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Research paper

# *SFRP1* repression in prostate cancer is triggered by two different epigenetic mechanisms

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ABSTRACT

Worldwide, prostate cancer (PCa) is the second cause of death from malignant tumors among men. Establishment of aberrant epigenetic modifications, such as histone post-translational modifications (PTMs) and DNA methylation (DNAme) produce alterations of gene expression that are common in PCa. Genes of the SFRP family are tumor suppressor genes that are frequently silenced by DNA hypermethylation in many cancer types. The SFRP family is composed of 5 members (*SFRP1–5*) that modulate the WNT pathway, which is aberrantly activated in PCa. The expression of *SFRP* genes in PCa and their regulation by DNAme has been controversial. Our objective was to determine the gene expression pattern of the SFRP family in prostatic cell lines and fresh frozen tissues from normal prostates (NP), benign prostatic hyperplasia (BPH) and prostate cancer (PCa), by qRT-PCR, and their DNAme status by MSP and bisulfite sequencing. In prostatic cancer cell lines, the 5 SFRPs showed significantly decreased expression levels compared to a control normal prostatic cell line ($p < 0.0001$). In agreement, *SFRP1* and *SFRP5* genes showed decreased expression levels in CaP fresh frozen tissues compared to NP ($p < 0.01$), while a similar trend was observed for SFRP2. Conversely, increased levels of SFRP4 expression were found in PCa compared to BPH ($p < 0.01$). Moreover, *SFRP2*, *SFRP3*, and *SFRP5* showed DNA hypermethylation in PCa cell lines. Interestingly, we observed DNA hypermethylation at the promoter of SFRP1 in the PC3 cell line, but not in LNCaP. However, in the LNCaP cell line we found an aberrant gain of the repressive histone post-translational modification Histone H3 lysine 27 trimethylation (H3K27me3). In conclusion, decreased expression by DNA hypermethylation of *SFRP5* is a common feature of PCa, while decreased expression of SFRP1 can be due to DNA hypermethylation, but sometimes an aberrant gain of the histone mark H3K27me3 is observed instead.


## 1. Introduction

Among men, prostate cancer (PCa) is the second most frequently diagnosed type of tumor and the fifth cause of death from cancer worldwide (Ferlay et al., 2015). The major cause of mortality from PCa is bone metastases (Siegel et al., 2015). However, the most common pathology of the prostate in elderly men is Benign Prostatic Hyperplasia (BPH), which is an enlargement of the prostate without invasion or migration capabilities. The etiology and pathology of BPH and PCa are still controversial. Both conditions share common features, such as increased levels of serum prostatic specific antigen (PSA) and the involvement of the Androgen Receptor (AR) pathway (Alcaraz et al., 2009). In the case of PCa, there are many epigenetic marks that have been described as relevant for its development (Jeronimo et al., 2011; Chinaranagari et al., 2015). The most well characterized epigenetic mechanisms are histone post-translational modifications (PTMs) and DNA methylation (DNAme) (Rothbart and Strahl, 2014). Histone PTMs determine the attachment of DNA to the nucleosome and regulate chromatin condensation for different DNA-dependent processes, such as transcription or replication. Among the best characterized histone PTMs, are acetylation and methylation, the former is enriched at euchromatic regions, including active enhancers and promoters, while methylation can be associated to both euchromatic and heterochromatic states. For example, marks on active promoters are defined by Histone

*Abbreviations:* 5meC, 5-Methylcytosine; AR, Androgen Receptor; BPH, Benign prostatic hyperplasia; DNAme, DNA methylation; H3K27me3, Histone H3 Lysine 27 trimethylation; H3K4me3, Histone H3 Lysine 4 trimethylation; H3K9me3, Histone H3 Lysine 9 trimethylation; NP, Normal Prostate; PCa, Prostate Cancer; PSA, Prostatic specific antigen; PTM, Post-translational modifications; TURP, Transurethral resection of the prostate.

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H3 lysine 4 trimethylation (H3K4me3), on the other hand, Polycomb repressed regions are enriched with Histone H3 lysine 27 trimethylation (H3K27me3), while heterochromatin has high occupancy of Histone H3 lysine 9 trimethylation (H3K9me3) (Roadmap Epigenomics et al., 2015). Many alterations in the patterns of histone PTMs have been described for PCa, e.g. some studies suggest global loss of chromatin repressive histone mark H3K9me3 in PCa compared to non-malignant prostate tissue, and many important genes have been found to be silenced by aberrant occupancy of H3K27me3 at their promoters (Kondo et al., 2008; Ellinger et al., 2010). Understanding these epigenetic modifications has allowed the discovery of new potential tools to improve diagnosis, prognosis, and even finding new targetable epigenetic modulators (Verma, 2015).

The most studied epigenetic mechanism in cancer is DNAme, the covalent addition of a methyl group to cytosines to form 5-methylcytosine (5meC). This reaction generally occurs in a CpG dinucleotide context and it is catalyzed by DNA methyltransferases (DNMTs). DNAme at CpG islands (CGIs) at promoter regions is generally associated with repression of gene transcription (Bogdanovic and Veenstra, 2009). In PCa a global genome DNA hypomethylation is observed when compared to normal prostate cells, this happens concomitantly with hypermethylation at certain regions, such as promoters of tumor suppressor genes, leading to their silencing (Kim et al., 2011; Zelic et al., 2015). Genes that encode the family of secreted frizzled-related proteins (*SFRPs*) have been studied in many cancer types and it has been reported that they are silenced due to aberrant DNA hypermethylation within their promoter regions (Suzuki et al., 2008; Lin et al., 2009; Liang et al., 2015).

In humans, the SFRP family of proteins encompasses 5 members (*SFRP1–SFRP5*) that are inhibitors of the wingless-type (WNT) pathway and participate in processes such as cell proliferation, differentiation, cell anchorage, apoptosis, and cell cycle regulation. *SFRPs* are extracellular soluble proteins with a cysteine-rich domain (CRD), also present in frizzled (FZ) receptors and wingless-type (WNT) proteins (Bovolenta et al., 2008). The activation of the WNT/β-catenin pathway is mediated by extracellular soluble WNT proteins that bind to a surface receptor. FZ, and to one of the co-receptors of the low density-lipoprotein-receptor-related protein (LRP) family, LRP-5 or LRP-6. A signaling cascade is then initiated to activate disheveled (DVL) which releases β-catenin from a complex with axin, adenomatous polyposis coli (APC), and glycogen synthase kinase 3B (GSK3B). Once β-Catenin is released, it is translocated to the nucleus, where in cooperation with transcription factors of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family, it initiates transcription of genes, including CCND1, MYC, JUN, and VEGFR, among many others (Undi et al., 2016). *SFRPs* are able to sequester WNTs to inhibit their interaction with FZ receptors. Moreover, *SFRPs* can bind directly to FZ receptors to form inactivated complexes. The WNT pathway is aberrantly activated in PCa and *SFRP*s decreased expression could be participating in its deregulation (Kawano and Kypta, 2003; Kypta and Waxman, 2012; Jung et al., 2013).

There are many discrepancies regarding *SFRP* expression and DNAme in PCa. Some reports indicate decreased *SFRP* expression by DNAme hypermethylation and a tumor suppressor activity, while others report an increased expression and an oncogenic role without DNA hypermethylation (Joesting et al., 2005; Lodygin et al., 2005; Zi et al., 2005; Horvath et al., 2007; Costa et al., 2010; Kilinc et al., 2012; Perry et al., 2013; Zheng et al., 2015). The molecular mechanisms underlying these inconsistencies have not been unraveled. Therefore, the aim of this study was to determine gene expression pattern of the *SFRP* gene family and its regulation by DNAme at their promoters in cell lines and prostatic tissues, using as control normal prostate (NP) tissues. Herein, we demonstrated a decreased expression by DNA hypermethylation of *SFRP5* in PCa. Moreover, *SFRP4* increased expression in PCa can discriminate between PCa and BPH. Finally, we described new insights in the epigenetic regulation mechanisms of SFRP1 in PCa, which can be silenced either by DNA hypermethylation or H3K27me3 occupancy.

## 2. Material and methods

### 2.1. Cell culture

We used normal epithelial cells RWPE-1 and PrEC, normal prostate stromal cells PrSC and as prostate cancer models, LNCaP, PC3, DU145 and 22Rv1. Human prostatic cell lines, RWPE-1, LNCaP, PC3, VCaP, DU145, and 22Rv1 were purchased from American Type culture collection (ATCC, Manassas, VA, USA). PrEC and PrSC primary cell cultures were obtained from LONZA (LONZA, Basel, Switzerland). All prostatic

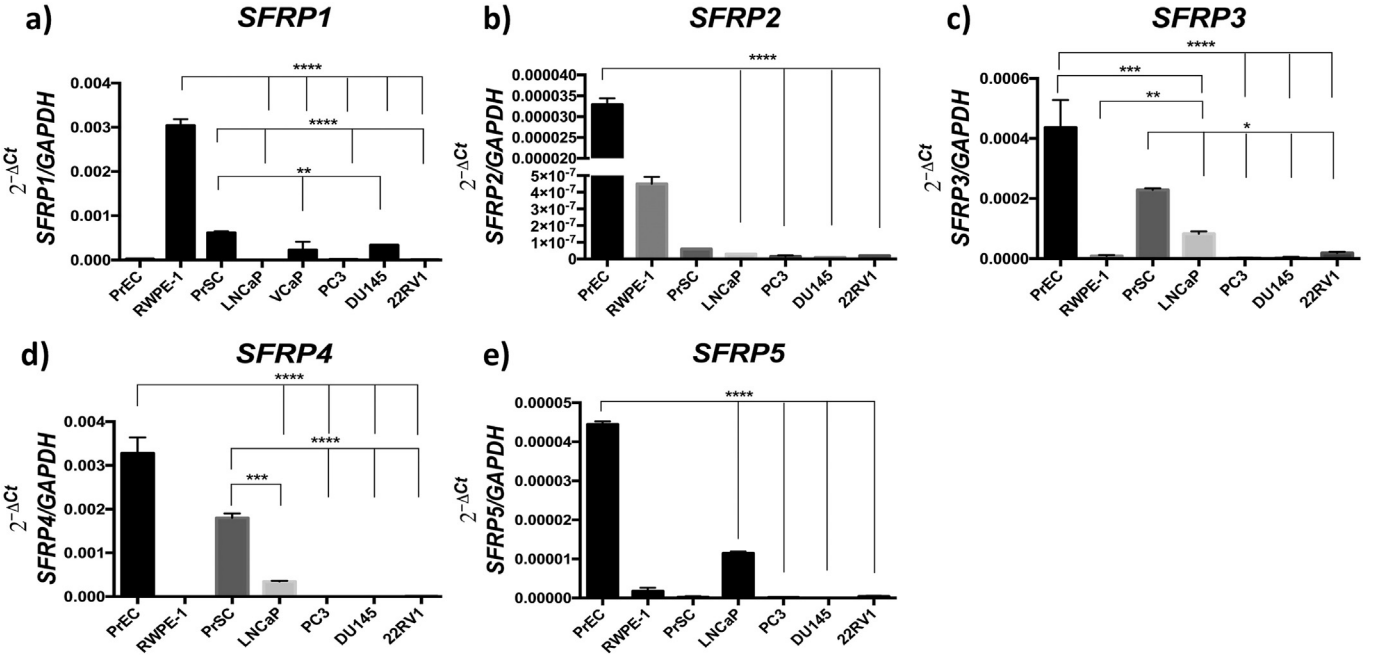


**Fig. 1.** *SFRP* family mRNA expression in prostate cell lines. qRT-PCR was conducted to measure the mRNA levels of *SFRP*s in prostate cell lines. Normalization was done against *GAPDH* Data represent $2^{-\Delta Ct}$ means ± SE of three independent experiments. All PCa cell lines were compared to PrEC, RWPE-1 and PrSC (****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$). (a) SFRP1 mRNA expression. (b) SFRP2 mRNA expression. (c) SFRP3 mRNA expression. (d) SFRP4 mRNA expression. (e) SFRP5 mRNA expression.

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