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Mini Review

Hypermethylation and Hypomethylation of DNA: Implication for Diagnosis and Prognosis of Prostate Cancer

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Abstract: Prostate cancer belongs to the most common cancers and it is the second leading cause of cancer death in men. A genetic predisposition with epigenetic changes in DNA contributes to the development of the disease. DNA hypermethylation and hypomethylation belong to the most studied epigenetic changes in prostate cancer development. Both forms may lead to chromosomal instability and transcriptional gene silencing. This article is aimed at brief review of information in respect to DNA hypermethylation and hypomethylation in the prostate cancer development. Moreover, we discuss their implication for diagnosis and prognosis of prostate cancer.

Keywords: Prostate Cancer, DNA Hypermethylation, DNA Hypomethylation, Diagnosis, Prognosis

Introduction

Prostate Cancer (PCa) belongs to the most common cancers and it is the second leading cause of cancer death in men (over 250.000 men dies worldwide every year). A genetic predisposition or acquired genetic and epigenetic changes with the effect of other factors, such as advanced age, race, diet, weight, physical activity, endogenous hormones and environmental factors contribute to PCa development (Demichelis and Stanford, 2014).

PCa is a very heterogeneous disease that is characterized by different clinical behavior, from indolent, slow-growing tumors to aggressive, fast-growing tumors with lethal progression. Early diagnostics and identification of PCa type are crucial prerequisites for efficient treatment of patients. Recently, the diagnostics of early stages of PCa is based mostly on evaluation of Prostate-Specific Antigen (PSA) in serum of patients (Lilja *et al*., 2008). Men with high levels of PSA undergo biopsy in order to determine histopatological grading of PCa-Gleason scoring which classifies tumors from 1 to 5 (most to least differentiated) (Epstein, 2010) as well as staging-determination of the status of their primary tumors (T1-T4; from bounded to fully invasive), with or without lymph node involvement (N0 or 1) (Cheng *et al*., 2012). The results from this screening diagnosis lead into conventional treatment, including radical prostatectomy and brachytherapy. In case of advanced PCa, conventional treatment continues with androgen deprivation therapy. However, in many cases the cancer recurs (Michaelson *et al*., 2008).

At present, the clinicians and researchers are forced to find more precise and sensitive biomarker suitable for PCa diagnostics as well as prognostics and therapy. The excessive study of epigenetical mechanisms, including DNA hypermethylation and hypomethylation provides promising results in this context (Chin *et al*., 2011).

The main goal of the present article is to offer brief review of information in respect to DNA hypermethylation and hypomethylation and their implications for PCa development, diagnosis and prognosis.

DNA Methylation in Prostate Cancer

Methylation of the DNA is its covalent modification by binding of the methyl group to the cytosine in dinucleotides containing Cytosine and Guanine (CpG). These dinucleotides are present in the DNA sequence and they are termed as CpG islands. They occur in about 60% of all genes in the human genome. They are responsible for controlling the gene expression and their

methylation lead into silencing of the transcriptional activity of the downstream genes (Irizarry *et al*. 2009). There are a lot of different genes, including tumor suppressor genes, genes involved in metabolism and genes responsible for cell invasion, which has been extensively studied in respect to cancer development and progression including PCa due to their methylation (Yang and Park, 2012). Aberrant DNA methylation can occur in two forms-hypermethylation and hypomethylation.

Hypermethylation

The most known genome alteration in PCa is hypermethylation of CpG at promoter of Glutathione S Transferase P1 (GSTP1). It occurs in majority (>90%) of PCa cases but it is not present in normal tissues (Lee *et al*., 1997). The lost of GSTP1 activity lead into increasing of oxidative damage of DNA which may lead in increase of PCa risk. GSTP1 hypermethylation is usually detected in high frequency in urine, blood and ejaculates of PCa patients.

Another frequently investigated is gene for Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) which is involved in PCa progression and metastasis. It was documented that hypermethylation of gene for TIMP-2 lead into its down-expression in PCa when compared with normal tissue (Pulukuri *et al*., 2007). On the contrary, there are several works which described the overexpression of TIMP-2 in PCa. Moreover, Ross *et al*. (2003) showed correlation between TIMP-2 expression and clinical stages and recurrence.

The lack of Retinoic Acid Receptor β (RARβ) expression is typical for tumor tissues. This is due to hypermethylation of promoter RARβ which occurs in 40 – 84% of PCs tissues (Nakayama *et al*., 2001; Zon *et al*., 2009). Moreover, moderate or high frequencies of RARβ promoter methylation were also documented in urine or blood samples (Bastian *et al*., 2008; Rouprêt *et al*., 2008). It makes RARβ promoter hypermethylation a promising biomarker for early PCa detection.

Recent studies, reported hypermethylation in promoter of Caveolin-1 (*CAV1*) gene in respect to PCa. *CAV1* belong to tumors suppressor genes which are involved in vesicular transport, cholesterol balance, transformation and tumorigenesis. Moreover, it acts as metastasis-promoting gene (Chang *et al*., 2013; Patra and Bettuzzi, 2007). It was shown that increased DNA hypermethylation of *CAV1* gene is associated with biochemical recurrence in patients with PCa (Bachmann *et al*., 2008). On other hand *CAV1* overexpression was recorded in patients with aggressive PCa recurrence (Karam *et al*., 2007). These conflicting data suggest that hypermethylation of *CAV1* may not be unfailing biomarker for detection of PCa.

The Table 1 provides overview of different genes which has been studied in respect to hypermethylation and PCa development.

Hypomethylation

DNA hypomethylation or demethylation can lead to structural and functional alternations of the genome. There are two forms of DNA hypomethylation-global (also known as genomic) and localized (also known as gene-specific) (Li *et al*., 2005).

Global hypomethylation is associated with reduced 5-methylcytosine content in the genome. For example, in case of some adenocarcinomas the genomic content of 5-methylcytosine is decreased by an average of 10%. Global hypomethylation was also reported in the pre-malignant or early stages of some neoplasia (Barciszewska *et al*., 2014; Ehrlich, 2009).

The pioneer work by (Bedford and van Helden, 1987) showed decreased content of 5-methylcytosine in patients with benign prostatic hyperplasia and metastatic tumors in comparison with non-metastatic prostate tumors. More recently, it was demonstrated that the repetitive DNA elements LINE-1 and SAT2 are heavily methylated in normal cells, but methylation levels of these elements in PCa cells are significantly lower (Cho *et al*., 2009).

Localized hypomethylation is related to a significant decrease in cytosine methylation level. It was demonstrated that some genes are substantially hypomethylated in cancer cells, but not in normal cells (Hammoud *et al*., 2013). Moreover, compared with normal cells, cancer cells possess two hypomethylated rasoncogenes, c-Ha-ras and c-Ki-ras and also c-jun and c-MYC protooncogenes (Li *et al*., 2005).

In PCa, PLAU gene responsible for expression urokinase plasminogen activator (protein involved in promotion the tumor invasion and metastasis) is hypomethylated (Banyard *et al*., 2014). Other hypomethylated genes in PCa are CAGE (a novel cancer/testis antigen gene) and heparanase. A methylation-specific PCR analysis revealed that hypomethylation of the CAGE promoter was present at frequency about 40% PCa, but not in normal prostate cells (Cho *et al*., 2003). It was also shown that heparanase (endo-β-D-glucuronidase) is overexpresed in PCa cells due to its substantial hypomethylation (Ogishima *et al*., 2005).

More recently, it was demonstrated that several other genes, including histone methyltransferase Enhancer of Zeste Homologue 2 (*EZH2*), Melanoma Antigen Gene Protein-A11 (MAGE-11), nsulin-like Growth Factor-2 *(IGF2)*, Cancer Testis Antigene (CTA) etc., are hypomethylated in PCa cells (Ribarska *et al*., 2012; Bhusari *et al*., 2011; Karpf *et al*., 2009; Yegnasubramanian *et al*. 2008).

Table 1. Overview of different genes studied in respect to hypermethylation and PCa development

Group	Examples
Tumor suppressor genes	Caveolin-1 Cyclin-dependent kinase inhibitors Death-associated protein kinase Fragile histidine triad Lipoprotein lipase Prostaglandin-endoperoxide synthase 2Tumor necrosis factor receptor superfamily
Genes involved in metabolism	Androgen receptor estrogen receptors Retinoic acid receptor β Glutathione S transferase P1 Cellular retinol-binding protein 1 Endothelin B receptor Gene Aldehyde dehydrogenase 1A2 and 1A3
Tumor cell invasion / metastasis	Adenomatous polyposis coli (APC) CD44 E-cadherin H-cadherin S100 calcium-binding protein A2 Tissue inhibitor of metalloproteinase-2 SRC family tyrosine kinase Neutral endopeptidase 24.11
DNA repair genes	Methylguanine-methyltransferase

Methylation of DNA as Prostate Cancer Biomarker

The "classical" diagnostics of PCa is based mainly on evaluation of PSA levels in serum and evaluation of needle biopsies. In some cases the results may be false negative. Recently, these standard approaches are supplemented by molecular diagnostics, including evaluation of DNA methylation, which has several advantages. First, it always occurs in defined DNA regions and can be detected using techniques of high sensitivity and resolution (e.g., methylation-specific PCR, bisulfite genomic sequencing). Second, hypermethylation occurs in every type of tumor. These characteristics make DNA methylation and superior biomarker for detection of cancer, including PCa (Li *et al*., 2005).

It was also shown that the utilization multigene methylation panel including GSTP1, APC, TIG1 and RARβ2 significantly increases the sensitivity of diagnostics of PCa in comparison with histological evaluation (Rosenbaum *et al*., 2005).

Especially hypermethylation in GSTP1 have significant importance for PCa diagnosis and prognosis. For example, Goessl *et al*. (2001) evaluated DNA hypermethylation in GSTP1 in urine after prostate massage and detected PCa with a specificity of 98% and overall sensitivity of 73%. Later, (Woodson *et al*., 2008) evaluated GSTP1 hypermethylation in urine collected after prostatic massage and in core needle biopsies from 100 men referred for diagnostic biopsy. Methylation of GSTP1 in urine specimens had 75% sensitivity and 98% specificity for prostate cancer. GSTP1 methylation in the biopsy had 88% specificity and 91% sensitivity. Interestingly they observed a higher frequency of GSTP1 methylation in the urine of men with stage III vs. II disease. In further study, Richiardi and co-authors used hypermethylation analysis not only in GSTP1 but also in APC and RUNX3. They showed that promoter methylation in APC is good marker of PCa progression and hyperethylation in RUNX3 is associated with prostate cancer mortality. On the contrary hypermethylation in GSTP1 was positively associated with Gleason score but did not predict mortality of patients (Richiardi *et al*., 2009).

It was also shown that by assessment of DNA hypermethylation it is also possible to distinguish primary PCa from benign prostate tissues, mainly when analyzed methylation pattern of multiple genes, such as GSTP1, RASSF1, APC, MDR1, PTGS2 and CD44 (Costa-Pinheiro *et al*., 2014; Kang *et al*., 2004).

Kollermann *et al*. (2003) demonstrated utilization of methylation in GSTP1 in detection of occult PCa cells in lymph nodes.

Yang *et al*. (2013) analyzed global DNA hypomethylation in human PCa and prostatic intraepithelial neoplasm tissues. They found that global DNA hypomethylation was low in most PCa compared with benign regions from the same individuals. Moreover, their results did not prove association of DNA hypomethylation in primary cancers with pathologic grade and clinical prognosis. On other hand, their results suggest that global DNA hypomethylation might play a pivotal role in the process of PCa initiation. These results were in stark contrast to finding obtained by (Yegnasubramanian *et al*., 2008) who provided evidence that DNA hypomethylation (mainly in LINE1 gene) occur not only in primary PCa but also later during or after metastatic dissemination.

For a more exact interpretation of the results there are available several scoring systems, such as Methylation Index (MI) which is defined as the ratio of methylated genes and the total number of analyzed genes (Tilandyová *et al*., 2010). The positive correlation betveen MI and Gleason score was demonstrated. It was also demonstrated that increased level of methylation in APC promoter seem to be good predictor of unfavorable prognosis. The higher level of methylation of GSTP1 and APC are in good correlation with high level of PSA. Moreover, hypermethylation of two genes, including GSTP1 and APC correlated with pT stage or Gleason score. The increased levels of their hypermethylation were also associated with progression of PCa and lead into biochemical recurrence after radical prostatectomy (Ellinger *et al*., 2008).

On the other hand, the utilization of hypermethylation and hypomethylation of selected genes

in clinical practice may be affected by biological variability (in selected population) as well as by preparation of material and methological approaches; but this may be overlapped by unification and standardization of selected methods.

Conclusion

A number of studies were established to check the capability of quantitative changes in DNA methylation, as measured either in PCa tissues or in serum and urine, to augment prediction of outcome for PCa patients. Although large trials are required prior clinical implementation, several of these studies suggest that hypermethylation and hypomethylation biomarkers may add value to existing models used for prediction of outcome in PCa.

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Author's Contributions

Lubos Danisovic: Wrote and revised manuscript.
Stanislav Ziaran: Wrote and revised manuscript.

Ethics

Both authors declare that there is no conflict of interest.

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