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Epigenetic markers for molecular detection of prostate cancer

Vera L. Costa[a,1], Rui Henrique[b,c,1] and Carmen Jerónimo[a,c,d,*]
[a]*Department of Genetics, Portuguese Oncology Institute – Porto, Rua Dr. Antonio Bernardino de Almeida, 4200-072 Porto, Portugal*
[b]*Department of Pathology, Portuguese Oncology Institute – Porto, Rua Dr. Antonio Bernardino de Almeida, 4200-072 Porto, Portugal*
[c]*Department of Pathology and Molecular Immunology, Institute of Biomedical Sciences Abel Salazar, University of Porto, Largo Prof. Abel Salazar 2, 4099-003 Porto, Portugal*
[d]*Fernando Pessoa University School of Health Sciences, Rua Carlos da Maia, 296, 4200-150, Porto, Portugal*

Abstract. Prostate cancer is a highly prevalent malignancy, which is clinically silent but curable while organ-confined. Because available screening methods show poor sensitivity and specificity, the development of new molecular markers is warranted. Epigenetic alterations, mainly promoter hypermethylation of cancer-related genes, are common events in prostate cancer and might be used as cancer biomarkers. Moreover, the development of quantitative, high-throughput techniques to assess promoter methylation enabled the simultaneous screening of multiple clinical samples. From the numerous cancer-related genes hypermethylated in prostate cancer only a few proved to be strong candidates to become routine biomarkers. This small set of genes includes *GSTP1*, *APC*, *RAR*$\beta 2$, *Cyclin D2*, *MDR1*, and *PTGS2*. Single and/or multigene analyses demonstrated the feasibility of detecting early prostate cancer, with high sensitivity and specificity, in body fluids (serum, plasma, urine, and ejaculates) and tissue samples. In addition, quantitative hypermethylation of several genes has been associated with clinicopathologic features of tumor aggressiveness, and also reported as independent prognostic factor for relapse. The identification of age-related methylation at specific loci and the differential frequency of methylation among ethnical groups, also provided interesting data linking methylation and prostate cancer risk. Although large trials are needed to validate these findings, the clinical use of these markers might be envisaged for the near future.

Keywords: Prostate cancer, epigenetics, methylation, molecular markers, biomarkers

1. Introduction

Prostate cancer has emerged as a leading health concern in recent years, standing as the most frequent non-cutaneous malignant disease in men and the second leading cause of cancer related mortality, with an estimated 232,090 new cases and 30,350 deaths for the year 2005 in the US [47]. Notwithstanding the dismal prognosis of locally advanced or metastatic disease, prostate cancer is a curable illness provided it is detected at its earliest stages, while still organ-confined. As effective prophylactic measures are not available and early-stage disease is often asymptomatic, highly efficient screening techniques are warranted.

The widespread use of serum prostate-specific antigen (PSA) determination constitutes a milestone event in early detection of prostate cancer [12]. The routine use of this cancer biomarker, in addition to digital rectal examination, resulted in an increase of organ-confined prostate cancer detection rate [8], and may account for the decreasing mortality rate related with this

[1]These authors contributed equally to this review and should be considered joint first authors.

*Corresponding author: Dr. Carmen Jerónimo, Department of Genetics, Portuguese Oncology Institute – Porto, Rua Dr. Antonio Bernardino de Almeida, 4200-072 Porto, Portugal. Tel.: +351 225084000 (ext. 5610); Fax: +351 225084016; E-mail: cjeroni@ufp.pt.

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disease [47]. However, the role of serum PSA levels as a screening tool for prostate cancer meets with important limitations. Although raised PSA levels (i.e., $>$ 4.0 ng/ml) are undoubtedly associated with the presence of prostate cancer, benign conditions, such as prostatitis and benign prostatic hyperplasia (BPH), which is common in the elderly, are also a cause of elevated serum PSA. Consequently, there is a relatively high frequency of unnecessary prostate sextant biopsies, an invasive procedure which is expensive and uncomfortable for the patients [14]. Moreover, a significant proportion of men with PSA levels within the normal range harbor prostate cancer (up to 22%) and a significant number of these show pathologic features of tumor aggressiveness [13,68,69,85]. Indeed, 21% of men with positive end of study biopsies enrolled in the Prostate Cancer Prevention Trial (PCPT) had serum PSA levels between 2.6 and 3.9 ng/mL and 15.4% of the tumors found in men with PSA levels $<$ 2.5 ng/mL were high grade cancers [88]. Furthermore, there are also ethnic variations which influence the performance of the serum PSA test, as only 25–35% of Caucasians and Hispanics with a PSA in the 2–10 ng/mL range have positive biopsies for prostate cancer, whereas up to 70–80% of African-Americans in this same range have a positive biopsy [57]. Because the sensitivity and specificity of the serum PSA test is at best 75%, this is a rather imperfect prostate cancer marker [70]. Although several PSA-derived indices have been developed (e.g., free PSA, complex PSA, PSA density, PSA velocity and doubling time), none has so far attained widespread acceptance. Finally, the PSA test is unable to accurately assess the threat that a prostate tumor poses to the patient's life. Usually, a patient diagnosed with localized prostate cancer will have his prostate surgically removed, although pathological findings indicate that approximately 15% to 30% of the tumors excised are clinically insignificant and, consequently, were unlikely to affect the patient lifespan or quality of life [61, 91].

Presently, ultrasound-guided needle sextant prostate biopsy is the standard method for prostate cancer diagnosis in its earliest stages [25]. This technique is safe, with a very low frequency of post-biopsy complications, and very accurate. Nonetheless, the tissue sample is rather small, which constitutes an important limitation for histopathological evaluation [25]. Indeed, interpretation of prostate core biopsies constitutes a difficult task for the pathologist because the diagnosis of cancer is often based on a limited number of malignant cells infiltrating among more numerous benign glands. Moreover, the limited tissue sampling poses two important problems to the pathologist. First, the morphology of malignant prostate glands might be difficult to assess when the representation in the biopsy is scarce. Immunohistochemical techniques might help circumvent this problem, but none has been proven to definitely confirm or deny the diagnosis of prostatic malignancy [19]. Consequently, an important proportion (about 24%) of men submitted to prostate biopsy because of increased serum PSA levels, suspicious ultrasonographic or clinical findings are found to harbor prostate cancer in repeat biopsies [55]. Second, in addition to cancer diagnosis, histopathological evaluation of prostate biopsies should also convey prognostic information that might guide clinical decision-making. However, from a purely statistical point of view, the sampled malignant glands might not be representative of the main tumor, resulting in frequent undergrading and understaging [86]. This problem is also augmented by the substantial interobserver variability of the most important prognostic parameter, i.e., the Gleason score [2]. Finally, even carcinomas with an identical morphology and stage may have quite different outcomes, thus limiting the usefulness of current prognostic indicators.

As it may be apparent from the previous statements, there is an urgent need for improving early detection, diagnosis and prognosis assessment in prostate cancer. Because routine histopathological evaluation has most probably reached its limits as a diagnostic and prognostic tool, the development of new, more sensitive and specific markers should be based on the identification of the actual mechanisms underlying prostate cancer, i.e., genomic alterations.

2. Epigenetic alterations in prostate cancer

Over the last few years, several genomic alterations have been consistently reported in prostate carcinoma (see [43] for a recent review). Many somatic alterations including mutations, gene deletions, gene amplifications, chromosomal rearrangements, and changes in DNA methylation are detectable in prostate cancer cells. These modifications probably accumulate over a period of several decades and the number of changes increases with disease progression. Although multiple alterations that appear to contribute to disease progression have been suggested, no single key change has been detected. Indeed, genetic alterations such as gene mutations are either infrequent (e.g., those that activate

the *Ras* oncogene or inactivate the *TP53* [44]) or occur late in disease progression (e.g., those that inactivate the tumor suppressor gene *PTEN* [10]). Hence, these alterations are not optimal candidates for cancer biomarkers in the clinic. Conversely, epigenetic events occur much more frequently and at earlier stages in prostate cancer, hypothetically providing more efficient tools for disease detection and management [51].

Two main categories of epigenetic alterations have been reported thus far in prostate cancer, i.e., histone modifications (including phosphorylation, methylation, and acetylation) and aberrant methylation (which includes both hypermethylation and hypomethylation) [63,66,83]. Most studies concerning the role of histone modifications in prostate cancer are based on the restoration of gene expression following treatment of cancer cells with histone deacetylases (HDAC) inhibitors. Although specific alterations have been reported for some genes (e.g., coxsackie and adenovirus receptor (CAR) and vitamin D receptor (VDR) [3,78]), it seems that alterations in the global levels of specific histone modifications are involved in prostate carcinogenesis and might also be independent outcome predictors [83].

DNA hypomethylation has also been seldom reported in prostate cancer. Interestingly, genomewide DNA hypomethylation was reported in 31% of prostate carcinomas and significantly correlated with aberrations in chromosome 8 and metastatic disease [82], thus linking hypomethylation with chromosome instability and disease progression. In addition, gene-specific methylation has been reported to affect several overexpressed genes in prostate cancer (e.g., *PLAU, Urokinase*, *Heparanase*, and *Cytochrome P450 1B1* [74,76, 77,89]) and might account for the activation of other proto-oncogenes in prostate carcinogenesis.

However, the most frequent and better characterized epigenetic alteration, not only in prostate carcinoma but also in other common human malignancies, is DNA hypermethylation [26], providing several promising cancer biomarkers [51]. A large number of cancer-related genes have been reported to be downregulated through promoter hypermethylation in prostate cancer (see reference [63] for a recent and comprehensive review) and several of these have shown promise for prostate cancer detection [5,22,35,42,48,52,53]). Besides the high frequency and early occurrence of this epigenetic alteration in prostate cancer, there are other important advantages in the use of DNA methylation as a biomarker for cancer detection. From a technical standpoint, DNA harboring methylation is more stable and easy to manipulate than RNA, and simpler to extract than proteins. Moreover, hypermethylation is a positive signal that is less likely to be masked through contaminant normal DNA, making it particularly suited for detection in clinical samples, where sensitive detection is necessary owing to scarce tumor DNA or dilution by excess normal DNA. Finally, detection of hypermethylation in a large number of samples is now feasible using recent standardized high-throughput technologies, thus enabling its use in clinical practice.

3. Methods for detection of DNA hypermethylation

Several methodologies might be used for the identification of DNA methylated sequences. Some of them (e.g., bisulfite-sequencing) provide important information concerning the methylation profile and the degree of methylation heterogeneity of cells [16]. However, these methods are expensive, time-consuming, and not amenable to routine clinical use. Indeed, if a widespread use of DNA methylation markers for cancer detection is envisaged, assays characterized by high sensitivity, specificity, reproducibility, homogeneity and high-throughtput capabilities must be chosen. For this purpose, the assays based on the analysis of sodium bisulfite converted DNA [75] offer many advantages. The most widely used of these assays is methylation-specific PCR (MSP) [40] because it usually requires only minute quantities of template DNA, it is simple, safe and easy to perform, and it shows considerable sensitivity, which is a key requirement for the analysis of clinical samples.

In the original MSP method (conventional MSP, CMSP), PCR products are run in a gel and the results are reported as methylated or unmethylated at the target DNA sequence [40]. Consequently, this method does not allow the identification of partial levels of methylation, a feature which is extremely relevant both biologically and clinically. Thus, quantitative methods have been developed in recent years to overcome this limitation of CMSP, and the most important of these was the fluorescence-based real-time quantitative MSP (QMSP) assay [20]. In this assay, locus-specific primers, designed to amplify methylated CpGs, flank an oligonucleotide probe labeled with a 5' fluorescent reporter dye and a 3' quencher dye. During amplification, the *Taq* polymerase cleaves the reporter from the probe, owing to its 5'-3' exonuclease activity, thus releasing it from the quencher. Then, the monitoring of fluorescence emissions during the PCR process al-

lows the quantitation of methylated alleles [37]. Because this quantity also depends on the input of template DNA, an internal reference should be used to normalize the assay. For this purpose, housekeeping gene (e.g., *MYOD1* or *ACTB*) without CpG islands has been used by most researchers. However, recent findings indicate that Alu-based control reactions might be the best strategy to measure the input levels bisulfite-converted DNA [92]. Several strategies might then be employed to express this quantity (e.g., a ratio calculated using the values obtained for the target gene and the reference gene in a given case) which represent the relative level of methylated gene in a given sample. The use of the probe improves the overall specificity of the assay owing to the more stringent amplification conditions. Moreover, the quantitative assay was shown to reliably detect promoter methylation in the presence of 10.000-fold excess of unmethylated alleles, i.e., a 10-fold increase in sensitivity over CMSP [20]. Consequently, high sensitivity and specificity are key features of QMSP. However, as incompletely methylated target sequences will not be amplified, the actual methylation levels might be greater than those determined. Notwithstanding this limitation of QMSP, its high-throughput characteristics are ideally suited for the analysis of large numbers of clinical samples.

4. Gene promoter methylation and prostate cancer detection/diagnosis

Among the relatively large number of cancer-related genes reported to be hypermethylated in prostate cancer, *GSTP1* is unquestionably the most widely studied and well documented. This gene encodes a π-class Glutathione S-transferase (GST-π) enzyme involved in the detoxification of reactive chemical species by catalyzing their conjugation to reduced glutathione [36]. Thus, *GSTP1* acts as a "caretaker" gene protecting prostate cells against genomic damage mediated by a variety of oxidants, including carcinogens [71]. Loss of *GSTP1* function would predispose normal prostatic cells to endure DNA damage motivated by inflammation and/or dietary intake thus leading to carcinogenesis [72].

Since the first reports linking *GSTP1* loss of expression with promoter methylation in prostate cancer [58, 59], many studies confirmed the high frequency of this epigenetic alteration in invasive tumor (36–94%), as well as in prostatic intraepithelial neoplasia (PIN) (30–76%) [9,22,23,28,35,48,49,52,54,64,67,93–95]. However, the tumor-specificity of *GSTP1* hypermethylation is not absolute as morphologically normal prostate tissue and BPH might carry this alteration [22,39,48,49]. This problem is solved by the use of quantitative assays, most notably QMSP, because non-cancerous prostate tissues display only very low levels of *GSTP1* promoter methylation [22,35,39,48,95,96].

Possibly, the most illustrative study concerning the power of *GSTP1* hypermethylation as a prostate cancer marker in prostate tissues was performed by Harden and co-workers, which managed to compare the diagnostic accuracy of the test with the standard histopathological evaluation by an expert uropathologist [35]. In their report, the quantitative assessment of *GSTP1* promoter methylation detected 11% more cases compared to histopathology, whereas a combination of both yielded a 15% (79% vs. 64%) increase over histopathology alone [35]. Thus, a QMSP assay for *GSTP1* might provide a valuable ancillary diagnostic tool for routine pathological assessment of prostate biopsies.

The better performance of the quantitative *GSTP1* hypermethylation assay compared to standard histopathology might be explained by the detection of minute foci of tumor cells that would be insufficient to raise morphological suspicion of malignancy. Alternatively, the methylated *GSTP1* alleles detected by the MSP assay might not only be derived from malignant cells but also from morphologically normal epithelial cells and/or stromal cells in the vicinity of the neoplastic glands. Thus, the epigenetic alterations detected in these morphologically normal cells would serve as surrogate markers for prostate cancer. This hypothesis is supported by a recent study which observed methylation at the *GSTP1* and *RARβ2* promoters in non-neoplastic cells of prostate tumor microenvironment [34]. These findings could be due to a "field-effect" phenomenon and raise the possibility that epigenetic alterations in prostate tissue are associated with the exposure to carcinogens (either endogenous or exogenous) which seem to affect both epithelial and mesenchymal components. However, owing to the high prevalence of prostatic epithelial neoplasms and the rarity of mesenchymal tumors, it seems reasonable to assume that the acquisition of *GSTP1* and *RARβ2* promoter methylation in stromal cells is not sufficient to induce a malignant phenotype. Hence, the intrinsic biology of prostatic epithelial cells is likely to render them more susceptible to endure genomic alterations and subsequent neoplastic transformation.

Although the performance of the *GSTP1* hypermethylation assay in tissue samples might perfect diagnostic accuracy in prostate biopsies, an important step

forward in prostate cancer detection would be the development of a body fluid-based screening test. Such a test could increase the sensitivity and specificity of the serum PSA test, thus diminishing the number of unnecessary prostate biopsies. Circulating DNA in the plasma and serum of patients with urological malignancies has been documented [27]. Prostate cancer DNA may be present in the circulation as a result of intravascular cell death of prostate cancer cells or circulating phagocytic cells that have ingested prostate cancer cells [69]. Moreover, the anticipated shedding of neoplastic prostatic cells into the prostatic ducts raises the possibility of detecting cancer-related epigenetic alterations in urine sediments. Thus, voided urine and serum/plasma are obvious candidates for non- or minimally-invasive screening and have been tested accordingly [11,28,29,31,50]. However, two major concerns arose from these studies. First, the sensitivity of the test is significantly hampered (18.8–38.9% for voided urine, and 13–72% for serum/plasma), although it might be augmented using prostatic massage prior to urine collection [29], collecting urine after prostate biopsy [31] or eventually by increasing the number of samples from each patient. Second, the test specificity is likely to be impaired when additional procedures are performed to increase the rate of detection [29,31]. The relatively high frequency of prostate cancer detection in ejaculates using a CMSP assay for *GSTP1* hypermethylation [28,30,87] might offer an additional source of test material. However, the nature of the sampling procedure, particularly in older men, meets with relevant ethical questions and practical limitations.

Several strategies might be anticipated to increase the sensitivity of prostate cancer detection in body fluids without compromising specificity. Thus, the combined use of an urine-based and a serum/plasma-based assay might increase the sensitivity of the test, as previously demonstrated [50]. Moreover, the use of a panel of genes surveyed for promoter methylation, in addition to *GSTP1*, might decisively increase both the sensitivity and the specificity of the test as verified in a recent report by Hoque and co-workers [42]. The gene panel, which included four genes (*GSTP1*, $p16$, *ARF*, and *MGMT*), allowed those researchers to detect 87% of prostate carcinomas with 100% specificity, using a QMSP assay on urine sediments [42]. Similar gene panels, which variably include *GSTP1*, *APC*, *MDR1*, *PTGS2*, *RARβ2*, and *RASSF1A*, have also been proposed to increase the sensitivity and specificity of prostate cancer detection in tissue samples [5,22,90, 95]. However, these panels should be viewed with some caution as the simultaneous use of more than 3 markers will only accomplish a minor increase in sensitivity at the cost of lowering the specificity of the test [95].

5. Gene promoter methylation and prognosis prediction in prostate cancer

Epidemiological data indicate that only a proportion of histologically diagnosed prostate carcinomas will progress to clinically significant and lethal disease whereas many patients die (from other causes) with an indolent form of prostate cancer [18]. Thus, tumors with overlapping morphological features may indeed be associated with a quite distinct disease outcome. As more effective screening tests are likely to increase the number of "clinically indolent" prostate cancers that would not benefit from aggressive forms of treatment, it is critical to develop markers that might identify the "clinically relevant" prostate carcinomas. Ideally, these markers would be tested in the clinical samples utilized for screening and/or diagnosis of prostate cancer and the results would guide patient management. Current prognostic indicators, including pre-treatment serum PSA level, clinical stage determined by digital rectal examination, and the tumor Gleason score on needle biopsy remain the mainstays used in clinical decision making [46]. However, the usefulness of these markers is limited as they do not allow for an accurate prognostic assessment in an individual basis. Consequently, and alongside with the reported up to 60% of clinically understaged prostate carcinomas [7], there is also an important proportion of prostate carcinomas treated by radical prostatectomy that might be better managed conservatively.

Several studies have documented statistical associations between clinical and pathological features of tumor aggressiveness (e.g., pathological tumor stage and Gleason score) and promoter methylation of single genes or gene panels including *APC*, *CDH1*, *EDNRB*, *GSTP1*, *MDR1*, *MT1G*, *PTGS2*, *RARβ2*, *RASSF1A*, and *RUNX3* [5,21,22,38,39,52–54,60,84,95]. Interestingly, in a recent report a methylation (M) score, derived from the quantitative assessment of promoter methylation at the *GSTP1*, *APC*, and *MDR1* promoters, was able to discriminate organ-confined from locally advanced disease with 72.1% sensitivity and 67.8% specificity [22]. Although these results are still suboptimal for clinical application, they provide sufficient preliminary evidence to support further testing is larger trials. Curiously, the same M-score was found

to be significantly higher in current smokers than in never smokers and to positively correlate with pack-years smoked [23]. This is a rather unexpected finding as smoking has not been previously associated with prostate cancer risk [33].

In addition to assist in the therapeutic triage of prostate cancer patients, methylation markers might also provide valuable information concerning disease relapse prediction. Indeed, *GSTP1* promoter methylation was found to be the most relevant predictor of early PSA recurrence in multivariate analysis in a recent study [4]. Moreover, *APC* and *Cyclin D2* hypermethylation also seem to predict time to recurrence in a defined set of prostate carcinomas (i.e., tumors with Gleason score $3 + 4 = 7$) and were shown to be independent prognostic factors [79]. Unexpectedly, lower levels of *GSTP1* promoter methylation were also reported to be associated with unfavorable outcome, eventually identifying a particular subset of prostate carcinomas that progress through alternative pathways [79].

It should be realized that the development of new prognostic markers for prostate cancer is difficult and complex owing to the heterogeneity of this neoplasm, which frequently manifests as multiple independent primary tumors within the same organ [73]. Histopathological assessment of radical prostatectomy specimens frequently reveals the existence of multiple, independent tumors, and this finding has been confirmed by genetic studies [6,15,56,65,80]. Thus, it is critical to determine whether independent prostatic tumor foci have comparable potential for invasion and metastasis. According to some reports, the characteristics of the "index" tumor in the prostatectomy specimen are those that more accurately predict the likelihood of recurrence and this is largely independent of the characteristics of the smaller, concurrent tumors [73]. Thus, the use of methylation markers for the pre-treatment assessment of prognosis in prostate cancer patients should obviously take in consideration the issue of prostate tumor heterogeneity.

6. Gene promoter methylation and risk-assessment in prostate cancer

Risk markers offer the benefit of providing an opportunity for early disease detection and diagnosis, thus augmenting the likelihood of curative treatment. Moreover, the use of these markers might allow the development of innovative prevention strategies, targeted to subjects at high risk, avoiding exposure of the whole population and thereby diminishing the potential problems related with the implementation of a screening test. In this vein, the refinement of the ability to identify individuals with high risk for prostate cancer would permit a less frequent screening in men at lower risk, consequently decreasing the false positive rate. The principal aim would be to augment the specificity of the screening and reduce the frequency of (unnecessary) prostate biopsies.

The search for risk-associated markers might then be guided by the known risk factors for prostate cancer, e.g., age, diet and genetic background [34]. Concerning age-related methylation, it is noteworthy that prostate cancer is primarily a disease of the elderly, with about 80% of the cases diagnosed after 65 years of age, and the average patient age at the time of diagnosis is 70 years. Moreover, the incidence is low prior to age 50 and increases exponentially until 80 years [47]. Previous studies documented that the acquisition of aberrant promoter methylation at several genes is an age-related phenomenon in normal human tissues [1]. Indeed, in colorectal mucosa, the methylation of a CpG island on the *ESR1* gene increases linearly with age [45]. Interestingly, methylation of *ESR1* has also been reported as both age-dependent and tumor differentiation-dependent in prostate cancer, thus representing a putative link between aging and prostate cancer [62]. Eventually, other cancer-related genes might become epigenetically inactivated in an age-dependent manner, thereby increasing the susceptibility of normal prostate cells to neoplastic transformation. Moreover, this hypothesis might substantiate the frequent, although at lower level, promoter methylation of specific genes reported in morphologically normal prostate glandular epithelium [39]. Hypothetically, individuals carrying these alterations in normal tissue might be at higher risk for developing prostate cancer and could benefit from more close surveillance.

Of all human cancers, prostate cancer shows the highest ethnic disparity in incidence and mortality [47]. When compared with Caucasians, African-Americans have a 1.7-fold higher incidence of cancer, twice the risk of developing advanced-stage prostate cancer, and a two- to threefold greater mortality rate [41]. Moreover, African-Americans are also more likely to have a family history of prostate cancer and are younger at the time of diagnosis. As socioeconomic, clinical, and pathological factors do not account for all these racial discrepancies [17], unknown race-related factors must be involved in prostate cancer susceptibility and progression. Conceivably, race-related patterns

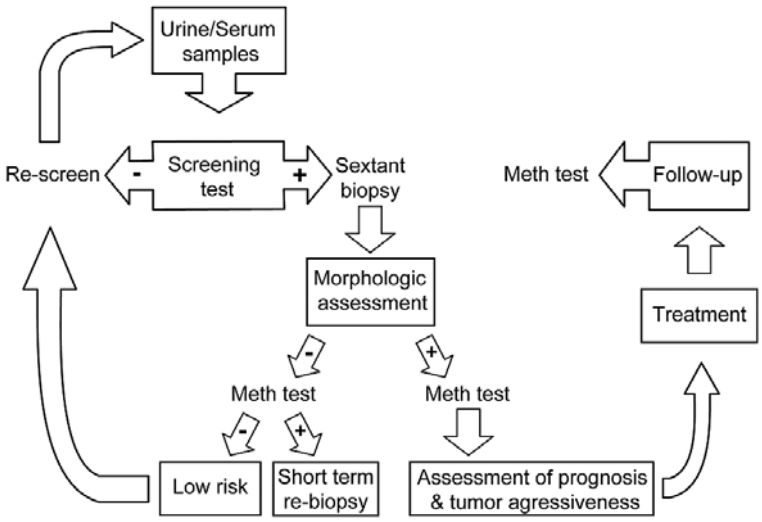

Fig. 1. Proposed algorithm for the management of prostate cancer suspects using epigenetic markers (Meth test, methylation markers-based test).

of gene methylation would be able to confer resistance or susceptibility to the development and/or progression of prostate cancer. Interestingly, racial differences in *CD44* gene promoter methylation have been reported, with a 1.7-fold higher frequency of methylation among African-Americans (43%) relative to Caucasians (25%) [93]. Differences in *GSTP1* promoter methylation in prostate cancer among African-American, Caucasian, and Asian populations have also been found. In a recent study, Enokida and co-workers reported that *GSTP1* methylation is a particularly good biomarker for prostate cancer in African-Americans whereas in Asian patients this epigenetic event correlates with pathological findings (higher pT categories and Gleason score) [23]. These findings suggest that *GSTP1* hypermethylation is likely to have different performances as a prostate cancer biomarker in distinct ethnic groups.

Thus far, no substantial evidence has been provided linking DNA methylation with environmental factors (e.g., diet) or with hereditary susceptibility in prostate cancer.

7. Application of epigenetic-based markers in prostate cancer detection and management: a proposal

While clinicians and patients wait for the upcoming availability of commercial tests based on current knowledge about the prostate cancer epigenome, we are tempted to propose an algorithmic approach for the use of those tests in the clinic (Fig. 1). Ideally, this assay should include several epigenetic biomarkers (probably from 2 to 4) to maximize the sensitivity of the test and also to provide relevant information concerning the clinical significance of a prostate tumor. The development of multiplex assays is likely to be a useful and cost-effective methodology for that purpose. Following clinical evaluation, the test should be initially performed as a screening tool in healthy individuals at risk for prostate cancer. Thus, urine and/or serum would be the ideal clinical samples because its collection is not or minimally invasive. Individuals with a negative test would be re-tested within a given time period, probably 1 or 2 years, depending on the clinical evaluation. Conversely, a positive test should lead to sextant prostate biopsy. In addition to standard histopathological evaluation, testing for hypermethylation of the same set of genes in tissue samples (ideally from each side) may add relevant information for clinical management when adenocarcinoma is diagnosed. Conversely, if there is no morphological evidence of malignancy, a positive test should be followed by close surveillance and repeat biopsy within a short period of time. Eventually, this biopsy might be more extensive on the side from which the tissue sample tested positive was collected. Double negative cases (i.e., without morphological and molecular evidence of malignancy) would re-enter the screening path after clinical evaluation. Furthermore, patients diagnosed with prostate cancer might be monitored after treatment with a periodical blood and/or urine methylation test. This procedure might identify disease progression almost from its inception and triage

patients for earlier and more adequate therapeutic procedures.

Please note that this is a purely speculative model and clinical evidence is still lacking to support many of the procedures. Moreover, the methylation test needs to be evaluated in large clinical trials to determine its usefulness and potential advantages over the currently available markers, such as serum PSA. However, this model may provide a rationale for the collection of the significant clinical and scientific evidence to back up the implementation of these new prostate cancer biomarkers in routine clinical practice.

8. Conclusions

Prostate cancer remains a significant challenge both to researchers and clinicians. Many questions concerning its biological characteristics and clinical significance await more definite answers. Clearly, current tools for prostate cancer screening, diagnosis, and management are limited, and innovative methodologies are needed. The hypermethylation of CpG islands represents a somatic, epigenetic event that almost uniformly arises during prostate carcinogenesis. Using modern detection assays, CpG island hypermethylation of multiple cancer-related genes has provided promising molecular markers for prostate cancer. Importantly, the detection of these alterations is readily feasible in routine clinical specimens such as urine, blood, and prostate biopsy samples, eventually providing critical information for risk-assessment, screening, diagnosis and treatment. Because epigenetic information is heritable but also has considerable plasticity, disease treatment and chemoprevention strategies might benefit from specific therapeutic methods that target epigenetic alterations. Hence, it is a reasonable expectation that epigenetic-based markers may decisively contribute for improved prostate cancer patient care in the future.

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