Narrative Review
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Genetics of Prostate Carcinoma

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Abstract
The aim of this review is to provide a brief overview of some current approaches regarding diagnostics, pathologic features, treatment, and genetics of prostate carcinoma (PCa). Prostate carcinoma is the most common visceral tumor and the second most common cancer-related cause of death in males. Clinical outcomes for patients with localized prostate cancer are excellent, but despite advances in prostate cancer treatments, castrate-resistant prostate cancer and metastatic prostate cancer patients have a poor prognosis. Advanced large-scale genomic studies revealed a large number of genetic alterations in prostate cancer. The meaning of these alterations needs to be validated in the specific prostate cancer molecular subtype context. Along these lines, there is a critical need for establishing genetically engineered mouse models, which would include speckle type BTB/POZ protein and isocitrate Dehydrogenase (NADP (+)) 1 mutant, as well as androgen receptor neuroendocrine subtypes of prostate cancer. Another urgent need is developing highly metastatic prostate cancer models, as only up to 17% of available models display bone metastases and exhibit a less typical neuroendocrine prostate cancer or sarcomatoid carcinoma. Moreover, androgen deprivation and relapse should be mimicked in the genetically engineered mouse models, as androgen independence may yield a better model for metastatic castrate-resistant prostate cancer. The development of such refined animal models should be guided by comparative genomics of primary versus corresponding metastatic tumors. Such an approach will have the potential to illuminate the key genetic events associated with specific molecular prostate cancer subsets and indicate directions for effective therapy. **Conclusion**. Despite excellent results in the treatment of localized prostatic carcinoma, castrate-resistant prostate cancer and metastatic prostate cancer have a poor prognosis. Advanced large-scale genomic studies revealed a large number of genetic alterations in PCa. Experimental models of prostate carcinoma in genetically modified mice could provide new data about the genetic changes in such cancers and help in developing better animal models for treatment resistant prostate carcinomas.

Key Words: Prostate Cancer ▪ Genetic Changes ▪ Molecular Subtypes ▪ Treatment.

Introduction

Prostate cancer (PCa) is the most common visceral tumor in males, with more than 1.1 million newly diagnosed cases each year worldwide. It is the second most common cancer-related cause of death in males due to malignant tumors, with approximately 300,000 deaths per year (1). It usually appears in persons older than 50 years. These days, most PCa are routinely diagnosed in asymptomatic patients by a simple and easily performed procedure that includes prostate-specific antigen (PSA) measurement and needle core biopsy. Such an approach enables adequate and timely treatment, leading to a good prognosis. In the pathogenesis of prostate cancer, various exogenous and endog-

enous factors are involved. The second one include inherited and acquired genetic and epigenetic changes. Understanding role of these factors concerning the occurrence and progression of PCa to a lethal outcome in some patients has a crucial translational impact on the detection, diagnosis, and prognosis of this frequently occurring cancer. Specifically, predicting men at risk for developing a lethal PCa vs. an indolent one is extremely important, but currently unmet clinical need (2, 3). This review will provide a brief overview of some current approaches regarding diagnostics, pathologic features, treatment, and genetics of PCa.

Diagnostics of Prostate Cancer

The suspicion of prostate cancer arises from digitorectal examination (DRE) and/or rising of the PSA levels. Most PCas are located in the peripheral zone and may be detected by DRE in conjunction with PSA value when the volume is >0.2 mL (4, 5). The use of PSA as a serum marker has revolutionized PCa diagnosis (4). This marker may be elevated in benign prostatic hypertrophy, prostatitis, and other non-malignant conditions. As an independent variable, PSA is a better predictor of cancer than either DRE or transrectal ultrasound (TRUS) (5). PSA is a continuous parameter, with higher levels indicating a greater likelihood of PCa. Currently, PSA is a gold-standard marker also used for assessing PCa risk and biochemical recurrence (BRE) (6). With respect to PCa, the PSA sensitivity and specificity of 60% and 79%, respectively, makes it the organ-specific marker but cannot be considered as a tumor-specific marker (7). One should be aware of high false-positive rates in indolent, low-risk localized PCa that may lead to overtreatment of PCa patients. On the other hand, many clinically significant PCa remain undetected until presented in the advanced stage (8).

The need for a better marker has been recognized and addressed timely. As a result, there are many tests currently available. They may be applied before and after taking the biopsy. Blood-based risk assessment tests that reduce unnecessary biopsies by ~ 40% are the Prostate Health Index (PHI) and 4Kscore. The first test relies on total PSA, free PSA, and precursor PSA, while the second one relies on total PSA, free PSA, intact PSA, and human kallikrein2 (7). In the modern era, genomic, epigenetic, and proteomic-based biomarkers are available to augment PSA and reduce unnecessary biopsies. They are all expected to improve diagnostics, staging, and monitoring. Finally, they can contribute to the knowledge needed to understand the basis of tumor aggressiveness and guide therapy decisions. Novel biomarkers are not necessarily restricted to proteins in a specific tissue or the blood. Instead, there may be other types of molecules (micro RNA (mi-RNA), for example) present in various biological specimens (9).

Urine-based noninvasive tests are useful for deciding whom to biopsy (SelectMDx, EndoDX Prostate IntelliScore) while the combination of urine- and tissue-based markers are useful for deciding whom to rebiopsy (SelectMDx, EndoDX Prostate IntelliScore, Progensa PCA3, The Michigan Prostate Score (MiPS), ConfirmMDx). The positive aspect of these tests should be considered for reducing the number of unnecessary biopsies and for improving a discrimination between clinically significant and indolent PCa. They are very useful for avoiding overdiagnosis (7). SelectMDx measures Homeobox C6 (*HOXC)* and Distal-Less Homeobox 1 (*DLX1)* mRNA in urine. In contrast, EndoDX Prostate IntelliScore measures Prostate Cancer-Associated 3 (*PCA3)*, ETS transcription factor *ERG* and SAM Pointed Domain Containing ETS Transcription Factor (*SPDEF)* mRNA in urinary exosomes (7). Progensa PCA3 is a urine-based assay that measures the level of prostate-specific long noncoding *PCA3* and *PSA* transcripts. The PCA3/PSA mRNA ratio is used to aid in repeated biopsy decision-making (10). The Michigan Prostate Score evaluates chimeric transcript Transmembrane Serine Protease 2 – *TMPRSS2:ERG* and *PCA3* mRNA in urine and normalizes it with serum PSA (11). ConfirmMDx, considering that non-tumors cells adjacent to the cancer are epigenetically changed, detects changes in DNA methylation of Glutathione S-Transferase pi1 (*GSTP1*), Ras Association Domain Family

Member 1 (*RASSF1*), and Adenomatous Polyposis Coli (*APC*) genes in histopathologically negative biopsy tissue (7, 12).

Currently, there are four commercially available tests for assessing PCa behavior and guiding therapy decisions for localized disease. ProMark quantifies eight protein markers in biopsy tissue of Gleason score 3+3 and 3+4. It is useful for predicting the risk of PCa aggressive behavior and helpful in the situation when the therapist must decide between active surveillance and active treatment (13). OncotypeDX Genomic Prostate Score determines mRNA levels of 17 genes in biopsy tissue of Gleason score 3+3 and 3+4 to predict aggressiveness, adverse pathology, and biochemical recurrence (7). Decipher measures RNA expression of 22 genes in biopsy or prostatectomy specimens. The test results predict a 5-year risk for clinical metastases and 10-year PCa-specific mortality risk from both specimens. It adds to the accuracy for predicting the existence of a high-grade PCa from the biopsy and is also helpful for making decisions related to therapy protocol (radiation therapy timing and hormone deprivation therapy) (14). Prolaris determines RNA expression of 31 cell cycle progression genes and 15 housekeeping genes in biopsy or prostatectomy specimens to predict cancer aggressiveness, PCa-specific mortality, and therapy decision-making (active surveillance or definitive treatment) (15).

Multiparametric-magnetic resonance imaging (mp-MRI) has shown promising results in diagnosis, localization, risk stratification, and clinically significant prostate cancer (16). Mp-MRI includes high-resolution T2-weighted imaging (T2WI) and at least two functional MRI techniques (17). Suspicious lesions in mp-MRI are graded using the Prostate-Imaging Reporting and Data Scoring System (PIRADS) version 2 (18). PI-RADS™ v2 assessment uses a 5-point scale based on the likelihood that a combination of mp-MRI findings correlates with the presence of a clinically significant cancer for each lesion in the prostate gland. There are five assessment categories (18) ranging from PI-RADS 1 – very low probability (clinically significant cancer is highly unlikely to be present) to PI-RADS 5 – very high probability (clinically significant cancer is highly likely to be present). Three methods based on MRI guidance are available for performing the targeted prostate biopsy: a) Cognitive fusion, in which the ultrasound operator positions the biopsy needle in the prostate area where the prior MRI demonstrated a lesion; b) Direct MRI-guided biopsy, performed within an MRI tube; and c) Software coregistration of stored MRI with real-time ultrasound, using a fusion device (19). Correlation with radical prostatectomy (RP) specimens shows that mp-MRI has good sensitivity for the detection and localization of the International Society of *Urological* Pathology (ISUP) grade > 2 cancers but is less sensitive in identifying PCas *ISUP* grade 1 (20). MRI-TBx (MRI-targeted biopsies) significantly out-performs systematic biopsy to detect ISUP grade > 2 in the repeat-biopsy setting. In biopsy-naïve patients, the difference appears less marked and not significant in all series, but it remains in favor of MRI-TBx in most studies (20).

Although DRE, TRUS, and MR are beneficial, the definitive diagnosis depends on histopathological verification of adenocarcinoma in prostate biopsy cores (20).

Pathology

On gross examination, prostate carcinoma is a gritty and firm, gray-yellow, poorly circumscribed tumor, which can be more easily felt than seen. Accurate identification of prostate cancer by gross inspection is possible in only 63% of cases, with a 19% false-positive rate (21). These days grading is performed according to the World Health Organization (WHO) 2016 recommendations based on the original Gleason grading system. Defined initially by Donald Gleason and published in the 1960s and 1970s (22, 23), the score is based on prostate adenocarcinoma histological patterns. It has been refined over the years and is nowadays the most widely used grading system (23). Grading should include primary and secondary Gleason grade, Gleason score as well as grade group that is determined on the basis of Gleason grades. Different architectural patterns have been as-

signed a number from 1 to 5 (from well to poorly differentiated). Gleason score is a sum of the two most prevalent Gleason grades and ranges from 2 to 10. However, in practice, only scores from 6 to 10 are usually used. There were some other inconsistencies in the Gleason system that led to some modifications and a grade group proposal that has been subsequently validated on a large number of patients (24). Today, grade groups from 1 to 5 and Gleason grading, are used according to WHO 2016 classification (23, 24).

Microscopically, prostate carcinoma is usually composed of small glands. However, medium to large papillary or cribriform glands or solid growth, as well as single cells, can be found. The cytoplasm is usually finely granular but may be clear/foamy due to intracellular lipid accumulation. Bluish luminal mucin and/or crystalloids can be seen in the lumina of neoplastic glands and peritumoral clefting around some glands (23, 25-27). There are nuclear enlargement, hyperchromasia, and prominent nucleoli. Mitotic figures are quite uncommon except in high-grade tumors (21, 25). However, diagnosis is based on at least three criteria, some of which are mentioned earlier. These criteria are favoring but not diagnostic of adenocarcinoma. Some features are associated with false-positive diagnoses, such as atrophic cytoplasm, atypical glands associated with inflammation, adenosis, and many others (21, 25, 26). Certain features are confirmative and diagnostic, including perineural invasion, mucinous fibroplasia, and glomeruloid structures (21, 25, 26). Diagnosis is occasionally difficult and for that reason some immunohistochemical methods should be used such as antibodies to p63, PSA, ERG, high-molecular weight cytokeratin, alpha-methyl CoA racemase (AMACR) and others.

Treatment of PCa

Approaches for managing localized PCa include active surveillance, brachytherapy, external beam radiation, radical prostatectomy and androgen deprivation therapy (28). Radical prostatectomy (as a surgical treatment of PCa) is the gold standard because hormone therapy and chemotherapy are usually not curative. Not all cancer cells can be eradicated consistently by radiation or other physical forms of energy, even if the tumor is localized within the prostate capsule. Moreover, if the prostate gland remains *in situ,* new prostate cancers can develop in the residual prostatic epithelium. There are four different approaches to radical prostatectomy: open perineal, open retropubic, manual laparoscopic, and robot-assisted laparoscopic approaches (29). Radical prostatectomy is indicated for the treatment (with curative intent) of patients in good condition with localized PCa whose life expectancy exceeds ten years (30).

While clinical outcomes for patients with localized PCa are excellent, metastatic PCa patients have a poor prognosis. The treatment of choice for metastatic PCa is androgen deprivation therapy (ADT) and, if ADT fails, cytotoxic chemotherapy. Phase III clinical trials investigating their combination are in progress (31). Hormone-sensitive PCa, even under low-androgen conditions, progress to lethal, castration-resistant PCa (CRPC). Therapy for castration-resistant PCa are androgen receptor (AR) signaling inhibitors and, in case of failure, AR-directed therapy, chemotherapy, Radium-223, poly (ADP-ribose) polymerase (PARP)-inhibitors (if harboring *BRCA1*, *BRCA2*, or *ATM* alterations) and immunotherapy are available (31-33). There are indications that androgen inhibition enhances cell sensitivity to PARP-inhibitors, and several clinical trials based on their combination are currently underway (33). Since the adverse effect of ADT is a decrease in bone mineral density, a combination of ADT and bisphosphonates is recommended but only in documented osteoporosis or androgen-independent prostate cancer with bone metastasis (34). Despite advances in CRPC treatments, it remains lethal. New strategies that would achieve long-term disease remissions are needed. Potentially targetable molecular targets may be p300/CBP, fibroblast growth factor (FGF), Wnt family member 5A (WNT5A)/receptor tyrosine kinase-like orphan receptor 1 (ROR1), tyrosine kinase ACK1, and STEAP1 (Six-transmembrane epithelial antigen of prostate-1).

Bipolar androgen therapy that combines a permanent ADT with high doses of testosterone applied monthly showed promising outcomes, which were even better when combined with checkpoint immunotherapy was applied (31).

Genetics of Prostate Carcinoma

Prostate cancer has an extraordinarily complex genetic makeup containing mutations, DNA copy-number changes, rearrangements, and gene fusions (2, 3, 35). These aberrations are associated with extensive changes in the epigenetic landscape (36). Multiple studies have shown a genetic component to the etiology of prostate cancer, which has been reviewed elsewhere (2, 3, 35-39). Epidemiological studies have shown that a family history of prostate cancer may significantly increase PCa occurrence risk (39). Twin studies have indicated a substantial heritability of prostate cancer (35). Large-scale genome-wide association studies (GWASs) have identified many prostate cancer susceptibility loci (3, 35), including 63 novel risk-associated single-nucleotide polymorphisms (SNPs), among which four SNPs (rs111599055, rs11859370, rs2788524, rs56366063) were shown to be clinically significant (40).

Several large-scale genomic studies in both primary malignant prostate tumors and metastatic castration-resistant prostate cancer (mCRPC) have identified recurrent DNA copy number changes, mutations, rearrangements, and gene fusions such as familial mutations in Homeobox B13 (*HOXB*13) and DNA repair genes, including *BRCA*2, *ATM*, Checkpoint Kinase 2 (*CHEK*2), *BRCA*1, DNA Repair Protein RAD51 Homolog 4 (*RAD*51D), and Partner and Localizer of BRCA2 (*PALB*2) (3, 35). It is known that germline mutations in BRCA genes are associated with increased risk for prostate cancer and a more aggressive phenotype and worse outcomes (3, 35).

Primary prostate tumors and mCRPC exhibit markedly increased genome-wide copy number alterations (35, 38). On the other hand, somatic point mutations are less common in prostate cancer than in most other solid tumors (41). The whole-exome sequencing analysis applied to 333 tumors revealed only 0.94 mutations per megabase (mut/Mb), corresponding to 19 non-synonymous mutations per tumor genome (median; 13–25, 25th, and 75th percentiles respectively) (41). As recently published, this contrasts with, for example, numbers related to small cell lung carcinomas of which 40% (N=122) was shown to contain a high mutation burden, defined as 10 mut/Mb (42). Prior exome sequencing of 112 prostate cancers identified 12 recurrently mutated genes through focused assessment of point mutations and short insertions and deletions (43). Moreover, differences in prostate cancer incidence and outcome have been observed in men from different racial/ethnic groups, with men of African descent having the highest incidence and mortality rates, which may partially be attributed to genetic factors (44). The heritability factor is crucial when mutations are present in DNA-repair genes (45).

Another important factor relates to the activity of the androgen pathway, as the signaling pathway mediated by AR plays a central role in the prostate gland's development and function. Studies using conventional approaches and next-generation sequencing (NGS) have revealed that a majority of primary and metastatic prostate cancers harbors genomic alterations in the androgen signaling pathway, including AR amplification/mutations, gain of AR nuclear receptor coactivator 1/2 (NCOA1/2), and loss of AR nuclear receptor corepressor 1/2 (NCOR1/2) which contributes to castration resistance (46). In addition, AR genomic structural rearrangements are present in one-third of mCRPC tumors, resulting in aberrant expression of diverse AR variant species lacking the ligand-binding domain and resulting in persistent activation of AR signaling, such as AR variant 7 (AR-V7), which appears to drive disease progression (47, 48).

Based on the previously mentioned study (41), it seems that a high proportion of all prostate cancers (74%) can be assigned to one of seven molecular classes based on oncogenic fusions: 1) *ERG*, 2) *ETV1*, 3) *ETV4*, or 4) *FLI1* (46, 8, 4, and 1% respectively), or mutations in 5) *SPOP*, 6) *FOXA1*, or 7) *IDH1* mutations (11, 3, and 1% respectively) (41).

Changes in the Number of Chromosomes and Copy Number Variations of Select Chromosomal Regions

Changes in Chromosomal Number There are few reports on chromosome number changes associated with prostate cancer progression (49-51). Braun et al. were explored 428 PCa, and PCa related specimens (186 localized, 75 lymph node metastasized, 125 lymph node metastases, 42 hormone-refractory distant metastases) and observed a significant increase in aneuploidy with advanced tumor stage (49). An increased expression of the mitotic marker Phosphorylated Histone H3 – PHH3 was significantly associated with aneuploidy and higher pT stage (49). Copy number gains were most commonly present on chromosomes X (26.6%), 21 (22.8%), Y (20.7%), 14 (19.2%), and 8 (17.7%), while the losses of chromosomes 20 (11.0%), 10 (4.1%), and 6 (4.0%) accounted for the most frequent monosomies. However, while overall ploidy status and PHH3 expression in primary tumors indicate advanced disease, a fluorescence in situ hybridization (FISH) – based test for distinct alterations did not seem to be beneficial for diagnostic or prognostic purposes (49). Celep et al. observed numerical aberrations in 41% of 19 analyzed prostate cancer cases (50). The most frequent aberration was a loss of chromosome 9 that was detected in 12 (63%) samples, followed by monosomic chromosomes 8, 7, and 17, which were present in 11 (58%), 9 (47%), and 6 (32%) tumors, respectively. The highest rate for trisomy was observed for chromosome 7 (three tumors, 16%) (50). There were no significant aberrations in benign prostate hyperplasia (BPH) samples. Visakorpi et al. studied 23 prostate cancer and 10 BPH specimens by FISH using pericentromeric repeat-specific probes for 10 chromosomes (51). All BPH specimens were diploid, without apparent chromosomal aberrations, as assessed by flow cytometry and FISH. In prostate carcinoma, flow cytometry and FISH revealed abnormal DNA content in 35% and 74% of tumors, respectively. Aberrant copy number of chromosomes 7, X, and 8 were found in approximately 40% of cases. Simple chromosome losses were uncommon. Still, in DNA tetraploid tumors, relative losses (trisomy or disomy) of several chromosomes were often found, with chromosome Y being most commonly affected, suggesting prostate cancer progression through tetraploidization, followed by losses of selected chromosomes. The most recent data have convincingly shown that, over a median follow-up of 15.3 years, increasing tumor aneuploidy strongly associates with an increased risk of lethal prostate cancer. When comparing the biological behavior of tumors with the same Gleason score, 23% of patients with five or more altered chromosome arms in their tumors had fivefold higher odds of lethal disease compared with those without aneuploidy (52).

Copy Number Alterations Copy number alterations (CNAs) are gains or losses in genetic material that affect a larger fraction of the genome. These alterations are found in nearly 90% of prostate cancers (2). Somatic tumor CNA burden (TCB) and genome-wide CNA patterns were shown to be associated with biochemical recurrence and metastasis in primary prostate cancer, especially in low and intermediate-risk prostate cancer patients (Gleason scores of 7 and less) (53). Tumor CNA burden as a continuous variable was also shown to be significantly associated with prostate cancer-specific death (53) in conservative treatment cohort, independent of Gleason sum score and Cancer of the prostate risk assessment (CAPRA) score (53). Copy number alterations (gains and losses) have an integral role in both the activation of oncogenes and inactivation of tumor suppressor genes. For example, the most common loss of 8p (the minimal region of deletion: 8p21.3-p21.2 harboring NK3 Homeobox 1 (*NKX*3-1)) was found in 304 of 546 (55.7%) and 105 of 116 (90.5%) cases of localized and advanced prostate carcinoma, respectively. Other common deletions in primary tumors were also on chromosomes 13q (13q13-q31.1; loss of RB transcriptional corepressor 1 (*RB*1)), 5q11.2-q23.3, 17p13.3-p11.2, 10q23.2-q26.12, 18q. On the other hand, the gain of chromosome 8q was identified in 114 of 546 (20.9%) and 97 of 116 (83.6%) primary and advanced cases, respectively (54). In metastatic tu-

mors, hundreds of aberrations can be found. This phenomenon may reflect increasing genomic instability, which relates to disease progression.

Recent genetic studies revealed that mCRPCs with neuroendocrine (NE) features commonly are *RB1* and *TP53* deficient and display attenuated AR signaling compared with non-metastatic CRPC (35, 51). On the other hand, castration-resistant metastatic tumors often show amplification of chromosomes X, 7, 8q, and 9q and include genes from the androgen receptor pathway and the *MYC* oncogene. Numerous studies have demonstrated an increase of *MYC* copy number in up to 50% of prostate cancers (55). Overexpression of Myc in mice resulted in prostatic intraepithelial neoplasia (PIN) with progression to invasive carcinoma (56). Besides, Myc functions as a driver in the metastatic Pten/Trp53-deficient Rapid CaP genetically engineered mouse models (GEMM) (57), in which *Myc* activation in combination with phosphatase and tensin homolog (*PTEN*) loss drives genomic instability and contributes to the occurrence of metastatic disease (58, 59).

It is challenging to detect CNAs in the sample obtained by prostatic needle core biopsy. Therefore, it was proposed that their identification should be performed in circulating and disseminated tumor cells from blood and bone marrow, respectively (3). However, the most recent data did not confirm circulating prostate cancer cells in the blood and bone marrow of patients with a localized prostate tumor (60).

Structural Rearrangements

Improper repair of double-stranded DNA breaks can result in both intra- and inter-chromosome rearrangements. The most common prostate cancer genomic alterations are translocations involving androgen-regulated promoters and the ETS family of transcription factors, such as ERG and the E twenty-six family of transcription factors (ETV) genes (61). In the previously mentioned study (41), 53% of tumors were found to have ETS-family gene fusions (*ERG*, *ETV1*, *ETV4*, and *FLI1*). A recurrent gene fusion of the 5' untranslated region of the androgen-responsive *TMPRSS2* to *ERG* (TMPRSS2:ERG) was the first translocation discovered by Tomlins and collaborators (62). This type of fusion is present in ~50% of localized prostate cancers (35). This chimera expression confers an increased risk of disease relapse after treatment for clinically localized prostate cancer due to the growth-promoting activity of the *ERG* oncogene under the control of the regulatory elements of *TMPRSS2* gene. The presence of TMPRSS2:ERG chimera varies concerning ethnicity and is more prevalent (~50%) in Caucasians than in African-Americans (31.3%) and Japanese patients (15.9%) (63). Several other rearrangements have been described in prostate cancer, including *ESRP1:CRAF*, the ETS family, and *RAF* kinase gene fusions (64). ERG-associated rearrangement has been associated with 10q, 17p, and 3p14 deletions (65). On the other hand, those tumors without ERG rearrangement exhibit 6q and 16q deletion and 7q amplification (65). The whole-genome sequencing of primary prostate tumors T2c or greater, and Gleason grade 7 or higher obtained from seven patients showed a median of 90 structural rearrangements (range 43–213) per tumor genome, highlighting the prevalence and complexity of these changes as well as the importance of chromatin structure. Further, in the tumors with *TMPRSS2:ERG*, rearrangement breakpoints were enriched near open chromatin, androgen receptor, and ERG DNA binding sites (66).

Single Nucleotide Polymorphisms and Point Mutations

The mutation rate is a crucial factor in determining a somatic cells risk of malignant transformation. Kan et al. have shown a low number of mutations (0.33 per Mb) in 58 analyzed prostate cancers, associated with a high number of *TMPRSS2–ERG* gene fusion transcripts, which were present in 75% of samples (67). Still, even with such a low mutation rate, there are crucial and clinically important genes for occurrence, development, and biological behavior of prostate carcinoma. It is thought that, on average, less than 20 mutations are likely

to affect protein stability or function. For example, in Kan's research, only three genes (*TP*53, speckle type BTB/POZ protein (*SPOP),* and A-kinase anchoring protein 9 (*AKAP*9) were shown to have a significant prevalence of protein-altering mutations (q-score ≥1.0) (67).

On the other hand, there are genes whose SNPs gained interest concerning PCa occurrence and progression. Some of these polymorphisms were thoroughly analyzed. However, in the *RNASEL (HPC1)* gene (1q25.3), elaC Ribonuclease Z 2 (*ELAC*2 or *HPC*2) (17p12), and macrophage scavenger receptor 1 (*MSR*1) (8p22), none of the polymorphisms analyzed was shown to be a strong prognostic/predictive and independent factor for prostate cancer (68, 69) (Table 1).

However, there is no doubt that prostate cancer is a polygenetic disease that is highly dependent on SNP–based genetic risk score (GRS). The first reported study assessing the association of a polygenic risk score derived from well-established risk-associated SNPs with patient age at PCa diagnosis was published in 2019 (70). The independent PCa risk-associated SNPs discovered through GWAS were defined through three standard criteria (70), which allowed for the profiling of 110 PCa risk-associated SNPs. The paper was shown a significant association of GRS with patient age at PCa diagnosis, especially when combined with family history. The highest risk allele frequency (RAF) value (0.93) was shown for rs12480328. This polymorphism is located in the intron of the activity-dependent neuroprotector homeobox gene (*ADNP*). So far, there are no data related to the importance of this gene in PCa.

Several GWAS have revealed the SNPs rs2292884 (missense mutation in melanophilin, *MLPH*; 2q37.3) and rs902774 in the noncoding region 12q13 (38). A large meta-analysis based on 78 PCa GWAS risk associations within 85 distinct genomic regions was recently published (71). Although numerous data have been published, the final confirmation of some SNPs as *bona fide* prognostic markers is yet to come. For example, the polymorphism rs2735839 in the *PSA* coding gene, kallikrein-related peptidase 3, (*KLK*3*)*, was crucial when estimating the occurrence and biological behavior of PCas in several populations (72, 73). A recently published meta-analysis, including 35,838 patients and 36,369 control subjects, did not find

Table 1. Common Genetic Changes in Prostate Carcinoma

Gene	Genomic alteration	Locus	Function	References
RNASEL (RNS4; PRCA1)	Mutation	1q25.3; HPC1 – hereditary-prostate-cancer (HPC)-predisposition locus; 8 exons	Innate immunity, part of the interferon-regulated 2-5A system	Alvarez-Cubero et al., 2016 (68); Liu et al., 2018 (69); Wallis et al., 2015 (3)
ELAC2 (HPC2; COXPD17)	Missense mutations	17p12; 25 exons	tRNA biosynthesis; interacts with activated Smad Family Member 2 (SMAD2)	Alvarez-Cubero et al., 2016 (68); Liu et al., 2018 (69); Wallis et al., 2015 (3)
MSR1(SRA; SR-A; CD204)	Mutation	8p22; 12 exons	The isoforms type 1 and type 2 mediate the endocytosis of modified low density lipoproteins (LDLs).The isoform 3 inhibits the function of isoforms type 1 and type 2	Alvarez-Cubero et al., 2016 (68); Liu et al., 2018 (69); Wallis et al., 2015 (3)
SPOP (TEF2; BTBD32)	Mutation	17q21.33; 16 exons	Modulation of the transcriptional repression activity of death-associated protein 6	Clark et al., 2020 (75); An et al., 2014 (76); Blattner et al., 2017 (77)
FOX1A (HNF3A; TCF3A)	Mutations	14q21.1; 3 exons	Binding to DNA	Zhou et al., 2020 (78); Adams et al., 2019 (79)
IDH1 (IDH; IDP)	Mutation	2q34; 2 exons	Oxidative decarboxylation	Kang et al., 2009 (80); Ghiam et al., 2012 (81); Dang et al., 2009 (82); Waitkus et al., 2018 (83)

an association between rs2735839 and the risk for PCa. It was, however, shown that there is a strong association between rs1058205 (T>C) and the decreased risk of PCa (74).

SPOP *SPOP* has been found to be mutated in prostate cancer in a range from 4.4% to 28.6% of cases (75). The inability of the mutant SPOP to induce degradation of full-length AR and inhibit AR-mediated gene transcription is of great importance for prostate cancer pathogenesis (76). However, the mutant SPOP (SPOP-F133V) was not confirmed as a strong prostate cancerogenesis driver in a transgenic mouse with prostate-specific conditional expression of the mutant allele, although it was associated with strong PI3K/mTOR signaling. However, when mice expressing mutant SPOP in a conditional *Pten* heterozygous background (Pb-Cre; PtenL/+; R26F133V) was generated, a highly penetrant phenotype with focal areas of high-grade prostatic intraepithelial neoplasia (HG-PIN) by six months of age was observed (77). The validation model presented by human *SPOP* mutant organoids revealed that *SPOP* mutations associate with specific changes, including genomic deletions at 5q21, 6q15, and 2q21 (77). On the other hand, *SPOP* mutant prostate cancers were shown to be exclusively negative for ETS rearrangement. All these facts point to the *SPOP* mutation-positive prostate cancers as a distinct molecular subtype. There is a high similarity in mRNA, copy-number, and methylation profiles in tumors with *FOXA*1 mutations and those with *SPOP* mutations.

Forkhead Box A1 – FOX1A Zhou et al. were recently shown that *FOXA*1 mRNA is consistently the most abundant mRNA in prostate tumors, ranking in the 95th percentile in 492 of 497 prostate tumors deposited in TCGA (78). By analyzing 3086 primary and metastatic prostate cancers, Adams et al. (79) were shown *FOXA1*-related aberrations in 11.4 % of samples. Among them, 3% were genomic amplifications, while 8.4% of tumors had somatic point mutations. Less than 1% of tumors were carrying both types of changes. Over 50% of *FOXA*1 mutations in the cited study were mapped to a specific hotspot in Wing2 of the forkhead (FKHD) DNA-binding domain, mainly between H247 and F266. These mutations were more prevalent in primary locoregional cases. Truncation mutations with consequential loss of the C-terminal transactivating domain were presented with 20% (79). Some functional consequences of these mutations will be explained later.

Isocitrate Dehydrogenase-1 (IDH1) In 2009, Kang et al. were shown the presence of the *IDH1* point mutations (R132H and R132C) in only two out of 75 analyzed prostate cancers (80). This finding was confirmed three years later; identical *IDH1* point mutations in codon 132 were discovered in two out of 158 analyzed prostate cancers (81). IDH1 is a cytoplasmic metabolic enzyme needed for oxidative decarboxylation of isocitrate to 2-oxoglutarate (α-ketoglutarate). When one allele is mutated, abnormally high 2-hydroxyglutarate (2-HG) production associated with an extensive reshape of cellular epigenome occurs (82). Targeting altered IDH1 in various tumors, including select prostate cancer patients, would be of the most significant interest (83). Although mutations in *IDH1* are the most common in gliomas, the first mutant IDH1 blocking drug (ivosidenib) was approved by the FDA in 2019 for treating adults with relapsed or refractory acute myeloid leukemia (AML) with an *IDH*1 mutation. Whether PCa may be targeted in a similar way remains to be seen.

General Epigenetic Landscape in Prostate Cancer and Micro-RNAs

Deregulation of genes controlling epigenetic processes involved in DNA modification (e.g., methylation and hydroxymethylation), histone modification, or nucleosome remodeling has been recognized as a tumorigenesis driver in many cancer types, including prostate cancer. Genomic DNA can be methylated by canonical DNA methyltransferase (DNMT) consisting of DNMT1, DNMT3A, and DNMT3B at the C-5 position of the cytosine within CpG dinucleotides, which are present in CpG islands (CGIs). There is app. 50,000 experimentally supported CGIs (eCGIs) in the human genome (84). Their length varies between 200 bps and 3.6 kbps. DNA methylation in normal cells

ensures that gene expression and gene silencing are adequately regulated. Aberrant level of DNA methylation is a part of disturbing epigenetics found in cancers. In the malignantly transformed cell, DNA is commonly hypermethylated in promoter regions of tumor suppressor genes, leading to decreased activity. Notwithstanding this fact, cancer is a disease of global hypomethylation, which is commonly present in non-coding regions of DNA. In prostate cancer, hypomethylation of noncoding long interspersed nuclear elements (LINE-1), which constitutes approximately 17% of the human genome, was shown in 1999 (85). As recently reviewed by Lam et al., the methylation rate of certain genes in prostate cancer may be considered a valid prognostic parameter for certain disease aspects (86). With respect to methylation, among all explored genes so far, none was shown to be undoubtedly predictive when all studies were taken into consideration (86). The reasons for these findings include diversity in sample type, cohort size, clinical endpoints examined, methylation profiling methodologies, analytical approach, and clinicopathological factors adjusted for in multivariate analyses. The methylation status of the *PITX*2 (paired-like homeodomain transcription factor 2)gene (4q25) seems to be highly conclusive for predicting biochemical recurrence-free survival in prostate cancer patients after radical prostatectomy (RP) (training cohort: hazard ratio (HR) = 1.83 (95 % CI 1.07–3.11), P=0.027; validation cohort: HR=2.56 (95 % CI 1.44–4.54), P=0.001 (87).

The only commercially available epigenetic test, ConfirmMDx, is made for diagnostic purposes. It is set up to produce binary results based on the methylation status of three genes: *APC*, *RASSF*1, and *GSTP1,* to detect cancer in histologically negative biopsies. Methylation-positive test result profiles men who are at increased risk of harboring occult (high-grade) cancer. Methylation negative samples spare the patient from unnecessary repeat biopsy due to the high accuracy of the test (negative predictive value (NPV) between 90% and 96%) (88, 89).

Previously described transcription factor FOXA1 is a "pioneer factor" that can bind to DNA in those segments where the chromatin is compacted. The role of FOXA1 is to contribute to increased accessibility of these regions for other transcription factors to bind (90). Specifically, in prostate tissue, FOXA1 plays a crucial role in AR-mediated gene regulation and signaling. AR chromatin binding is dependent on FOXA1 (91). The molecular basis of this process is not simple. Gao et al. were recently shown that lysine-specific histone demethylase 1A (LSD1) may regulate FOXA1 chromatin binding through directly demethylating its lysine 270, *in vitro* (91). The complexity of this process should be kept in mind when considering LSD1 inhibitors for treating tumors with mutated or highly expressed FOXO1. In prostate cancer, this may be problematic because the loss of FOXA1 can result in transdifferentiation from AR/FOXA1-driven adenocarcinoma to neuroendocrine prostate cancer (NEPC). This aggressive subtype is AR-ligand independent (92, 93).

Micro-RNA and Prostate Cancer Mi-RNAs are a class of small noncoding RNAs (22-25 nucleotides long) that bind to messenger RNA (mRNA). Consequently, they negatively influence protein expression through cleavage of specific target mRNAs or inhibition of their translation (94). Thus, a specific mi-RNA's functional role depends on the role of the specific mRNA, which is a miRNA binding partner (95). If specific mi-RNA targets mRNA originating from a tumor suppressor gene, then it has a strong potential to act as oncogenic mi-RNA (96). If, on the other hand, miRNA targets mRNA originating from an oncogene, it may be considered a tumor-suppressive molecule in a specific tissue. Mi-RNAs are highly promiscuous molecules as one miRNA may bind to and control numerous target mRNAs simultaneously. Currently, 2654 mature human miRNA sequences are known (97). Many miRNAs are located in genetically unstable sites where they are prone to deletion or rearrangement, which occur in cancer (98). Accordingly, the miRNAs located in the chromosomal regions of deletion show the lowest expression level.

In contrast, the miRNAs located in the regions of amplifications show the highest expression levels (99). Many miRNA genes are located next to CpG islands, where they may be prone to epigenetic silencing through methylation. In prostate cancer, this miRNA silencing mechanism was shown in 2014 through analysis of 74 formalin-fixed, paraffin-embedded (FFPE) clinical specimens: 24 normal prostate samples and 50 PCa samples from radical prostatectomies (100). Methylation occurred in 0%, 5%, and 13% of cancers for miR-18b, miR-148a, and miR-450a/542-3p, respectively, while no methylation was present in control samples. The same research study has shown that low levels of miR-132 correlated with a higher rate of metastatic events, lymph node invasion, and shorter recurrence-free time (Table 2). There was a negative correlation of miR-132 expression levels with the overall Gleason score and tumor stage (100). Two years later, Qu et al. demonstrated that decreased miR-132 levels in prostate cancer cells positively regulate the Warburg effect through inhibiting solute carrier family 2 member 1 *SLC*2A1/*GLUT1* expression (101).

In 2007, Porkka et al. published a seminal paper describing miRNA expression in 4 BPHs, 5 untreated prostate carcinomas, and four hormone-refractory prostate carcinomas (99). Their work has shown a unique profile of 51 differentially expressed miRNAs (PCa vs. BPH: 37 and 14 miRNAs downregulated and upregulated, respectively). Among these miRNAs, 22 and 8 were decreased and increased in all carcinoma samples, respectively, whereas 15 and 6 of them were downregulated and upregulated, respectively, only in the hormone-refractory carcinomas compared with BPH samples. These early data pointed out on mi-RNAs as unique molecules, which are mechanistically involved in prostate cancer development. They were confirmed in recent studies.

Table 2. Some miRNAs with confirmed clinical value associated with Prostate Carcinoma

miRNA	Locus	Clinical Significance	Mode of Action	References
miR-132	17p13.3	Downregulated in human PCa tissues; miR-adverse correlation with Gleason score	In vitro: inhibition of TGF-β (transforming growth factor-β)-induced EMT)	Formosa et al., 2013 (100); Qu et al., 2016 (101); Liu et al., 2016 (102)
miRNA -146a	5q33.3	Downregulated in androgen-independent prostate cancer (AIPC) tissue, suppressive role	Regulation of ROCK/Caspase 3 signaling pathway	Xu et al, 2015 (103)
miR-141	12p13.31	Associated with increased risk of biochemical PC recurrence.	In vitro: Suppression of prostate cancer stem cells and metastasis by targeting a cohort of pro-metastasis genes, including Enhancer of Zeste Homologue 2 (EZH2)	Richardsen et al., 2019 (105); Liu et al., 2017 (106)
miR-375-3p	2q35	Prediction of time to progression in mCRPC patients treated with docetaxel or abiraterone	Predicted targets: CCND2, MAP3K2, MXI1, PAFAH1B1, YOD1, ZFYVE26	Zedan et al., 2020 (107); Ciszkowicz et al., 2020 (108)
miR-331-3p	12q22	High expression is associated with advanced PC stage and distant metastases	Suppressive role through targeting NACC1, ERBB-2 expression and androgen receptor signaling. Oncogenic role through stimulation of epithelial-to-mesenchimal transition (EMT)	Epis et al., 2009 (111); Morita et al, 2018 (112); Fujii et al 2016 (113)
miR1792 cluster (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a)	13q31.3	Oncogenic role in majority of prostate cancer models.	Specific targeting of SERTAD3 with miR-92a;enhancement of migration and invasion in vitro, attributed to the induction of integrin β-1	Zhang et al 2020 (114); Zhou et al 2016 (115)

For example, the difference between androgen-dependent- (ADPC) and androgen-independent prostate cancer (AIPC) tissues was shown to be dependent on miR-146a and its influence on Rho-associated coiled-coil containing protein kinase 1 (*ROCK*1) kinase (102, 103). The presence of miRNAs in plasma of PCa patients was for the first time described in 2008 (104). The potential discriminatory miRNA was shown to be miR-141, which had the most significant differential expression and could detect individuals with cancer with 60% sensitivity at 100% specificity (104). In PCa tissues (N=535), expression of miR-141 in the epithelial part of the tumor significantly correlates to Gleason score ≥8 (P=0.040) and large tumor size (≥20 mm, P = 0.025). In contrast, its overall expression (defined as both epithelial and stromal expression) strongly relates to Gleason grade (P=0.001) (105). It was recently shown that plasma levels of miR-141-3p and miR-375-3p might predict time to progression in mCRPC patients treated with docetaxel or abiraterone; their high baseline levels were significantly associated with shorter overall survival (OS) in the abiraterone and in docetaxel treated patients (106-108).

There are also efforts to explore the clinical value of mi-RNAs in the urine. A novel logistic regression model based on five urine miRNAs (miR-151a-5p, miR-204-5p, miR-222-3p, miR-23b-3p, and miR-331-3p) and PSA were recently shown as a strong predictor for biochemical recurrence (109).

The crosstalk between miRNAs and molecules belonging to various signaling pathways may be established through strong networks containing transcription factors and various protein kinases. Erb-b2 receptor tyrosine kinase 2, ERBB2 (Her-2/neu), is a tyrosine kinase receptor that is overexpressed in abiraterone-resistant prostate cancer. It was recently shown to be included in the activation of the PI3K/AKT signaling and stabilization of AR protein. Accordingly, it was hypothesized that combination therapy with abiraterone and ERBB2 antagonists might be effective for treating the subset of CRPC with increased ERBB2 activity (110). In 2009, miR-331-3p expression was shown to be reduced in prostate tumors relative to normal adjacent tissue and is inversely correlated with *ERBB*2 mRNA expression (111). Thus, in this specific scenario, miR-331-3p may be considered to be a suppressive molecule (112, 113). At least ten different miRNAs have been found to be involved in apoptosis. In many cases, their way of acting follows a cascade pattern. Up-regulation of the miR-17-92 cluster leads to overexpression of miR-20a, which subsequently targets E2F1-3 transcription factors (114-116). Then, depending on the cell cycle phase, reduced E2F1-3 results either in cellular proliferation or reduced apoptosis via TP53 and caspase activity, thus creating an auto-regulatory feedback loop as E2F1-3 controls miR-20a expression. MiR-21 also contributes to apoptosis through the mechanism, which includes TP53 and is preserved in various malignant tumors (117). In prostate cancer specifically, miR-21 acts as an oncogenic factor, as it targets both *PDCD*4 (programmed cell death 4) (118) and *PTEN* mRNA (119). Numerous biological processes can be significantly affected by mi-RNA molecules. For example, MiR-15a and miR-16-1 are down-regulated in most prostate tumors (120).

Conclusions

Advanced large-scale genomic studies revealed a large number of genetic alterations in PCa. The meaning of these alterations needs to be validated in the context of the specific PCa molecular subtype. Along these lines, there is a critical need for establishing GEMMs, which would include *SPOP* and *IDH*1 mutants and AR−NE− subtypes of PCa. Another urgent need is the development of highly metastatic PCa models, as less than 20% of available models display bone metastases and exhibit a less typical NEPC or sarcomatoid pathology. Moreover, androgen deprivation and relapse should be mimicked in the GEMMs models, as androgen independence may yield a better model for metastatic CRPC. The development of such refined animal models should be guided by comparative genomics of primary PCa and corresponding metastases. Such an approach will potentially illu-

minate the critical genetic events associated with specific molecular Pca subsets and indicate directions for effective therapy.

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