase II trial showed potential clinical benefit of cituximab in combination with paclitaxel in patients with metastatic urothelial cancer [46]. Lapatinib, a dual kinase inhibitor of EGFR and HER2, did not meet its primary end point for treatment as a single agent for recurrent transitional cell carcinoma [47].

The identification of well-studied signaling pathways that are altered in bladder carcinogenesis is vital to understanding disease development and progression. The role of targeted therapy in the neoadjuvant or adjuvant setting remains unknown. Most clinical trials looking at the effect of small molecular inhibitors in the metastatic setting are currently of unclear benefit. Currently, these altered pathways can be used to better characterize more indolent from aggressive disease within the known categories of muscle-invasive or nonmuscle-invasive disease via a process called *molecular grading* or *staging*.

Molecular Grading

Several groups have identified molecular profiles to help predict recurrence and overall survival. *TP53* mutations have been associated with muscle-invasive disease, whereas

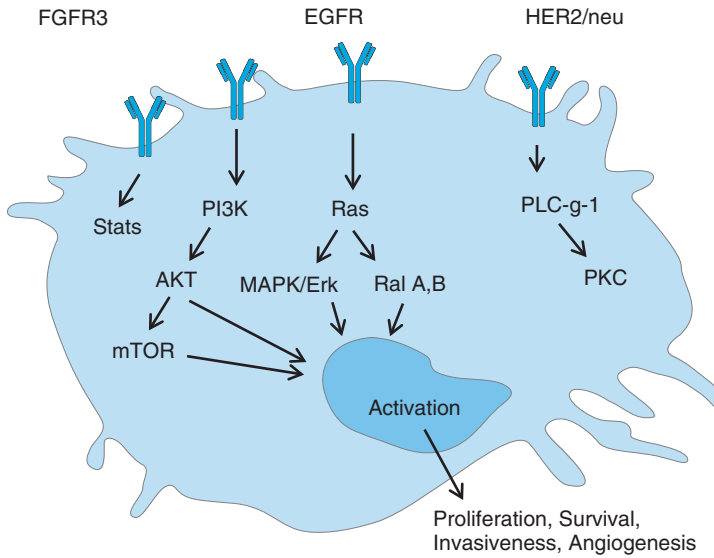


Figure 1.2 Activated signaling pathways in bladder cancer. Mutations and overexpression of FGFR3, EGFR, and HER2 result in activation of downstream pathways shown and drive cell cycle progression, growth, and angiogenesis. PI3K, MAPK, and mTOR inhibitors are clinically available and may attenuate downstream signaling. Adapted from Thomas CY, and Theodorescu D. "Molecular Pathogenesis of Urothelial Carcinoma and the Development of Novel Therapeutic Strategies." In Lee CT, and Wood DP. *Bladder Cancer: Diagnosis, Therapeutics, and Management*. New York, NY: Humana Press, 2010.

FGFR3-activating mutations are thought to result in lower stage/grade tumors (Figure 1.3) [45]. Lindgren *et al.* developed a molecular signature defining two molecular subtypes of tumors within both low/high grade as well as invasive versus noninvasive categories [48]. *TP53/MDM2* alterations were seen in the more aggressive MS2 subtype. The MS1 group was defined by *FGFR3/PIK3CA* mutated tumors and conveyed a better prognosis across grade and stage. Sjobahl *et al.* defined five molecular subclasses of urothelial cell carcinoma: urobasal A, genomically unstable, urobasal B, SCC-like, and heterogeneous infiltrated [49]. Urobasal A was characterized by high FGFR3 and TP63 expression as well as a normal pattern of cytokeratin expression and conveyed the best prognosis. Genomically unstable tumors had *TP53* mutations, ERBB2 expression, and decreased cytokeratin staining, all of which portended a worse prognosis. Urobasal B shared FGFR3 overexpression and *TP53* mutation with several alterations

in cytokeratin expression to suggest an evolution from urobasal A. Other groups have shown increased expression in lysosomal cysteine proteases, matrix metalloproteinases, and genes involved in angiogenesis in muscle-invasive tumors [50]. Choi *et al.* designated three major clusters for bladder tumors: basal, luminal, and P53-like [51]. The basal phenotype had overall shorter survival and was characterized by p63 activation, squamous cell differentiation as well as the presence of EMT biomarkers. Luminal type showed a similar pattern of expression to luminal breast cancers, including the activation of ER pathways, ERBB2 expression, and activating *FGFR3* mutations, which respond more favorably to therapy. *TP53* mutations were distributed equally among all three classes, but the P53-like group had "normal" expression of P53 regulated genes but still conveyed a resistance to chemotherapy.

These findings lay the groundwork for using "molecular staging" of bladder cancers

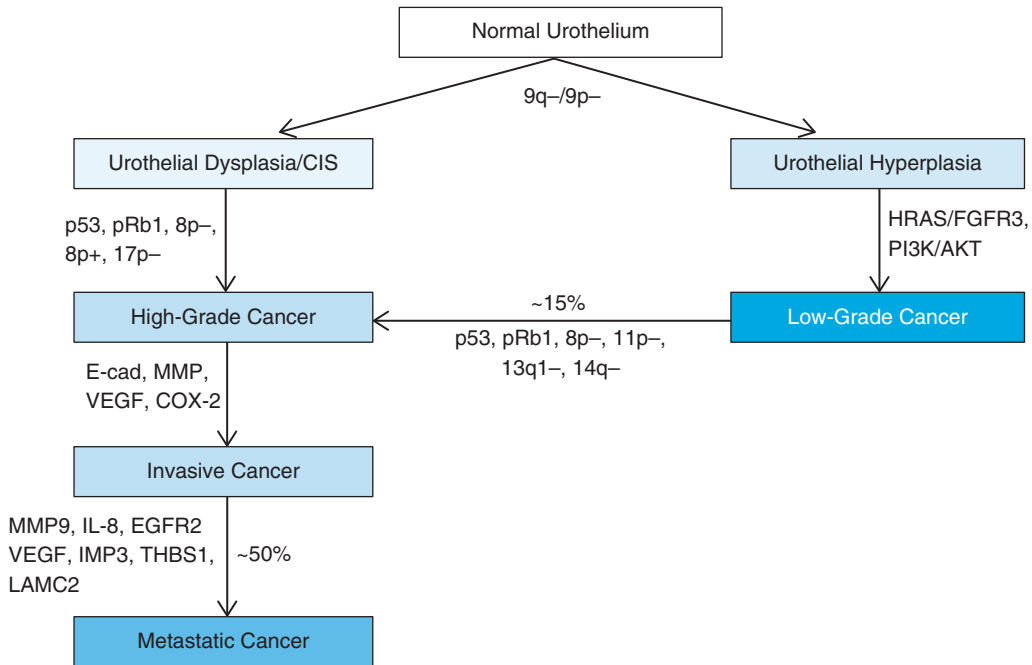


Figure 1.3 Molecular pathogenesis of bladder cancer. Clear patterns of dysregulation are observed in low-grade (LG) urothelial cancer (UCa) versus high-grade (HG) and invasive disease. LG UCa have a high rate of recurrence and can progress to HG UCa in 15% of cases, which may lead to invasive and metastatic disease. Adapted from *Nat Rev Urol*. 2011 Dec 13;9(1):41–51.

to better define high- and low-risk groups within invasive and noninvasive tumors. This may provide a clearer basis for recommendations regarding surveillance cystoscopies and early intervention in certain nonmuscle-invasive cancers. More research needs to be done to define the “invasiveness” of bladder tumors. Alterations in P53 may lead to a more aggressive phenotype through an unclear mechanism, whereas FGFR3 mutation may facilitate growth without the necessary dysregulation for invasion and eventually metastasis.

Prostate Cancer

Epidemiology

Prostate cancer is the most-common epithelial cancer in males with a lifetime risk of more than 15% for developing the disease. Despite the high incidence of the disease in

the United States, only 4% of prostate cancers are metastatic at the time of diagnosis. The large majority of new cases are confined to the prostate or regional lymph nodes, which confers a favorable prognosis. The SEER database (2003–2009) estimates the 5-year relative survival for newly diagnosed prostate cancer to be above 99%. Based on epidemiology alone, these data suggest that many prostate cancers have an indolent phenotype.

On autopsy, prostate cancer was incidentally found in 34.6% of U.S. Caucasian men older than the age of 50 [52]. Younger men in their 40s (34%) and 30s (27%) also had a considerable likelihood of harboring foci of prostate cancer [53]. The high prevalence of clinically insignificant disease in young individuals underscores the notion that many cases of prostate cancer do not need to be treated. Precancerous lesions such as prostatic intraepithelial neoplasia (PIN) and proliferative inflammatory atrophy (PIA) were also identified in these men. PIN lesions

contain proliferating epithelial cells characterized by an enlarged nucleus and prominent nucleoli found within a ductal structure [54]. High-grade PIN (HGPIN) is more common in the aging prostate and contains similar genetic and molecular alterations to that of carcinoma [55]. Although atrophic in appearance, PIA lesions have high levels of Bcl-2, increased Ki-67, and reduced levels of cell-cycle inhibitors [56]. A chronic inflammatory infiltrate is commonly found associated with these lesions and has been implicated in carcinogenesis. PIA is found adjacent to or in close proximity to HGPIN in 46% of samples analyzed in one study, which may suggest an evolutionary relationship between the two [57]. The progression from normal epithelium to precursor lesions and eventual prostate cancer may take years or even decades. In many of these men, their disease will remain subclinical and untreated without consequence. The difficulty for the urology and oncology communities is to identify the aggressive, lethal forms of the disease. Decades of research have been dedicated to studying the tumor biology of precursor lesions, hormone sensitive PCa, castration resistant PCa, and metastatic disease in the hope of identifying those patients who will require treatment.

Molecular Pathogenesis: Inflammation and Genomic and Protein Alterations

Nelson *et al.* studied and characterized the molecular pathogenesis of prostate cancer [58]. Similar to the findings in colon cancer [59], prostate cancer progresses from normal epithelium to carcinoma through a series of common molecular alterations (Figure 1.4). Inherited mutations and early somatic changes have been discovered in genes mediating the body's inflammatory response.

A study of prostate cancer families identified a region of chromosome 1 (1q24-25) involved in cancer susceptibility [60]. Germline mutations in *RNASEL/HPC1* (1q25) cosegregated with prostate cancer within these families. *RNASEL/HPC1* encodes

RNASEL, an interferon regulated endoribonuclease, which degrades both cellular and viral RNA [61]. Casey *et al.* noted that an Arg462Gln variant was implicated in 13% of prostate cancers [62]. Impaired apoptosis has resulted from mutations in *RNASEL* and is a proposed mechanism for tumorigenesis [63]. Macrophage scavenger receptor 1 (*MSR1*) has also been implicated in hereditary prostate cancer and has a role in the innate immune response [64]. This gene maps to chromosome 8p22, a region that undergoes loss of heterozygosity in 69% in cases of prostate cancer [65]. A mutation in the receptor may impair the ability of the cell to remove reactive oxygen species leading to increased level of oxidative DNA damage [66]. *NKX3.1*, located at 8p21, is an androgen-regulated, prostate-specific homeodomain protein essential for normal prostate development and thought to be a key tumor suppressor in prostate carcinogenesis [67,68]. *NKX3.1* levels are decreased in proliferative inflammatory atrophy and downregulated in response to inflammatory cytokines [69,70]. Decreased levels of *NKX3.1* have been shown to increase growth, decrease apoptosis, and affect DNA repair [71,72]. Moreover, the loss of *NKX3.1* correlates with disease progression [73]. *GSTP1* undergoes somatic inactivation via promoter methylation in approximately 90% of prostate cancers [74,75]. This “caretaker” gene encodes a glutathione S-transferase that is responsible for neutralizing electrophilic carcinogens and reactive oxygen species [76]. Loss of *GSTπ* protein expression was seen in more than 90% of prostate cancer specimens in one study [74]. It is thought that *GSTπ* has a key role in maintaining genetic integrity. Many of the inherited mutations/deletions as well as acquired somatic changes implicated in prostate carcinogenesis, involve genes regulating inflammation, oxidative DNA damage, and cellular immunity.

The ultimate tumor-sparing approach to prostate cancer would be chemoprevention. The preceding data generated significant interest in antioxidants and nonsteroidal anti-inflammatory drugs (NSAIDs) as potential

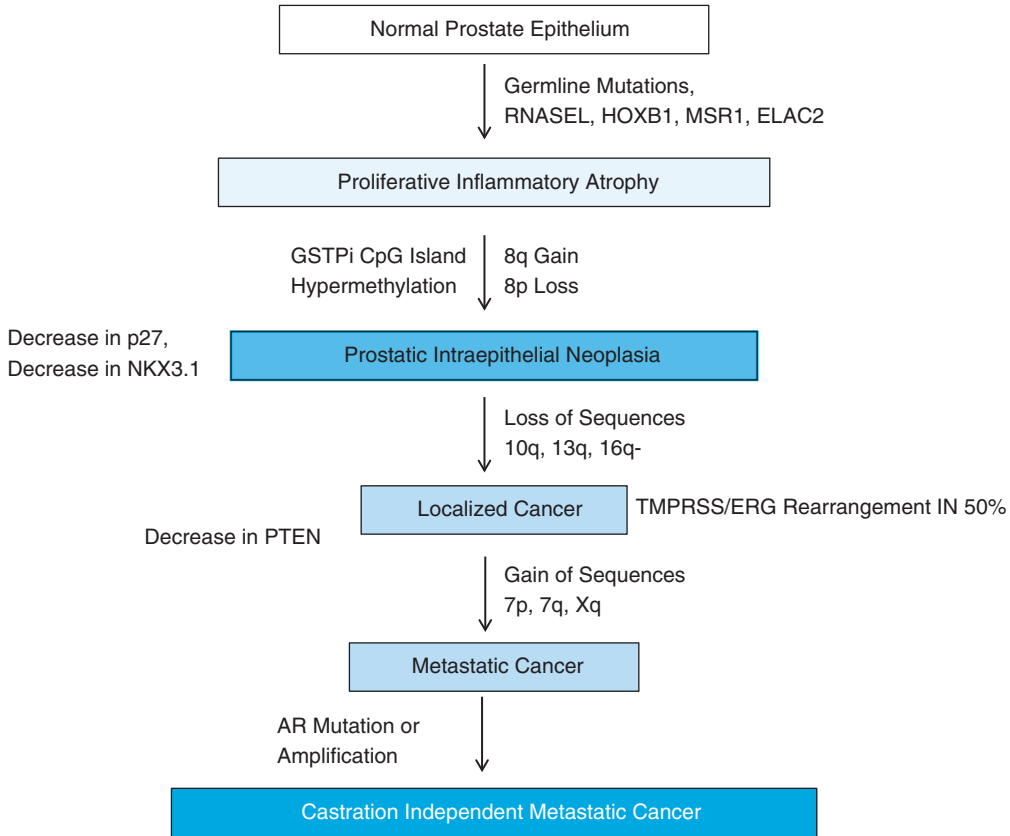


Figure 1.4 Pathogenesis of prostate cancer. Several common genetic insults have been observed throughout the progression of normal epithelium to malignancy. Inherited and somatic mutations are found in key genes regulating the immune response. Loss of the tumor suppressor genes, *NKX3.1* and *PTEN*, are early events in tumorigenesis predisposing the epithelium to malignant change. Loss of response to androgen withdrawal can occur later in disease development. Adapted from N Engl J Med. 2003 Jul 24;349(4):366–81.

agents to reduce cancer incidence. Several meta-analysis studies have not definitively shown any benefit of NSAIDs to protect against prostate cancer [77]. Unfortunately, the side-effect profiles of these drugs preclude their long-term daily use, particularly without compelling evidence of therapeutic benefit. Antioxidants such as selenium and vitamin E are common over-the-counter supplements. The SELECT trial sought to examine the effect of selenium alone, vitamin E alone, or the combination of both on prostate cancer incidence. The initial data, released in 2009, showed no benefit in any of the trial arms [78]. However, after longer follow-up, the arm using vitamin E

alone had a statistically significant increase in the amount of prostate cancer compared with the placebo group [79]. This data was discouraging, particularly given the strong interest in inflammation-induced carcinogenesis. Although it is widely accepted that intraprostatic, chronic inflammation is a risk factor for development of prostate cancer, a novel means to reduce cancer incidence by taking advantage of this mechanism remains unknown.

Amplification of chromosome 8q, which contains the gene, *c-MYC* (8q24), is a known finding in both HGPIN and carcinoma. Increased copy numbers of *c-MYC* and 8q correlate with increasing Gleason score,

disease progression, and poor prognosis [80–82]. MYC is a known oncoprotein upregulated in a variety of cancers and regulates cell proliferation, protein synthesis, and metabolism. Both mRNA and protein levels of c-MYC are elevated in prostate cancer as well as PIN, which suggests a key role of the protein in tumorigenesis [83]. Gain of MYC expression in murine models resulted in both PIN and adenocarcinoma formation recapitulating human prostate cancer [84].

Both amplification of 8q24 and c-MYC overexpression has been shown to predict biochemical recurrence after prostatectomy [85]. Fluorescence in situ hybridization (FISH) detection of 8q copy number may be a useful strategy to better risk stratify low-grade tumors. One study using RNA interference showed that MYC might be a downstream target of AR signaling [86]. Additionally, MYC has been shown to be a mediator of ligand-independent AR signaling, suggesting a mechanism for castration resistance [87]. The development of an effective inhibitor of MYC function may have clinical use to treat early hormone refractory prostate cancer. For now, MYC is a useful marker for disease progression and tumor aggressiveness.

PTEN is a known tumor-suppressor gene that encodes a phosphatase, which can target both protein and lipid substrates. By inhibiting the PI3k-Akt pathway, PTEN is thought to have a key role in inhibiting cellular growth. The gene undergoes somatic mutation during prostate cancer progression and reduced protein expression is observed in higher grade and advanced disease [88,89]. In mouse models, *Nkx3.1* loss cooperates with *Pten* loss to form murine PIN and invasive adenocarcinoma of the prostate [90,91]. NKX3.1 can upregulate IGFBP3 leading to decreased AKT phosphorylation and cell growth [72]. In the presence of decreased levels of NKX3.1, loss of PTEN activity may lead to unregulated activity of AKT and downstream targets such as p27. *CDKN1B* encodes p27, a cyclin-dependent kinase inhibitor. Decreased levels of p27 has been

shown to be a negative predictor of survival in organ-confined PCa treated with radical prostatectomy [92]. Inhibition of the androgen receptor has been shown to increase AKT signaling, which suggests a novel mechanism for resistance to androgen resistance [93]. Androgen ablation in conjunction with abrogation of the AKT signaling axis may be a potential therapeutic intervention for high-risk, localized prostate cancer. The Eastern Cooperative Oncology Group (ECOG) is studying the effect of AKT inhibition in combination bicalutamide in patients with biochemical recurrence. The results of this Phase II study are pending.

In 2005, *ERG* was discovered to be overexpressed in prostate cancer specimens [94]. Using cancer outlier profile analysis (COPA), *ERG* and *ETV1*, both members of the ETS transcription factor family, were noted to be outliers in prostate cancer [95]. Further analysis showed that both genes were found as fusion products with the 5' untranslated region of the androgen responsive *TMPRSS2* gene. More than 20 members of the ETS family have been found in gene rearrangements with *ERG* being the most-common and implicated in approximately 50% of prostate cancers [96]. Interestingly, the *TMPRSS2-ERG* fusion has not been found in normal prostate epithelium but has been shown in HGPIN [97]. This finding suggests a key role for this fusion product in carcinogenesis. The prognostic significance of the gene fusion is less clear. Although higher levels of ETS transcription factor expression were found in cancers with a lower Gleason score, a population cohort study found an association between the presence of the *TMPRSS2:ERG* fusion and prostate cancer-specific death in patients managed with watchful waiting [98,99]. In 2012, a cohort of 1,180 men treated with radical prostatectomy found no significant association between *TMPRSS2:ERG* and biochemical recurrence or mortality [100]. There remains conflicting evidence regarding the prognostic implication of the gene fusion and how best to use this information. However, the presence of the fusion at

early stages of disease may aid with more accurate and earlier diagnosis particularly when combined with prostate-specific antigen (PSA) screening [101].

Gene Expression and Molecular-Modeling Concepts

The link between high PSA, Gleason score, and clinical staging with PCA outcomes is well established. Yet within each category, there is a large degree of heterogeneity resulting in uncertainty about which cancers will remain clinically irrelevant or aggressive and fatal. As research techniques have become more sophisticated, a better understanding of the genetic and molecular dysregulation has resulted. The clarification of tumor biology may serve to complement the conventional risk stratification criteria for treatment.

Several studies have examined the gene expression profiles within prostate cancers at different stages of both development and progression. Clarifying the mechanism of how prostate cancers are initiated and eventually evolve may explain how some cancers can be managed with a tissue-sparing approach compared with definitive resection.

Microarray expression profiling became popularized in the late 1990s and early 2000s. This technique allowed researchers to survey the differential expression of a large number of genes. Common molecular changes in prostate cancer were already known including overexpression of c-MYC and loss of p27 and PTEN. Several groups investigated the gene expression profiles of tumor compared to normal prostate tissue [102,103]. These early studies validated the technique and identified a number of genes differentially expressed in malignant tissue. Singh *et al.* compared microdissected tumor cells to normal prostate and sought to identify a high-risk signature that could be correlated with outcome [104]. A 5-gene signature that included, IGFBP3, PDGFRb, and Chromogranin A was predictive of

disease-free survival. Traditional markers of aggressiveness, PSA, and Gleason score did not significantly correlate with disease-free survival in this study. In 2003, Best *et al.* analyzed high- and moderate-grade tumors to identify a different 21-gene signature to predict high-grade cancers (Gleason 9-10 vs. 5-7) [105]. This signature was not tested to predict clinical outcome and showed little commonality with prior studies. Biochemical recurrence was also predicted based on microarray analysis, but little overlap was noted in comparison to the Singh data [106].

A consensus pattern defining high-risk disease has remained elusive. It is this heterogeneity that has limited attempts to define a meaningful molecular signature that may be used in the clinical setting to guide therapy. LaPointe *et al.* made the observation that low-grade tumors had a profile closer to normal prostate epithelium, perhaps suggestive of a more differentiated state [106]. Genes involved in cellular invasion and angiogenesis defined higher grade cancers. Because there is not a defining gene “signature” across multiple studies, a potential use of microarray analysis may be to identify themes of molecular changes.

Tomlins *et al.* used “molecular concepts,” which are defined as a set of biologically connected genes to characterize prostate carcinogenesis [107]. This approach seeks to identify patterns of dysregulation rather than changes in individual genes. In their study, more than 14,000 molecular concepts were analyzed to define progression signatures from benign epithelium through metastatic disease (Figure 1.5). Interestingly, Tomlins *et al.* found only subtle differences in gene expression between low- and high-grade tumors as in LaPointe *et al.* No clear pattern of differential gene expression was identified. Using molecular concept mapping analysis, the group found a strong enrichment of decreased androgen signaling in high-grade tumors. Genes known to be upregulated in

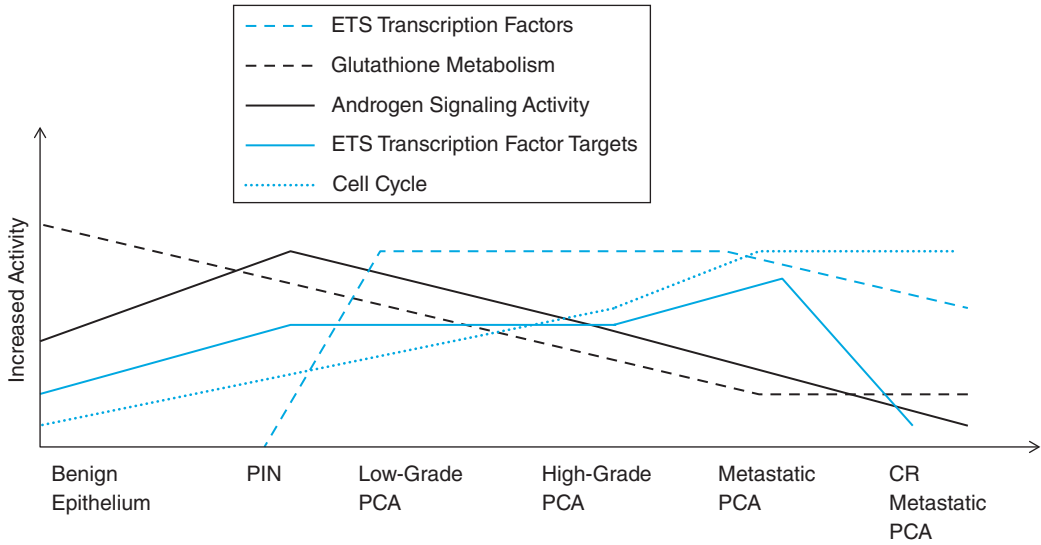


Figure 1.5 Molecular concept mapping of prostate cancer. The relative expression of molecular concepts are mapped according to prostate cancer progression from benign epithelium to hormone naïve (HN) and hormone refractory (HR) metastatic disease. Seven of the highest enriched concepts are shown. Adapted from Nat Genet. 2007 Jan;39(1):41–51.

the presence of androgens had lower levels of expression with more advanced disease. This trend was most significant in metastatic disease and may highlight the selection of cell populations that can survive with low levels of androgen signaling. Also noted throughout disease progression was an increase in ETS target genes, protein biosynthesis, and amplification of MYC, which are known markers of advanced disease.

The studies using microarray and, later, molecular concept mapping, highlight the evolutionary changes in prostate cancer. Teasing out the key regulator genes and proteins that define both low- and high-risk disease has been difficult. What is clear is that prostate cancer remains largely an indolent disease. Several alterations in gene expression, mutation, and copy number are required to progress from normal epithelium to prostate cancer, which can take decades to occur.

As shown in Tomlins *et al.* decreases in androgen signaling may be a hallmark of

higher risk disease and eventually metastasis. The dedifferentiation that defines higher Gleason scores may reflect the ability of the tumor to progress with less reliance on the androgen signaling axis. Perhaps, the efficiency of the tumor to use alternative pathways for survival may be a key determinant for further disease progression. Such an example may involve MYC, which also becomes amplified in progression of disease as well as correlating with higher Gleason score. Moreover, the transformation to androgen independence may be reflective of changes in sugar metabolism or the ability to metabolize sex hormones [108]. As tumors become less responsive to hormonal therapies, new targeted therapies are needed to further prolong survival and delay chemotherapy. Molecular concept mapping has identified novel pathways that may be key to further understanding androgen independence and lead to clinically relevant interventions.

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