Molecular Classification of Prostate Cancer Progression: Foundation for Marker-Driven Treatment of Prostate Cancer

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ABSTRACT

Recently, many therapeutic agents for prostate cancer have been approved that target the androgen receptor and/or the prostate tumor microenvironment. Each of these therapies has modestly increased patient survival. A better understanding of when in the course of prostate cancer progression specific therapies should be applied, and of what biomarkers would indicate when resistance arises, would almost certainly improve survival due to these therapies. Thus, applying the armamentarium of therapeutic agents in the right sequences in the right combination at the right time is a major goal in prostate cancer treatment. For this to occur, an understanding of prostate cancer evolution during progression is required. In this review, we discuss the current understanding of prostate cancer progression, but challenge the prevailing view by proposing a new model of prostate cancer progression, with the goal of improving biologic classification and treatment strategies. We use this model to discuss how integrating clinical and basic understanding of prostate cancer will lead to better implementation of molecularly targeted therapeutics and improve patient survival.

Significance: Rapid development of drugs with efficacy against prostate cancer now makes it possible to consider applying these agents with curative intent in men with currently incurable cancers. However, when to apply these new drugs, as well as those under development, to obtain the best outcomes is a challenge that must be addressed. To meet this challenge, better classification of the disease based on the underlying molecular mechanisms of progression will facilitate the implementation of current and emerging therapies.

CURRENT CLINICAL MODEL OF PROSTATE CANCER PROGRESSION

The pathologic classification of prostate cancer is defined by Gleason sum score (Gleason, Union for International Cancer Control), which is based on morphologic criteria. The Gleason score is the major method for prostate cancer tissue grading (1) and the most important prognostic factor in prostate cancer (2). A high Gleason score predicts more rapid progression and suggests that aggressive treatments are needed. However, the Gleason score does not provide information on therapy selection. As a result, patients are currently grouped by clinical stage or treatment status (e.g., with or without bone metastasis, resistance to androgen ablation therapy or not, with or without chemotherapy). This framework categorizes patients with similar prognoses (3, 4). Thus, these factors currently dictate clinical trial design. However, this approach does not have a mechanistic foundation that can guide the proper sequences or combinations of molecularly targeted agents. The current model of prostate cancer progression also fails to account for the observation that the state of cancer progression determines drug-specific efficacy. For example, androgen ablation, but not chemotherapy, is more efficacious when given at early-stage prostate cancer progression (5). Paradoxically, chemotherapy is more effective at the later stages of prostate cancer progression (6–8). This stage-dependent response to treatments indicates that prostate cancer undergoes an evolution into different states during disease progression. Furthermore, prostate cancer shows site-specific preference of progression, in that prostate and bone are two preferred areas of persistent or recurrent cancer. Although prostate cancer also metastasizes to lymph nodes, these metastases are usually not resistant...
to therapy. These observations suggest that prostate cancer has a unique relationship with the microenvironment in the prostate and bone (9, 10). Each of these features is therapeutically relevant, but they do not provide a guideline for therapy selection.

WHY A NEW CLASSIFICATION IS NEEDED

The above problems indicate that a new classification system will be needed to guide therapy selection. Rather than using tumor morphology as a criterion for therapy selection, molecular markers that define a specific stage of progression would be preferable in choosing therapy, independent of the tumor stage. Our recent understanding of the molecular mechanisms of prostate cancer progression has determined that the androgen receptor (AR), oncogenes/tumor suppressors, and microenvironment are the major mechanisms that lead to prostate cancer progression and will provide such biomarkers to guide therapy approaches. Therefore, these mechanisms need to be incorporated into a classification system designed to guide therapy.

Multiple lines of evidence show that AR function plays a central role throughout the entire process of prostate cancer progression. The prostate is an androgen-dependent organ, and androgen ablation is commonly used in the treatment of advanced prostate cancer. However, nearly all patients with advanced cancers will develop progressive disease despite castrate levels of androgens (testosterone level <50 ng/mL), that is, castration-resistant prostate cancer. Recent studies have suggested multiple “escape mechanisms” that lead to AR signaling under “castration levels of androgens” (11–13), which will be discussed below. Thus, the complex alterations in AR signaling need to be considered and potentially targeted throughout nearly the entirety of prostate cancer progression (14, 15).

Loss of tumor suppressor genes is also critical in prostate cancer progression. The loss of PTEN, p53, and RB (16–19) is common in prostate cancer progression. Loss of PTEN, observed in some cancers at diagnosis, is linked to shorter progression-free and overall survival (20). However, to date, PTEN loss has not been predictive of response to specific therapies. Similarly, the loss of p53 and RB, while linked to more advanced stages of the cancer, is also not predictive of response to specific therapies.

Aberrant activation or expression of oncogenes, for example, Src, MET, Axl, and FGFR, is prevalent in the late stage of prostate cancer progression. Several inhibitors of tyrosine kinases have been used in clinical trials. Each seems to have some efficacy on subsets of patients, supporting their roles as drivers of prostate cancer progression in a limited subset of tumors. Although activation of oncogenes may play an important role in prostate cancer progression, AR signaling and oncogene activation are not independent mediators of this process. Decreased androgen levels have been shown to induce MET oncogene expression (21), and the aberrant expression of oncogenes also affects AR function through AR phosphorylation and/or direct association of AR with oncogenes (22–25). Therefore, a biologic classification scheme needs to consider both AR status and the expression and activation of oncogenes.

The salient feature of advanced prostate cancer progression is the bone-forming and bone-homing nature of metastases. These findings led investigators to hypothesize that the prostate tumor microenvironment in bone plays a role in prostate cancer progression. This hypothesis is supported by the clinical trial in which Sr89, a radionuclide that targets bone environment following chemotherapy, exhibited an increase in survival, which concurs with a more recent report with Radium 223, a bone-homing alpha-emitter with a favorable therapeutic index (26–28). Though one cannot attribute the effects of Sr89 and Radium 223 exclusively to treatment effects on the tumor microenvironment, the bone-targeting nature of these radiopharmaceuticals implicates bone in the lethal progression of prostate cancer and serves as an impetus to focus mechanistic and clinical studies to understand the growth of bone metastases. These factors should be put in a disease progression context that can be exploited therapeutically.

BIOLOGIC CLASSIFICATION OF PROSTATE CANCER

On the basis of the above considerations, the purpose of this review is to propose a new molecular classification of prostate cancer that incorporates AR, oncogenes/tumor suppressors, and the tumor/bone microenvironment in the disease model. Because of the heterogeneity of prostate cancer, responses to therapies have given us better insights into which of these players are drivers of specific stages of progression. This “responses-to-therapies” concept forms the basis of a new molecular classification of prostate cancer and should help to determine at which stage of progression specific inhibitors can be most effectively applied.

In our alternative model, prostate cancer progression is grouped into three categories: endocrine-driven, microenvironment-dependent, and tumor cell-autonomous. Androgen signaling plays a central role in this model of tumor progression. In the early phase of prostate cancer, androgen signaling responds to dihydrotestosterone (DHT) depletion (endocrine-driven; Fig. 1). A portion of low-grade and low-stage cancers are in this “DHT-dependent” stage. However, upon “escaping” DHT dependence, cancers are characterized by paracrine-driven progression where androgen signaling remains important, albeit by different mechanisms (detailed below). The transition from an endocrine- to a paracrine-driven prostate cancer is a milestone that signals the potential lethal progression of the cancer. In this phase of prostate cancer progression, the cancer enters into what we term a “progression spiral,” in which numerous changes in androgen signaling are accompanied by altered microenvironment/tumor interactions. In the last phase of the disease, the cancer cells lose AR dependence, exit the “paracrine progression spiral,” and become tumor cell-autonomous. This model serves as a framework to group prostate cancers into therapeutically relevant subsets.

ENDOCRINE-DRIVEN PHASE

The endocrine-driven phase of prostate cancer depends on the presence of 5α-DHT, occurring by 5α-reduction of
Biology-Based Prostate Cancer Classification

Figure 1. DHT-dependent phase of prostate cancer. In this phase of prostate cancer, some tumors rely mainly on DHT. The source of testosterone (T) is mainly from the testis, and adrenal glands provide dehydroepiandrosterone (DHEA). Testosterone and DHEA are converted to DHT by 5-α-reductases (SRD5A) present in the prostate. AR has higher affinity for DHT than testosterone. For this reason, finasteride and dutasteride, which inhibit type I or both type I and type II 5-α-reductase activity, respectively, can suppress tumor development or growth, accounting for suppression of low-grade cancers, but not high-grade cancers, in a proportion of men with prostate cancer.

testosterone (see Fig. 1). Although testosterone and DHT both bind to AR and activate AR and its downstream-targeted genes, DHT has a lower dissociation rate and therefore a greater effect on AR signaling than does testosterone (29, 30). Thus, inhibition of DHT formation should reduce AR activity. Indeed, in the endocrine-driven phase of prostate cancer, androgen signaling responds to DHT depletion. The 5-α-reductase inhibitors finasteride and dutasteride are used to prevent the conversion of testosterone to DHT to interrupt DHT’s growth-promoting signaling. However, the Prostate Cancer Prevention Trial (31) and Reduction by Dutasteride of Prostate Cancer Events (32) trial showed that finasteride and dutasteride reduced the rate of low-grade cancers but did not have an effect on high-grade cancers. In addition, the Reduction by Dutasteride of Clinical Progression Events in Expectant Management trial (33) showed that a significant number of patients with low-grade cancer at initial diagnosis did not have detectable cancer on subsequent repeat biopsies after dutasteride treatment compared with placebo. These findings support the hypothesis that some low-grade cancers are dependent on DHT (34). The grade-dependent effects of finasteride or dutasteride point to therapeutically relevant heterogeneity of androgen signaling networks that are different between low-grade and higher-grade cancers.

Molecular Mechanisms of Endocrine-Driven Phase

Differential effects of DHT and testosterone on AR signaling were observed by several groups. In characterizing the different roles of testosterone and DHT on signaling, Lin and Chang (35) found that the expression of TDD5, an androgen target gene, was differentially repressed by testosterone as compared with DHT. In animal studies, Dadras and colleagues (36) reported that castrated rats treated with testosterone and finasteride expressed higher levels of genes responsible for prostate growth inhibition and differentiation than those treated with testosterone alone. Because finasteride treatment led to high intraprostatic testosterone levels, the studies concluded that the effects of DHT on proliferation and differentiation were not the same as those of testosterone (36).

In another set of experiments, Li and colleagues (37) found that finasteride or dutasteride caused an overall reduction in intraprostatic DHT levels. However, the central axis of androgen signaling is subject to multiple regulatory pathways. Their results indicated that regardless of the AR status, 5-α-reductase genotypic variants, and varying expression levels of 5-α-reductase in prostate cancer cells are also responsible for modulating AR signaling. They found that 5-α-reductase expression varied among prostate cell lines, and, further, the expression of each of the three 5-α-reductase enzymes in response to androgen was also cell type specific. Recent studies suggest that type I 5-α-reductase is increased in most prostate tumors (relative to type II, which is more predominant in the normal prostate), leading the authors to argue that the efficacy of dutasteride, a dual 5-α-reductase inhibitor, would be superior to finasteride, a type II 5-α-reductase inhibitor (38).

More recently, laboratory studies have been undertaken in an attempt to explain the clinical trial results with 5-α-reductase inhibitors. Using samples from men with prostate cancer who had undergone treatment with one of two dutasteride dosages for 4 months before prostatectomy, Mostaghel and colleagues (39) microdissected normal prostate epithelial cells and analyzed the expression of 90 androgen-regulated genes. The results showed that treated samples could be grouped into high- or low-AR gene activity groups based on their gene-expression profiles. The relationship between AR gene activity and treatment response to dutasteride has not yet been clarified.

Marker and Therapy for Endocrine-Driven Phase

The grade- and stage-dependent differential responses of prostate epithelial cells to DHT depletion by 5-α-reductase
inhibitors support the conclusion that a portion of early prostate cancers can be identified by the development of markers that point to DHT dependence. The differences between the DHT-dependent and -independent groups are most likely due to the changes in AR sensitivity to DHT. However, the biochemical basis for these changes remains unclear. Understanding the androgen signaling networks, including the expression of the 5α-reductase enzymes, during early stages of prostate cancer progression will be important in determining whether the cancer is in the endocrine-driven stage and whether the patient will respond to treatment with 5α-reductase inhibitors.

**MICROENVIRONMENT-DEPENDENT PHASE (ENDOCRINE-TO-PARACRINE TRANSITION)**

The transition from endocrine- to paracrine-driven prostate cancer signals potential lethal progression of the cancer. For patients with high-grade prostate cancer, androgen ablation (depletion of gonadal androgen), for example by Lupron, is more effective than 5α-ablation (depletion of gonadal androgen), for example by Lupron, is more effective than 5α-ablation (depletion of gonadal androgen), for example by Lupron, is more effective than 5α-ablation (depletion of gonadal androgen)

For patients with high-grade prostate cancer, androgen ablation (depletion of gonadal androgen), for example by Lupron, is more effective than 5α-reductase in inhibiting high-grade or metastatic prostate cancer (40). However, the response to androgen ablation is heterogeneous in that some patients have a sustained suppressive effect, whereas others are refractory to the treatment within a few years. When the disease advances to the metastatic stage, only a minority of men with prostate cancer will have sustained control of cancer with androgen ablation. This heterogeneity in response to androgen ablation indicates a clinically and biologically meaningful difference in the role of AR signaling among different stages of prostate cancers. These clinical observations suggest that different mechanisms of AR signaling need to be considered in categorizing patients (Fig. 2).

Associated with AR changes is the activation of oncogenes and loss of tumor suppressor genes, which has been discussed in numerous reviews (41, 42). Loss of the PTEN tumor suppressor gene, leading to the activation of the phosphoinositide 3-kinase/Akt pathway, is one of the earliest genetic changes detected during prostate cancer progression (43). Several oncogenes, as described above, are upregulated in advanced prostate cancer (42). Interestingly, androgen depletion leads to upregulation of genes associated with epithelial-to-mesenchymal transition (44), as observed with the expression of mesenchymal cadherins, for example, N-cadherin (45) or cadherin-11 (46), in castrate-resistant prostate cancer. The combination of these changes leads to prostate cancer progression and metastasis (see Fig. 2).

Acquired resistance to androgen deprivation coincides with progression of cancer in bone, the preferred area of recurrent cancer, pointing to the presence of a specific bone–epithelial interaction that drives the striking organ-specific progression (see Fig. 2).

Together, these observations indicate that under the selective pressure of androgen ablation, prostate cancers evolve from an endocrine-driven (by the gonadal steroid hormones) to a paracrine-driven (by the factors present in the tumor microenvironment) cancer. The endocrine-to-paracrine transition of prostate cancer often signals the presence of disseminated cancer that is incurable with current standard therapy.

During the microenvironment-dependent phase, cancer progression is dominated by tumor adaptation over time. These adaptations comprise continuous vicious cycles, in which the microenvironment alters the tumors and the tumors in turn alter the microenvironment. We propose the term “progression spiral” to illustrate these serial changes over time (see Fig. 2). The serial molecular mechanisms that drive vicious cycles are reflected in the “turns” of the spiral. The interval between each turn, the “pitch,” reflects the rate of adaptation of the tumor. Although there are many changes that may occur in a “turn,” we refer to the changes that are most critical in driving the tumor progression as the key player in each turn.

Because prostate cancer is heterogeneous, with varied “drivers” for different tumors at different times of tumor progression, the “turns” may be identified through the response to specific therapy. For example, a tumor that is responsive to abiraterone, a Cyp17 (a steroid synthesis enzyme) inhibitor, may comprise one such turn (Fig. 2). The duration during which the tumor remains responsive to abiraterone therapy is the “pitch.” When the tumor is no longer responsive to androgen depletion therapy, it indicates that another turn has occurred and the tumor has progressed into another phase of the progression spiral, which signals that additional alterations in the tumor and its microenvironment have occurred. Tumors in this new “spiral” will require different therapeutics that specifically target the altered properties that define this phase of the spiral. At present, the early “turns,” which are detected by responses to specific targeted therapy, have been identified (Fig. 2). Because novel antiandrogens targeting AR signaling as initial therapy of prostate cancer are more effective in early rather than in later stages of progression, altered androgen biosynthesis and changes in the AR are the drivers of the initial turns of the progression spiral. Candidate drivers of the later turns in the spiral have been identified, but their sequence remains unclear (Fig. 2, shaded area). Examination of the molecular changes before and after challenge with targeted agents will clarify the pathways to help guide targeted therapy to specific turns in the spiral.

**Molecular Mechanisms of Microenvironment-Dependent Phase**

The progression spiral is characterized by a combination of signaling through AR and the tumor microenvironment. With respect to AR signaling, recent studies have suggested multiple “escape mechanisms” by which AR can sustain signaling under “castrate level” of androgen (11, 14). These escape mechanisms are important in driving tumor progression. Intracellular production of androgen by upregulation of the steroid synthesis enzymes, for example, Cyp17, induces the transition from endocrine to intracellular androgen dependence (11). AR copy number consistently increases after prolonged castration (47). The involvement of these mechanisms in prostate cancer progression is shown by a recent report that therapeutic agents, for example, abiraterone (12) or MDV3100 (48), that deplete tumor-produced androgens or inhibit the function of the androgen receptor, respectively, lead to improved clinical outcomes (Fig. 2). It was also shown that mutations of AR broaden AR ligand...
Figure 2. Proposed spiral model for prostate cancer progression. The model proposes 3 main phases in prostate cancer progression. The first phase is the DHT-dependent phase, during which the tumor is responsive to 5α-reductase inhibitor treatments, as indicated by the yellow arrow on the left side of the figure. When the tumor is no longer responsive to inhibitors of 5α-reductase, it enters the progression spiral, as marked by a broad up arrow, where multiple factors, including AR signaling changes, oncogene activation, tumor suppressor gene downregulation (not shown), and microenvironment changes, affect tumor progression. Each "turn" is defined by a predictive marker(s) that can be targeted. The pitch in each spiral reflects the duration that the tumors remain responsive to a specific therapy. The adaptive changes in tumors in response to therapy account for resistance, leading the tumor to progress to the next turn of the progression spiral, which signals additional alterations in the tumor and its microenvironment. Tumors in this new "turn" will require different therapeutics that specifically target the altered properties that define this turn. Markers that reflect the biology that drives each turn can be used to guide timely therapy application in anticipation of progression. Exit from the spiral occurs when a series of mutations arise, including the loss of AR, RB, or p53, upregulation of polo-like kinase 1 (PLK1), Aurora kinase A (AURKA), and amplification of MYCN. At this stage, the prostate cancer cells are no longer regulated by the microenvironment and become tumor cell autonomous, as indicated by the red arrow on the right side of the figure. Targeted therapies that may affect candidates that drive turns in the spiral are indicated in the figure. Possible disease stages corresponding to the spiral are also indicated.
specifcity such that glucocorticoids besides androgen are able to activate AR (49). Another mechanism that accounts for castration resistance and occurs frequently after therapy is the expression of AR isoforms (AR-Vs) that lack the AR ligand-binding domain but nevertheless can transduce signals (50). Importantly, an increasing number of studies show that these AR-Vs induce the transcription of additional genes (51–53). The AR-V signature is composed of numerous genes involved in mitosis, including UBE2C, whose expression correlates with AR-V7 expression in clinical specimens (52). Several groups showed that in androgen-independent prostate cancer cells, AR regulates a distinct transcriptional program that is different from AR-dependent prostate cancer cells, notably the upregulation of mitotic cell-cycle genes (13, 54). Androgen depletion has also been reported to lead to an increase in the expression of mesenchymal cell adhesion molecules, including N-cadherin (45, 55) and cadherin-11 (46), which increase the migratory property of tumor cells as well as contribute to the interaction of tumor cells with osteoblasts in bone microenvironment. Thus, as androgen becomes further and further depleted, AR signaling becomes altered, favoring metastasis and growth-promoting functions over differentiation functions (54). These alterations may ultimately signal the final turn in the progression spiral, that is, poorly differentiated neuroendocrine carcinoma, which is devoid of AR.

The changes in “turns” in the “progression spiral” are determined not only by AR alterations but also by activation of oncogenes and changes in the tumor microenvironment. As a result of these interactions, numerous oncogenes are activated. As this topic has been discussed in Gallick and colleagues (42), only a few illustrative examples are given here. When and how these oncogenes are activated is complex and not well understood. Examples of oncogenes that may play a role in turns of the spiral include Src, IGF-IR, FGF-R, MET, Ad, and ACK. At later stages of the spiral, polo-like kinase 1 (PLK1), Aurora kinase A (AURKA), UBE2C, and MYCN are upregulated due to androgen depletion (Fig. 2). These may drive late stages of disease progression.

Activation of oncogenes and androgen depletion are not independent functions. Emerging evidence suggests that there is bidirectional crosstalk between oncogenes and AR. Resistance to AR blockade has been shown to be associated with an increase in Src activity, as evidenced by phosphorylated Src family kinase expression (Efstathiou and colleagues, manuscript in preparation); thus, activation of Src family kinases (SFK) may be used as a marker, but the mechanism is not known. Androgen depletion has been shown to increase the expression of MET (21). Hence, this may contribute to the response seen by cabozantinib in castration-resistant prostate cancer (56). Oncogene kinases, for example, Src, Her2, AKT, and ACK, have been shown to phosphorylate AR, which may change the functions of AR as well as affect oncogene activation (57). Each of these changes may represent an individual “turn” in the spiral, or may work in conjunction or sequence to lead to progressive turns. Because we do not have detailed knowledge of how many of the identified AR changes affect prostate cancer progression, nor do we know the time and sequence of oncogene activation, it is not yet possible to assign the specific “AR signaling changes” and “oncogene changes” to specific turns in the spiral progression (hence, the ambiguity represented by the shaded box in Fig. 2). The combination of AR signaling blockers and molecular targeting agents will allow us to define these “turns,” which represent critical changes in disease status. It is likely that the factors that determine the “turns” may be different among individuals, as the specific oncogene and sequence of oncogene activation may vary among tumors. Nevertheless, if targeted therapy against a given oncogene is successful, it will prolong the time a patient remains in a specific turn as dictated by the activation of that oncogene (Fig. 3). Subsequent identification of markers for patients entering a specific “turn” in the spiral will likely determine future therapy selection.

Castration-resistant prostate cancer progression is also dependent on paracrine factors from the tumor environment. Gleave and colleagues (58) showed that by coinoculating the low tumorigenic androgen-responsive LNCaP cells with human bone fibroblast, a castration-resistant cell line C4-2 was generated, indicating that stromal cells are able to modify the tumor cell properties and render LNCaP cells castrate resistant (58–60). The tumor cell–environment interactions are mostly driven by paracrine factors. It has been shown that prostate cancer cells secrete bone morphogenetic protein (61–63), VEGF (64), or fibroblast growth factor 9 (FGF9; ref. 65), which affect the proliferation of stromal cells and increase tumor angiogenesis. In turn, tumor-educated stromal cells provide factors, such as osteonectin (66, 67), that increase tumor cell invasiveness, survival, or proliferation. In addition to bone formation, proliferation of tumor cells in the bone marrow frequently induces an osteolytic response, as reflected in the increase in receptor activator of nuclear factor-kappa B ligand (68, 69) and N-telopeptide (70). Increased bone resorption leads to the release of TGF-β from the bone matrix (71), further altering tumor cell properties. These vicious cycles are also important in driving “turns” in the spiral. In addition, both the tumor and the microenvironment adapt under selection pressure of therapies, and such adaptations can lead to turns into different phases of the spiral. A combination of all these factors amplifies the bidirectional interactions to multidimensional, multilevel communication, representing another example of a vicious cycle. Thus, the combination of alterations in AR signaling, oncogene activation, and environmental factors all contribute to the spiral model presented in Fig. 2.

Markers and Treatment Strategies for Microenvironment-Driven Phase

The spiral model suggests that therapy should be applied before complex interactions between a tumor and its microenvironment occur. Some patients with primary prostate cancer have a sustained suppressive effect in response to androgen ablation, suggesting that there is a stage of prostate cancer that is sensitive to gonadal androgen. These patients eventually become refractory to treatment within a few years, and this stage of prostate cancer defines an initial “turn” in the spiral. The markers that point to the entry into the initial turn may be an increase in Cyp17 expression in prostate cancer cells, an indication of intracrine androgen biosynthesis. If this were the case, application of abiraterone may lead to
prolongation of the “Cyp17 turn” (see Fig. 3). In support of this possibility, recent studies from our group showed that application of abiraterone at the early stage of prostate cancer significantly improves clinical outcome (unpublished data). AR amplification and/or mutations may lead to a lack of response to abiraterone treatment. Increased SFK activity may be developed as a marker for predicting abiraterone resistance (Efstathiou and colleagues, manuscript in preparation) and entry into the next phase of the spiral. Detection of an increase in SFKs would suggest that an inhibitor such as dasatinib may be applied in preventing the disease from advancing into the next turn of the spiral. These are potential examples of how the proposed model may be used to predict disease progression and implement targeted therapy at the right time with the right sequence. However, some men with prostate cancer may have very short pitches, indicating very rapid disease progression. These short pitches indicate multiple alterations occur in a very short period of time. In these instances, a single therapy may be unable to elicit a response. As of yet, no predictive markers exist that can guide treatment for this subset of men with prostate cancer. In this case, a combination therapy based on the emergence of multiple markers may be considered.

As shown in the model in Fig. 3A, it is possible that no further “turns” in the spiral will occur, suggesting that the therapy may be curative. This possibility is likely to occur through earlier application of therapy to a known predictive marker. Alternatively, the turn may be elongated, suggesting that the targeted therapy has been effective against the predictive marker (Fig. 3B). Following the treatment, tumor adaptation may occur, leading to the next turn in the spiral. Successful application of the correct therapeutic agent at this stage of the spiral will elongate the next turn. Application of targeted therapy does not have a positive therapeutic effect. This result would suggest that the tumor is in a more complex phase of the disease.

**Figure 3.** Possible outcomes of therapeutic treatments using the spiral model. A turn in the spiral is defined through the expression or alteration of a predictive marker. Three possible outcomes of therapeutic treatment may occur based on a predictive marker that defines a turn in the spiral. Abiraterone is used as an example. A, after application of abiraterone, no further “turns” in the spiral occur, suggesting that the therapy may be curative. B, the turn may be elongated, suggesting that abiraterone is effective in reducing androgen generated from Cyp17. Following the treatment, tumor adaptation may occur, leading to the next turn in the spiral. Successful application of the correct therapeutic agent at this stage of the spiral will elongate the next turn. C, application of targeted therapy does not have a positive therapeutic effect. This result would suggest that the tumor is in a more complex phase of the disease.

**TUMOR CELL AUTONOMOUS PHASE**

The exit from the microenvironment-dependent progression spiral is heralded by a distinct clinical manifestation characterized by a large tumor mass in the prostate or lymph nodes, and visceral metastases without a commensurate increase in serum prostate-specific antigen (PSA). If bone metastases occur, they are predominantly lytic bone metastases. These findings reflect the emergence of a rapidly proliferating androgen-independent cancer that will require different treatment. In contrast to the microenvironment-dependent phase, prostate cancer responds to chemotherapeutics, including docetaxel, by reducing the tumor volume, an indication that the tumor is no longer regulated by its microenvironment, a stage we term the “tumor cell autonomous” phase.

This form of prostate cancer can be distinguished from other castrate-resistant prostate cancer because it does not
express the AR and/or secrete PSA, thus its growth is truly androgen-independent. The clinical features of the “tumor cell autonomous” phase of prostate cancer mimic small cell carcinomas. Pathologically, most of these cancers share some neuroendocrine features and, in their pure form, are termed neuroendocrine prostate cancer (NEPC) (72–74). NEPC is rare at de novo diagnosis, as less than 1% of prostate cancer at initial diagnosis comprises this histologic/morphology subtype (75). However, NEPC seems to occur most commonly after the failure of hormone therapy and is thus almost certain to increase in frequency as better androgen deprivation therapies are used clinically. It was noted that some tumors may possess a morphologic spectrum ranging from acinar adenocarcinoma to a small cell phenotype (19, 75, 76). Rearrangement of TMPRSS2–ERG is found in NEPC (77, 78), providing additional support that NEPC progresses from prostate cancer adenocarcinoma. These findings are in line with clinical observations that most NEPC are detected upon sudden and rapid progression in the setting of advanced adenocarcinoma of the prostate. NEPC is therefore predicted to be responsible for an increasing percentage of lethality from prostate cancer, estimated to be as high as 30% (19, 76, 79, 80).

**Molecular Mechanisms of Cell Autonomous Phase**

The mechanism by which the “tumor cell autonomous” phase arises is under intense study but is yet unclear, including how the tumor becomes enriched with AR-negative cells. The hallmark of this transition is loss of RB and loss or regulation of the anaphase-promoting complex, and eventual exit from the microenvironment-dependent phase. NEPC or SCPC markers such as chromogranin A, synaptophysin, and neuron-specific enolase are also characteristic markers of this phase (88), as is MYCN amplification.

In the “tumor cell autonomous” phase of the disease, inhibitors that affect mitotic function may be efficacious, as opposed to earlier stages when AR signaling affects more “classic” AR-mediated pathways. Currently, first-line treatment for this phase is chemotherapy, but patients become rapidly resistant to this approach. As the molecular basis for SCPC becomes better understood, individualized therapy may be possible. For example, AURKA inhibitors such as PHA-739358 (danusertib) have been tested in clinical trials. However, danusertib failed to achieve the primary endpoint of PSA response (89). On the basis of our analysis, PSA as an endpoint is unlikely to be suitable for tumors that are in the “tumor cell autonomous” phase. In addition, therapeutic treatment in this trial was not directed specifically to patients with amplified AURKA; hence, it is not certain whether better response would have been achieved by focusing on NEPC patients with amplified AURKA.

The result of genetic instability leads to additional changes, many of which affect cell-cycle genes, especially those related to M-phase transition, including AURKA and PLK1 (82). Wang and colleagues (13) showed that “cell cycle” (52 transcripts) and “mitotic cell cycle” (24 transcripts) are the 2 top upregulated transcripts by Gene Ontology analysis. AURKA, PLK1, UBE2C, as well as MYCN are all examples of potential markers. AURKA regulates entry into mitosis, as well as assembly of the mitotic spindle apparatus, thereby affecting chromosome separation (83). MYCN amplification is frequently associated with AURKA amplification. In addition, Otto and colleagues (84) also showed that AURKA stabilizes MYCN.

Another regulator of M-Phase overexpressed in prostate cancer is PLK1. PLK1 mediates entry into mitosis as well as centrosome maturation, spindle checkpoint activity, activation of the anaphase-promoting complex, and eventual exit from the M-phase with the initiation of cytokinesis (85). PLK1 is overexpressed in prostate cancer, with higher expression in high-grade tumors (86). PTEN loss upregulates PLK1 (87). Recently, Deerkas and colleagues (82) showed increases in PLK1 expression in androgen-independent LNCaP cells. Importantly, these cells respond to the PLK inhibitor BI2536 by undergoing necroptosis (82). Thus, numerous recent results suggest the possible importance of inhibitors of M-phase gene products as therapies for NEPC, discussed below.

**Markers and Treatment Strategies for Cell Autonomous Phase**

In the late stage of the microenvironment-dependent phase, AR is either not present or has undergone changes as to regulate a distinct transcriptional program, for example, mitotic cell-cycle genes, that is different from AR-dependent prostate cancer cells (13). Thus, detection of the increase in mitotic markers, for example, UBE2C, AURKA, PLK1, and proliferation markers, for example, Ki-67, may indicate exit from the microenvironment-dependent phase. NEPC or SCPC markers such as chromogranin A, synaptophysin, and neuron-specific enolase are also characteristic markers of this phase (88), as is MYCN amplification.

At present, very few markers have been identified that predict each turn in the prostate cancer progression spiral. In our proposed model, each of the turns in the spiral occurs in
response to a specific "driver" of prostate cancer progression (Fig. 2). These "turns" are identified by the patient's response to a specific therapy. Identification of specific biomarkers is critical to determining appropriate therapy for a given turn and predicting transit to the next turn. Several targeted therapeutic agents have been approved for prostate cancer treatment based on their proven effectiveness in prolonging patients' survival, and others are in active clinical trial. However, only a fraction of treated patients benefit from these targeted therapies. Patients who do benefit comprise true responders, and biopsies collected from this group of patients will be invaluable in identifying markers for each "turn." For example, expression of Cyp17, AR, nuclear localization of AR in the tumor, and detectable levels of plasma testosterone predict the likelihood of response to abiraterone treatment (14). In contrast, a decrease of AR nuclear localization to a more cytoplasmic distribution during abiraterone therapy may indicate changes in AR function that render abiraterone ineffective. Such a result might predict that an AR inhibitor with a different mechanism of action, such as MVD3100, might be useful in therapy. Markers may also provide clues that a more progressive stage of the disease is occurring or is about to occur. For example, irrespective of Gleason score, PTEN status or TMPRSS2-ERG-fusion proteins, if markers of the cell-autonomous stage of prostate cancer are increased, for example, Aurora A, MYCN, or PLK1, these results would suggest that patients should be treated with chemotherapy. Markers can also help predict impending resistance to targeted therapy. For example, increase in phosphorylation of Src family proteins was found in specimens from patients who were not responsive to abiraterone treatment (Efstatthiou and colleagues, manuscript in preparation). These examples suggest that new therapies or combination therapy could be applied when specific markers allow us to predict that a new target (or driver of progression) will emerge, rather than waiting until resistance to a given inhibitor occurs. Most importantly, identification of markers that predict therapy response will enrich the subset of patients who will benefit from specific inhibitors or combinations of inhibitors.

Our model, then, requires multiple and repetitive tumor samplings during the course of treatment for determining the "turn" for therapy selection. This may not be practical in the clinical setting. A likely solution to this problem lies in the recent advances in "liquid biopsy," where the circulating tumor cells (CTC) provide the information on tumor status (stage of progression). Analysis of CTCs is valuable as multiple samplings of blood for marker determination is possible.

Factors in the microenvironment may also "drive" progression, and many of these factors are released into the blood. Therefore, development of antibody arrays that capture these paracrine factors in the circulation will provide information on the changes in tumor microenvironment that predict tumor progression (or therapy failure). A comprehensive secretome analysis of the paracrine factors that mediate the bidirectional interaction between prostate cancer cells and bone microenvironment at various stages of tumor progression in bone and the changes of secretome components in response to targeted therapies will augment the effort to identify specific markers that predict "turns" in the spiral.

A major contribution to understanding key molecular markers of tumor initiation and progression will almost certainly arise from next generation sequencing (NGS) of tumors, an ongoing endeavor. This approach has already identified several genetic changes that may "drive" tumor progression, as discussed by Beltran and Rubin (95). For example, in ETS-negative prostate cancer in which PTEN has not been lost, SPINK (serine peptidase inhibitor, Kalal type 1) is overexpressed in 10% of tumors. Mutations in the speckle-type POZ gene (SPOP) occur in 6% to 13% of tumors. In a very small fraction (<1%) of tumors, NGS has shown rearrangements in the BRAF gene. Predictive and prognostic implications of these genetic alterations are uncertain but under intense investigation, and whether development of inhibitors that target these molecules specifically will be of therapeutic value remains to be determined.

NGS studies suggest that some prostate progression may occur through clonal evolution, and this is further supported by recent studies from Sowalsky and colleagues (96) that show that Gleason 3 to Gleason 4 prostate tumors have identical TMPRSS2 gene rearrangement. Although the initial driver may be clonal, experience with all drugs currently in clinical use shows that resistance to them will arise, leading to the prediction that the initiating clone will further evolve to express additional drivers described in our spiral model. This prediction is borne out by the great majority of tumors that express, for example, activated oncogenes and altered AR. Whether the clonal origin of the tumor will help predict the order in which these additional "drivers" occur will require further study.

**HOW WILL THE NEW MODEL ALTER THERAPY SELECTION?**

Therapy selection that incorporates molecular drivers that promote tumor progression in heterogeneous tumors, as proposed in Fig. 2, should improve clinical outcome. Current prognostic-based models do not include the factors described above, that is, AR alterations, microenvironment factors, and oncogene activation. In our proposed biology-based model, we use predictive markers; some that have been clearly identified and others that will emerge as targeted therapies continue to be used in the clinic. This model will also lead to more effective sequences or combinations of molecularly targeted agents. For example, in the prevailing prognostic model in which Gleason grade and extent of disease dictate treatment strategy, chemotherapy is reserved for treatment of hormone refractory patients with impending or symptomatic progression. In our proposed model, we suggest that chemotherapy will benefit those patients whose cancer is in the cell-autonomous state, regardless of whether they are detected in an early or late stage of cancer progression. In current prognostic models, the newly approved inhibitors of intracrine/paracrine androgen signaling are reserved for patients with castrate-resistant progression. In our proposed
model, we suggest that these inhibitors will be more effective when applied earlier, that is, in the initial androgen signaling driven turns of the endocrine-driven stage of progression, rather than in the later microenvironment-driven stage. The rationale for this approach is that when the tumor enters a more microenvironment-driven stage, the diseases are in a complex progression spiral where AR inhibitors will be less effective or insufficient to inhibit tumor progression due to activation of oncogenes and complex tumor/microenvironment interactions. As an example, we know that in the prognostic model, patients respond to androgen-ablation therapy with variable duration. However, in our model of progression, we may be able to better predict the duration of therapy by examination of specific markers. For example, our data predict that androgen ablation therapy will be durable until increase of phosphorylation of SFKs occurs. When SFK increases occur, a combination therapy that targets both the androgen receptor axis and tyrosine kinases may improve outcome, regardless of the stage the tumor would be classified by the prognostic model. As an understanding of molecular markers in the increasingly complex turns of the spiral advances, this marker-based approach will provide further guidance as to what therapeutic combinations are likely to yield the greatest success.

CONCLUDING REMARKS

Currently, new drug development for treatment of prostate cancer outpaces our understanding of the biology of the disease. Although several recently approved U.S. Food and Drug Administration agents have prolonged patient survival, at present, we lack knowledge on how best to apply these agents. Clinical trials using the crude “grouping by disease stage” method showed each drug results in a modest prolongation of life (approximately 3 months for most agents) for patients with metastatic late-stage prostate cancer. The 3-month gain is an average, and likely an underestimate for many patients, due to a large portion that fail to respond to the treatments. Selection of the patients who will be responsive will significantly improve the outcomes of targeted therapy. Furthermore, applying the targeted therapy at the right time before the disease enters the progression spiral we describe, that is, anticipating the need for therapy before clinically apparent disease progression has occurred, will be critical in prolonging the effectiveness of the therapy. The development of markers that predict the disease progression will not only be prognostic, indicating the impending changes in tumor properties (i.e., a “turn” in the spiral model), but also will suggest the application of additional targeted therapy to counter the specific changes. Our model will also help select combination therapy for an even more effective outcome. Marker-guided individualized cancer therapy will have a tremendous impact on prostate cancer treatment. We expect that our proposed prostate cancer progression model will help guide the development of markers, resulting in logical therapy selection. We hope that with the many targeted drugs that have been developed as well as those currently under development, we will be able to change the current “modest gain” from each of the targeted agents into the possibility of prostate cancer becoming a manageable chronic disease.

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