INTRODUCTION

Hardly any modern study nowadays does not claim that prostate cancer should be classified on the basis of molecular or genetic features. It could almost be said that progress in all areas of prostate cancer research is impossible until we determine the basic principles of this classification. Indeed, the number of fundamental research programs in prostate cancer molecular biology and genetics is overwhelming. However, a significant gap appears to exist between the huge number of studies on the genetic characterization of prostate cancer, which often have limited translation into clinical practice or simply were not conceived to be so translated, and clinical practice. From a clinical point of view, this balance should be urgently shifted towards rapid translation into urological practice. However, prostate cancer is characterized by prominent genetic heterogeneity, which could be a very difficult barrier to overcome. In this review, we discuss the possible clinical applications of scientific data from fundamental studies of prostate cancer genetics, the main problems with the translation of these data to clinics, and future perspectives.

Keywords: Biological markers; Molecular biology; Prostate neoplasms; Translations

GENE EXPRESSION STUDIES AND GENETIC SIGNATURES

Gene expression analysis (mRNA analysis) is an attractive method of tumor tissue analysis (for example, after biopsy or during the final pathology examination). Current technologies (DNA microarrays, quantitative polymerase chain reaction, RNAseq) provide the possibility for streamed analysis of thousands genes in a relatively small volume of extracted tissue. Key issues for gene expression analysis are the quality of the analyzed material (better
with fresh-frozen tissues), the amount of tumor tissue in the probe (a certain amount of stromal tissue contaminates the material), and the necessity to use reference genes (as a rule, housekeeping genes, which are supposed to be expressed equally in all tissues) and normalization to account for differences in the RNA quantity in the probes. Recent studies have shown that formalin-fixed, paraffin-embedded (FFPE) tissues can also be used for analysis despite fixation-related RNA degradation if special extraction and preparation techniques are used [2]. In view of the large amount of generated data, certain demanding bioinformatics approaches are necessary for the analysis and to compensate for possible errors.

Gene expression studies in the area of prostate cancer have been carried out for more than 10 years. The principle idea of all these studies is to develop a gene expression signature, which could be useful for characterization of the tumor (mainly the aggressive, metastatic, lethal, or latent nature of the tumor) and for prognosis of outcomes or sensitivity to certain therapeutic modalities [3]. Different groups of scientists have undertaken efforts to extract these data from the analysis of gene expression [4-14]. Some signatures were developed in cooperation with genetic companies, some almost to the stage of being ready for clinical application [15-21]. Some of the signatures also used noncoding regions in the genome and not only protein-coding mRNAs with clear cellular functions [17, 18].

Nevertheless, all these signatures have some common problems that hamper their rapid integration into clinical practice. First, they do not account for intra- and interfocal heterogeneity, because the sampling for investigation included only the highest grade tumors. Second, some interpretation errors are possible owing to the use of FFPE archive tissues (the quality of which is lower than that of fresh or fresh-frozen tissues, which are hard and expensive to handle). Third, the use of these genetic expression signatures with biopsy tissues is difficult owing to the undersampling issue and therefore entails possible bias due to characterization of less aggressive tumors. Fourth, the performance of these signatures is only slightly better than that of clinical variables and it is hard to estimate whether the prospective translation of these assays and their implementation for certain clinical cases will preserve this advantage. Fifth, the signatures were not compared to other important contemporary diagnostic modalities, e.g., multiparametric magnetic resonance tomography or some other biomarker [22] to see whether they will maintain their value.

Moreover, the method (gene expression analysis) itself is questionable for the purposes of high-resolution molecular characterization of prostate tumors. In the modern era, more than 60 studies of gene expression in prostate carcinoma have been published (e.g., in Oncomine Database, a publicly available database of stream gene expression data for various types of cancers). In almost all of these studies, thousands of genes were analyzed in a streamed fashion with regard to their mRNA expression. Two important conclusions stem from there. It was often found that the genes with altered expression in these studies did not correspond with the genes in other studies [6,10-12,16,17,21]. Second, the prognostic value in terms of clinical risks was never overwhelmingly high but rather low, with the primary endpoint of prostate-specific antigen recurrence not being a good surrogate of other prostate cancer outcomes [16-21]. The question arises of whether the expression of multiple genes is adequate for the aforementioned purposes. It seems that the gene expression analysis detects only virtually randomly mediated transcriptional reactions in the tissues, affecting thousands of genes, which are compensatory owing to changes in the cells and microenvironment but not a direct consequence of tumor growth. Behind this prominent transcriptional reaction lie some limited genetic oncogenic changes, which cannot be seen now because of this genetic chaos.

Nevertheless, selected signatures tested in real clinical practice showed some promising results in a postsurgical setting in patients with high risk of recurrence, which should be further prospectively evaluated [23]. However, the problem of valuable genetic characterization of a tumor at the time of biopsy (first minimally invasive contact with the tumor) is to date not solved.

GENETIC CHARACTERIZATION AND CLASSIFICATION OF PROSTATE TUMORS

A key finding in prostate tumor biology was the identification of the recurrent gene fusion (TMPRSS2:ERG, short abbreviation T2:ERG) in prostate cancer tumors [24]. This fusion is present in approximately 50% of all prostate adenocarcinomas [25] and is considered to be the early initial rearrangement, being also present in precancerous lesions (high-grade prostatic intraepithelial neoplasia) [26,27] and in a small percentage of benign prostatic hyperplasia tissues [28]. Several types of structural rearrangements leading to the T2:ERG fusion are
The results of attempts to link T2:ERG fusion to T2:ERG is the most common type of ETS– tumors are common. It could also be that there are other fusion partners from the ETS family of genes ETV5 being most common [29]. Other genes with strong should provide some differences in tumor phenotype at approximately 85% of all fusions [25,31,33].

The role of the aforementioned fusions for oncogenesis is currently understudied. Importantly, some functional studies show that overexpression of ERG as a single rearrangement is not enough for tumor formation, despite being obviously oncogenic, which suggests that additional mutations or rearrangements are necessary [34-36]. The results of attempts to link T2:ERG fusion to cancer aggressiveness and clinical outcomes have to date been unremarkable [37-40], especially in a postdefinitive therapy setting (for a review of studies, see reference [29]).

From a logical point of view, T2:ERG fusion is a relatively early event and gives rise to the two big molecular branches of prostate cancer. Correspondingly, the T2:ERG fusion represents two ways of cancer evolution and should provide some differences in tumor phenotype at late stages. The resulting late genetic aberrations, which are important for tumor formation, in these ETS+ and ETS– tumors are common. It could also be that there are many unique sprouting pathways in both tumor groups, which could lead to both aggressive and unaggressive entities independent of ETS status. The evidence for ERG-dependent aberrations is emerging (see below), but to date there are not enough data to make any final conclusions. However, given the high prevalence of these fusions, they could already be used as potent diagnostic biomarkers alone or together with other assays [41].

With the introduction of next-generation sequencing technologies into prostate cancer research, it became possible to gain deep insight into tumor genetics. On the one hand, it became possible to obtain important information in recurrently mutated genes and to classify the tumors by some of these genes. On the other hand, the mechanisms underlying the genetic rearrangements in prostate cancer were elucidated.

Some investigations show that the rate of somatic mutations in prostate tumors is very low, with many gene dysfunctions being a result of gene rearrangements [42-44]. The prominent article by Baca et al. [42] indicates that these complex genetic rearrangements are caused by the phenomenon called “chromoplexy,” which entails multiple breaks of DNA chains with newly arising interconnections and copy-number variations owing to inadequate reparation. Importantly, the breakpoint distribution and assembling are not random and involve adjacent fragments of the broken DNA chain. Chromoplexy is thereby responsible for the punctuated accumulation of genetic rearrangements. Nevertheless, the tempo of chromoplexy is not known. Thus, the pace of progression and time to clinically significant tumor formation remain obscure.

One remarkable success has been the identification of common recurrent genetic aberrations in prostate cancer. Many genes and altered pathways have been related to prostate cancer [42-44; for review see also reference 1]. The main genes recurrently affected in prostate tumors are ERG and the genes of the ETS family, TMPRSS2, Ki67, MYC, NKK3-1, PTEN, CHD1, Ras/Raf/MAPK pathway, PI3K pathway, NCOA2, SPINK1, EZH2, P53, RB1, HOXC6, CDKN2A, BMI1, SPOP, MED12, FOXA1, MLL2, CDKN1B, KDM6A, and MAGI2 [1,42-44]. Importantly, a single gene alteration in the pathway is enough to cause a pathway dysfunction [43]. This points at the importance of assessing genetic rearrangements with regard to pathways and their multiple interconnections and not in reference to selected genes [9].

A significant point is that localized and treatment-naive prostate cancers carry a relatively small number of genetic rearrangements and mutations. Thereby, castration-refractory lethal cancers are highly mutated [42-44], indicating that hormonal therapy itself is a significant promoter of the mutational processes, which is a major object of contemporary research.

Information obtained from the aforementioned studies provides a first basis for a highly desired molecular classification of prostate tumors. It seems that, being an early event, T2:ERG fusion and other ETS fusions are major classification criteria that can be used to divide all tumors into ETS-positive or ETS-negative. Such division could in turn have an almost unique set of further associated mutations, which again proves that the mutational process
is not random [42]. The genes common for ETS+/– tumors are discussed in detail elsewhere [1,45].

The main question is how to use this information on recurrent genetic aberrations for clinical purposes. The only way is to assess these aberrations in all patients in the clinic prospectively with strict respect for multifocality and intra- and interfocal heterogeneity. Such prospective evaluation will provide us with valuable information on the phenotypic properties of the tumors with regard to their genotype. Importantly, this genetic and molecular information from the primary tumor should be linked to outcomes and to the molecular genetic features of the metastatic lesions to understand the patterns of evolution to metastatic disease. This could give insight into which genetic alterations are responsible for invasion, metastasis, and progression and provide important data for clinical stratification of risks. Indeed, newly emerging prostate cancer–related genes and pathways should be included in this prospective model with time.

The first steps are already being taken in this direction. For example, PTEN loss and C-MYC gains are considered to be good tissue markers (fluorescence in situ hybridization analysis or immunohistochemistry) to rule out tumors with more aggressive phenotypes [46,47]. This is particularly important for Gleason stage 3 tumors, which could be considered more invasive and unfit for active surveillance in the presence of PTEN loss. Being a later and decisive genetic rearrangement by many prostate carcinomas [1,42-44,48,49], PTEN loss seems to have less prognostic significance in high Gleason score tumors.

Whereas copy number variations (CNVs) are the most common type of genetic rearrangement in prostate carcinoma, array comparative genomic hybridization (aCGH) can be used to detect the loci of CNVs and to cluster the cases in terms of aggressiveness and prognosis given that high-resolution arrays are available [43,47,50]. Overall, rough CNV burden estimation also seems to be a promising tool for identification of aggressive tumors [50,51], nevertheless, the technique is still far from clinical application. Other successful examples of molecular subclassification have also been published recently [8,52-58]. The new examples will warrant development of a new molecular classification model (analogous to the Gleason score) in the next few years with possible applications at the biopsy stage and in the post–radical-therapy setting.

However, some tumors will probably always be outstanding. For example, some tumors, according to several studies [43,44], have no typical prostate cancer mutations, meaning that prostate carcinoma can be developing in ways other than genetic regulation and that some important genetic or epigenetic rearrangements, which could explain the oncogenesis in those tumors, are to date not in scope.

**MULTIFOCALITY AND INTERFOCAL AND INTRAFOCAL HETEROGENEITY**

Multifocality is a well-known feature of prostate cancer and is found in from 60% to 90% of prostate tumors [59]. Therefore, at the time of biopsy, tissue sampling may be inconclusive with regard to the index (dominant, most aggressive) lesion. Moreover, multifocal tumors within one prostate arise independently (interfocal heterogeneity), therefore having different sets of genetic rearrangements and representing separate issues with diverse behaviors [60,61]. In simple words, two tumors in one patient could be as different as two tumors in two different patients. This should always be accounted for in research and in the clinical setting.

The other emerging issue is intrafocal heterogeneity. This term represents two different conditions: intrafocal heterogeneity due to the merger of two independent tumor loci in the process of their growth and intrafocal heterogeneity due to clonality of the cell populations within one focus. The latter seems to be an understudied issue and could be a major obstacle for clinical translation of genetic information.

Emerging evidence [61-64] shows that substantial interfocal heterogeneity is present in the individual tumor foci with regard to TMRSS2:ERG fusion formation and its structural type, to PTEN loss, CNVs, and epigenetic alterations across the whole genome. In most cases, these genetically different tumors seem to be identical in terms of Gleason grade and visual appearance.

By contrast, certain intratumoral heterogeneity with regard to Gleason grade (presence of Gleason grade 3 and 4 tumors in one focus) often mirrors the clonality issue (with Gleason 3 being a predecessor of Gleason 4 tumors) with shared genetic rearrangements between these tumors [49,65]. This is an interesting yet understudied component of contemporary research outlining the evolution of low-grade cancers. When we compare mutations present in Gleason 3 and Gleason 4 tumors, which could be partly common and partly different, we can gain insight into which mutations the tumor progresses through to the next stage.

The main questions that arise with the reports of intrafocal heterogeneity and which may significantly
influence the clinical application of genetic analyses are as follows: how many clones can reside within one tumor focus? Which spatial relations are typical for the clones (are the cells from different clones lying in layers, zones, or mixed)? Is the dominant clone advantage in terms of growth the biggest advantage (volume) in the tumor focus? How can we detect the most aggressive or the most important clones in terms of progression? These questions should be answered in the next few years.

**EPIGENETICS AND FIELD EFFECT**

Epigenetic alterations are typical for many cancers [66]. Three of the most important epigenetic regulators are DNA methylation, histone modifications, and micro-RNAs [67]. Methylation of DNA in the promoter region, which blocks the expression of the affected genes, is the most studied epigenetic alteration. The epigenetics of prostate cancer is a newly developing area of research with a limited amount information on the significance of epigenetic events for oncogenesis, progression, and important clinical issues (diagnosis, prognosis, treatment selection). Information is also lacking on the manifestation of these events in the natural course of prostate cancer. Nevertheless, the importance of some of these alterations for prostate tumors is confirmed by many relevant studies (for review, see reference [67]). A detailed review of the epigenetic events in prostate cancer was not the aim of this review; nevertheless, one issue with significant impact on everyday practice is worth discussing here. One of the most controversial issues in the genetics and epigenetics of prostate cancer is a field effect: the possibility of the cancer focus being associated with changes in the surrounding normal tissues, which appear visually as nonimpacted. The field effect is a cumulative concept consisting of several issues that must be clearly distinguished:

1. Germline genetic or molecular changes in the tissues, which predispose to prostate cancer development owing to the altered functioning of intracellular pathways (for example, as the consequence of germline mutations or single-nucleotide polymorphisms in key genes).

2. The field effect as a consequence of systemic actions of the prostate (viral/mycotic or bacterial infection, urine reflux, aging, etc.) that could lead to changes in the tissues that predispose to the development of prostate cancer.

3. A real field effect associated with the presence of the tumor (as a hypothesis, the affected cells could be the primary precursor clones of tumor cells with a visual appearance indistinguishable from normal cells but already with certain genetic/epigenetic traits of the tumor or as a result of extracellular transport of genetic or epigenetic material to the normal cells with subsequent changes).

4. A microenvironment response to the tumor, which is likely most important in the clinical setting, which is a real field effect with the only difference being that the changes in the cells may not predispose to the development of new tumors as they would with the real field effect.

The evidence of a field effect in prostate tumors stems from studies that investigated morphologically, genetically, proteomically, and epigenetically the tissues adjacent to the prostate cancer (for review of these studies, see reference [68]). The most promising data emerged from the assessment of the following epigenetic DNA methylation markers: GSTP1, APC, RASSF1A, and RARB [69-72]. Also, some studies showed that gene expression in benign tissues near the tumors could be altered [73]. These latter findings are hard to interpret, because modified genetic expression could be the result of the microenvironment response (the function of the affected genes is mainly understudied).

The main clinical application of the field effect concept is the prediction of prostate cancer in patients with an initial negative biopsy result via analysis of normally appearing prostate tissues in the obtained samples. Prominent results were achieved by Partin et al. [74] with the use of DNA methylation assessment for the GSTP1, APC, and RASSF1 genes. That study showed that the epigenetic assay could be readily implemented in clinical practice with a potentially high impact. The application of the epigenetic assay resulted in a negative predictive value of 88%. In simple words, when the assay does not detect methylation of three genes in the normal tissue from biopsy cores after an initial negative biopsy result, the probability that this patient has prostate cancer is as low as 12%. This is a prominent result and a ready solution for the clinical dilemma of whether to perform a repeat biopsy in a patient with an initial negative biopsy result. The disadvantage of the assay, although the study presented the clinical implications of the field effect concept, is that it is not clear which dimensions have this field effect. Important information, such as whether a tumor was detected in a repeat biopsy in the area of the previously identified epigenetic changes and whether any correlation between these issues persisted, was also lacking.

Another similar study by Truong et al. [75] investigated the methylation of the other gene set: EVX1, CAV1, and FGF1. Those authors reported that the combination of
EVX1 and FGF1 had a negative predictive value of approximately 91%, which is a little bit more than in the study of Partin et al. [74]. These assays will obviously have a significant place in clinical practice in the next several years.

**CONCLUSIONS AND FUTURE DIRECTIONS**

It is obvious that genetic characterization of prostate cancer is a mainstream component of contemporary translational prostate cancer research (Fig. 1). Nevertheless, the extremely heterogeneous nature of prostate tumors sets up substantial obstacles on the path to the clinical integration of the numerous findings.

Gene expression analysis is an interesting and simple implementation tool, but the contemporary evidence shows that the current state of this method does not reach the desired aim. Some obvious limitations are inevitably present.

The genetic characterization of prostate cancer with the use of contemporary molecular genetic methods is progressing unbelievably. There is nevertheless a substantial gap between the fundamental studies and the clinic. However, the emerging evidence shows that step by step, gene by gene, we are moving towards the clinically relevant genetic characterization of prostate cancer. The obvious limitations are the necessity of prospective evaluation of all findings and the outstanding interpatient and inter- and intrafocal heterogeneity of the prostate carcinoma. Given that the genetic characterization of the tumors by use of next-generation sequencing technologies is a labor-intensive task coupled with the analysis of huge amounts of data, international initiatives with division of tasks should play an important role in future progress (e.g., the International Cancer Genome Consortium, The Cancer Genome Atlas, projects similar to AURORA initiative for metastatic breast cancer [76]). One extremely important breakthrough has emerged from the epigenetic studies, which intended to solve the problem of repeat biopsies for most patients.

Importantly, a significant breach is now evident between the huge amount of studies of the genetic characterization of prostate cancer, which have limited translation to clinical practice or simply were not conceived to be so translated, and clinical practice. From a clinical point of view, this balance should be urgently shifted towards translation. Nevertheless, strict control of the significance of the new markers is necessary against the common clinical and pathological variables (e.g., Gleason score). This will guarantee protection from the enormous volume of insignificant data generated in the fundamental studies.

**CONFLICTS OF INTEREST**

The authors have nothing to disclose.

**REFERENCES**

1. Barbieri CE, Tomlins SA. The prostate cancer genome: perspectives and potential. Urol Oncol 2014;32:53.e15-22.
2. Klopfleisch R, Weiss AT, Gruber AD. Excavation of a buried treasure: DNA, mRNA, miRNA and protein analysis in for-
malin fixed, paraffin embedded tissues. Histol Histopathol 2011;26:797-810.
3. Sorensen KD, Orntoft TF. Discovery of prostate cancer biomarkers by microarray gene expression profiling. Expert Rev Mol Diagn 2010;10:49-64.
4. Febbo PG. Genomic approaches to outcome prediction in prostate cancer. Cancer 2009;115(13 Suppl):3046-57.
5. Bismar TA, Demichelis F, Riva A, Kim R, Varambally S, He L, et al. Defining aggressive prostate cancer using a 12-gene model. Neoplasia 2006;8:59-68.
6. Glinsky GV, Glinskii AB, Stephenson AJ, Hoffman RM, Gerald WL. Gene expression profiling predicts clinical outcome of prostate cancer. J Clin Invest 2004;113:913-23.
7. Kosari F, Munz JM, Savci-Heijink CD, Spiro C, Klee EW, Kube DM, et al. Identification of prognostic biomarkers for prostate cancer. Clin Cancer Res 2008;14:1734-43.
8. Gasí Tandefelt D, Boormans JL, van der Korput HA, Jenster GW, Trapman J. A 36-gene signature predicts clinical progression in a subgroup of ERG-positive prostate cancers. Eur Urol 2013;64:941-50.
9. Tomlins SA, Mehra R, Rhodes DR, Cao X, Wang L, Dhanasekaran SM, et al. Integrative molecular concept modeling of prostate cancer progression. Nat Genet 2007;39:41-51.
10. Varambally S, Yu J, Laxman B, Rhodes DR, Mehra R, Tomlins SA, et al. Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. Cancer Cell 2005;8:393-406.
11. Sethi S, Kong D, Land S, Dyson G, Sakr WA, Sarkar FH. Comprehensive molecular oncogenic profiling and miRNA analysis of prostate cancer. Am J Transl Res 2013;5:200-11.
12. Bismar TA, Alishalifa M, Petersen LF, Teng LH, Gerke T, Bakkar A, et al. Interrogation of ERG gene rearrangements in prostate cancer identifies a prognostic 10-gene signature with relevant implication to patients’ clinical outcome. BJU Int 2014;113:309-19.
13. Bishoff JT, Freedland SJ, Gerber L, Tennstedt P, Reid J, Welsbourn W, et al. Prognostic utility of the cell cycle progression score generated from biopsy in men treated with prostatectomy. J Urol 2014;192:409-14.
14. Chandran UR, Ma C, Dhir R, Biscegilia M, Lyons-Weiler M, Liang W, et al. Gene expression profiles of prostate cancer reveal involvement of multiple molecular pathways in the metastatic process. BMC Cancer 2007;7:64.
15. Wu CL, Schroeder BE, Ma XJ, Cutie CJ, Wu S, Salunia R, et al. Development and validation of a 32-gene prognostic index for prostate cancer progression. Proc Natl Acad Sci U S A 2013;110:6121-6.
16. Klein EA, Cooperberg MR, Magi-Galluzzi C, Simko JP, Falzarano SM, Maddala T, et al. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. Eur Urol 2014;66:550-60.
17. Erho N, Crisan A, Vergara IA, Mitra AP, Ghadessi M, Buerki C, et al. Discovery and validation of a prostate cancer genomic classifier that predicts early metastasis following radical prostatectomy. PLoS One 2013;8:e66855.
18. Karnes RJ, Bergstralh EJ, Davicioni E, Ghadessi M, Buerki C, Mitra AP, et al. Validation of a genomic classifier that predicts metastasis following radical prostatectomy in an at risk patient population. J Urol 2013;190:2047-53.
19. Cuzick J, Swanson GP, Fisher G, Brothman AR, Berney DM, Reid JE, et al. Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study. Lancet Oncol 2011;12:245-55.
20. Cuzick J, Berney DM, Fisher G, Mesher D, Moller H, Reid JE, et al. Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. Br J Cancer 2012;106:1095-9.
21. Cooperberg MR, Simko JP, Cowan JE, Reid JE, Djaliilvand A, Bhatnagar S, et al. Validation of a cell-cycle progression gene panel to improve risk stratification in a contemporary prostatectomy cohort. J Clin Oncol 2013;31:1428-34.
22. van den Bergh RC, Ahmed HU, Bangma CH, Cooperberg MR, Villers A, Parker CC. Novel tools to improve patient selection and monitoring on active surveillance for low-risk prostate cancer: a systematic review. Eur Urol 2014;65:1023-31.
23. Badani KK, Thompson DJ, Brown G, Holmes D, Kella N, Albala D, et al. Effect of a genomic classifier test on clinical practice decisions for patients with high-risk prostate cancer after surgery. BJU Int 2014 Apr 30 [Epub]. http://dx.doi.org/10.1111/bju.12789.
24. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644-8.
25. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer 2008;8:497-511.
26. Zhang S, Pavlovitz B, Tull J, Wang Y, Deng FM, Fuller C. Detection of TMPRSS2 gene deletions and translocations in carcinoma, intraepithelial neoplasia, and normal epithelium of the prostate by direct fluorescence in situ hybridization. Diagn Mol Pathol 2010;19:151-6.
27. Mosquera JM, Perner S, Genega EM, Sanda M, Hofer MD, Mertz KD, et al. Characterization of TMPRSS2-ERG fusion high-grade prostatic intraepithelial neoplasia and potential clinical implications. Clin Cancer Res 2008;14:3380-5.
28. Velaeti S, Dimitriadis E, Kontogianni-Katsarou K, Savvani A, Sdrolia E, Pantazi G, et al. Detection of TMPRSS2-ERG
fusion gene in benign prostatic hyperplasia. Tumour Biol 2014;35:9597-602.

29. Tomlins SA, Bjaartell A, Chinnaiyan AM, Jenster G, Nam RK, Rubin MA, et al. ETS gene fusions in prostate cancer: from discovery to daily clinical practice. Eur Urol 2009;56:275-86.

30. Lucas JM, Heinlein C, Kim T, Hernandez SA, Malik MS, True LD, et al. The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. Cancer Discov 2014;4:1310-25.

31. Han B, Mehra R, Dhanasekaran SM, Yu J, Menon A, Longro RJ, et al. A fluorescence in situ hybridization screen for E26 transformation-specific aberrations: identification of DDX5-ETV4 fusion protein in prostate cancer. Cancer Res 2008;68:7629-37.

32. Barros-Silva JD, Paulo P, Bakken AC, Cerveira N, Lovf M, Henrique R, et al. Novel 5' fusion partners of ETV1 and ETV4 in prostate cancer. Neoplasia 2013;15:720-6.

33. Mehra R, Tomlins SA, Shen R, Nadeem O, Wang L, Wei JT, et al. Comprehensive assessment of TMPRSS2 and ETS family gene aberrations in clinically localized prostate cancer. Mod Pathol 2007;20:338-44.

34. Klezovitch O, Risk M, Coleman I, Lucas JM, Null M, True LD, et al. A causal role for ERG in neoplastic transformation of prostate epithelium. Proc Natl Acad Sci U S A 2008;105:2105-10.

35. King JC, Xu J, Wongvipat J, Hieronymus H, Carver BS, Leung DH, et al. Cooperativity of TMPRSS2-ERG with PI3-kinase pathway activation in prostate oncogenesis. Nat Genet 2009;41:524-6.

36. Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, et al. Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. Nat Genet 2009;41:619-24.

37. Eguchi FC, Faria EF, Scapulatempo Neto C, Longatto-Filho A, Zanardo-Oliveira C, Taboga SR, et al. The role of TMPRSS2:ERG in molecular stratification of PCAs and its association with tumor aggressiveness: a study in Brazilian patients. Sci Rep 2014;4:5640.

38. Steurer S, Mayer PS, Adam M, Krohn A, Koop C, Ospina-Klinck D, et al. TMPRSS2-ERG fusions are strongly linked to young patient age in low-grade prostate cancer. Eur Urol 2014;66:978-81.

39. Pettersson A, Graff RE, Bauer SR, Pitt MJ, Lis RT, Stack EC, et al. The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta-analysis. Cancer Epidemiol Biomarkers Prev 2012;21:1497-509.

40. Hoogland AM, Jenster G, van Weerden WM, Trapman J, van der Kwast T, Roobol MJ, et al. ERG immunohistochemistry is not predictive for PSA recurrence, local recurrence or overall survival after radical prostatectomy for prostate cancer. Mod Pathol 2012;25:471-9.

41. Dijkstra S, Mulders PF, Schalken JA. Clinical use of novel urine and blood based prostate cancer biomarkers: a review. Clin Biochem 2014;47:889-96.

42. Baca SC, Prandi D, Lawrence MS, Mosquera JM, Romanel A, Drier Y, et al. Punctuated evolution of prostate cancer genomes. Cell 2013;153:666-77.

43. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11-22.

44. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012;487:239-43.

45. Lorente D, De Bono JS. Molecular alterations and emerging targets in castration resistant prostate cancer. Eur J Cancer 2014;50:753-64.

46. Van der Kwast TH. Prognostic prostate tissue biomarkers of potential clinical use. Virchows Arch 2014;464:293-300.

47. Zafarana G, Ishkanian AS, Malloff CA, Locke JA, Sykes J, Thombs J, et al. Copy number alterations of c-MYC and PTEN are prognostic factors for relapse after prostate cancer radiotherapy. Cancer 2012;118:4053-62.

48. Lapointe J, Li C, Giacomini CP, Salari K, Huang S, Wang P, et al. Genomic profiling reveals alternative genetic pathways of prostate tumorigenesis. Cancer Res 2007;67:8504-10.

49. Sowalsky AG, Ye H, Bubley GJ, Balk SP. Clonal progression of prostate cancers from Gleason grade 3 to grade 4. Cancer Res 2013;73:1050-5.

50. Lalonde E, Ishkanian AS, Sykes J, Fraser M, Ross-Adams H, Erho N, et al. Tumour genomic and microenvironmental heterogeneity for integrated prediction of 5-year biochemical recurrence of prostate cancer: a retrospective cohort study. Lancet Oncol 2014;15:1521-32.

51. Hieronymus H, Schultz N, Gopalan A, Carver BS, Chang MT, Xiao Y, et al. Copy number alteration burden predicts prostate cancer relapse. Proc Natl Acad Sci U S A 2014;111:11139-44.

52. Markert EK, Mizuno H, Vazquez A, Levine AJ. Molecular classification of prostate cancer using curated expression signatures. Proc Natl Acad Sci U S A 2011;108:21276-81.

53. Nagle RB, Algotar AM, Cortez CC, Smith K, Jones C, Sathy-anarayana UG, et al. ERG overexpression and PTEN status predict capsular penetration in prostate carcinoma. Prostate 2013;73:1233-40.

54. Grupp K, Wilking J, Prien K, Hube-Magg C, Sirma H, Simon R, et al. High RNA-binding motif protein 3 expression is an independent prognostic marker in operated prostate cancer and tightly linked to ERG activation and PTEN deletions. Eur J
Genetic characterization of prostate tumors

55. Grupp K, Kohl S, Sirma H, Simon R, Steurer S, Becker A, et al. Cysteine-rich secretory protein 3 overexpression is linked to a subset of PTEN-deleted ERG fusion-positive prostate cancers with early biochemical recurrence. Mod Pathol 2013;26:733-42.

56. Gumuskaya B, Gurel B, Fedor H, Tan HL, Weier CA, Hicks JL, et al. Assessing the order of critical alterations in prostate cancer development and progression by IHC: further evidence that PTEN loss occurs subsequent to ERG gene fusion. Prostate Cancer Prostatic Dis 2013;16:209-15.

57. Stumm L, Burkhardt L, Steurer S, Simon R, Adam M, Becker A, et al. Strong expression of the neuronal transcription factor FOXP2 is linked to an increased risk of early PSA recurrence in ERG fusion-negative cancers. J Clin Pathol 2013;66:563-8.

58. Krohn A, Seidel A, Burkhardt L, Bachmann F, Mader M, Grupp K, et al. Recurrent deletion of 3p13 targets multiple tumour suppressor genes and defines a distinct subgroup of aggressive ERG fusion-positive prostate cancers. J Pathol 2013;231:130-41.

59. Andreou M, Cheng L. Multifocal prostate cancer: biological, prognostic, and therapeutic implications. Hum Pathol 2010;41:781-93.

60. Wolters T, Montironi R, Mazzucchelli R, Scarpelli M, Roobol MJ, van den Bergh RC, et al. Comparison of incidentally detected prostate cancer with screen-detected prostate cancer treated by prostatectomy. Prostate 2012;72:108-15.

61. Ibeawuchi C, Schmidt H, Voss R, Titze U, Abbas M, Neumann J, et al. Genome-wide investigation of multifocal and unifocal prostate cancer—are they genetically different? Int J Mol Sci 2013;14:11816-29.

62. Yoshimoto M, Ding K, Sweet JM, Ludkovski O, Trotti G, Song KS, et al. PTEN losses exhibit heterogeneity in multifocal prostatic adenocarcinoma and are associated with higher Gleason grade. Mod Pathol 2013;26:435-47.

63. Minner S, Gartner M, Freudenthaler F, Bauer M, Kluft M, Salomon G, et al. Marked heterogeneity of ERG expression in large primary prostate cancers. Mod Pathol 2013;26:106-16.

64. Brocks D, Assenov Y, Minner S, Bogatyrova O, Simon R, Koop C, et al. Intratumor DNA methylation heterogeneity reflects clonal evolution in aggressive prostate cancer. Cell Rep 2014;8:798-806.

65. Kevtun IV, Cheville JC, Murphy SJ, Johnson SH, Zarei S, Kosari F, et al. Lineage relationship of Gleason patterns in Gleason score 7 prostate cancer. Cancer Res 2013;73:3275-84.

66. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer 2011;11:726-34.

67. Chiam K, Ricciardelli C, Bianco-Miotto T. Epigenetic biomarkers in prostate cancer: Current and future implications. Cancer Lett 2014;342:248-56.

68. Nonn L, Ananthanarayanan V, Gann PH. Evidence for field carcinization of the prostate. Prostate 2009;69:1470-9.

69. Van Neste L, Herman JG, Otto G, Bigley JW, Epstein JI, Van Criekinge W. The epigenetic promise for prostate cancer diagnosis. Prostate 2012;72:1248-61.

70. Mehrotra J, Varde S, Wang H, Chiu H, Vargo J, Gray K, et al. Quantitative, spatial resolution of the epigenetic field effect in prostate cancer. Prostate 2008;68:152-60.

71. Stewart GD, Van Neste L, Delvenne P, Delree P, Delga A, McNell SA, et al. Clinical utility of an epigenetic assay to detect occult prostate cancer in histopathologically negative biopsies: results of the MATLOC study. J Urol 2013;189:110-6.

72. Trock BJ, Brotzman MJ, Mangold LA, Bigley JW, Epstein JI, McLeod D, et al. Evaluation of GSTP1 and APC methylation as indicators for repeat biopsy in a high-risk cohort of men with negative initial prostate biopsies. BJU Int 2012;110:56-62.

73. Risk MC, Knudsen BS, Coleman I, Dumpit RF, Kristal AR, LeMeur N, et al. Differential gene expression in benign prostatic epithelium of men with and without prostate cancer: evidence for a prostate cancer field effect. Clin Cancer Res 2010;16:5414-23.

74. Partin AW, Van Neste L, Klein EA, Marks LS, Gee JR, Troyer DA, et al. Clinical validation of an epigenetic assay to predict negative histopathological results in repeat prostate biopsies. J Urol 2014;192:1081-7.

75. Truong M, Yang B, Livermore A, Wagner J, Weeratunga P, Huang W, et al. Using the epigenetic field defect to detect prostate cancer in biopsy negative patients. J Urol 2013;189:2341-45.

76. Zardavas D, Maetens M, Irrthum A, Goulioti T, Engelen K, Fumagalli D, et al. The AURORA initiative for metastatic breast cancer. Br J Cancer 2014;111:1881-7.