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MicroRNA Regulating Glutathione S-Transferase P1 in Prostate Cancer

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Abstract Glutathione S-transferase P1 (GSTP1), an enzyme involved in detoxification process, is frequently inactivated in prostate cancer due to epigenetic modifications. Through in silico analysis, we identified a subset of microRNAs (miRNAs) that are putative targets in regulating GSTP1. miRNAs are small endogenous non-coding RNA that are critical regulators of various physiologic and pathologic processes, and their level of expression may play a precise role in the early diagnosis and prognosis of cancer. These small molecules have been detected in a wide variety of human biological specimens including blood, serum, urine, ejaculate, and tissues, which could be utilized as clinically useful biomarker in early detection and prognosis of prostate cancer. The chapter summarizes the current knowledge about miRNA involved in GSTP1 regulation in prostate cancer and their potential as useful biomarkers of the disease for early detection and prognosis, along with challenges and limitations in this development.

Keywords miRNA · GSTP1 · Prostate cancer · Epigenetics · Oxidative DNA damage

Abbreviations

3′UTR	3′ Untranslated region
8-OHdG	8-Oxo-2′-deoxyguanosine
CDC42	Cell division cycle 42
CDK	Cyclin-dependent kinase
FOXO	Forkhead box O
GSTP1	Glutathione S-transferase P1
GSTs	Glutathione S-transferases
HDAC	Histone deacetylases
HGPIN	High-grade intraepithelial neoplasia
miRNA	MicroRNA
mTOR	Mammalian target of rapamycin
PIA	Proliferative inflammatory atrophy
PIN	Prostatic intraepithelial neoplasia
PSA	Prostate-specific antigen
PTPRN	Protein tyrosine phosphatase receptor type N
RBPs	RNA-binding proteins
RHOA	Ras homolog gene family member A

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Introduction

Prostate cancer remains the most common form of cancer in males in the USA [1]. According to an estimate by the American Cancer Society, in 2014 approximately 233,000 new cases of prostate cancer will be diagnosed and about 29,480 men will die from this disease [1, 2]. Early diagnosis of tumor

and timely detection of prostate cancer progression following either surgery or radiation therapy are critical for its effective and beneficial clinical outcome. The widespread use of serum-based total prostate-specific antigen (PSA) has led to detection of prostate cancer at a potentially curable stage; however, its use as a screening tool remains controversial due to the absence of a true tPSA cutoff point for identifying prostate cancer risk [3, 4]. First, approximately one third of prostate cancers detected at a PSA level at or above 4 ng/mL have already spread to the prostate capsule or beyond, and approximately 15 % of men with a PSA level <4 ng/mL have prostate cancer that is detectable by needle biopsy [5–7]. Specifically, the Prostate Cancer Prevention Trial reported that in men who underwent an empiric biopsy at PSA levels of ≤0.5 ng/mL, 0.6–1.0 ng/mL, 1.1–2.0 ng/mL, 2.1–3.0 ng/mL, and 3.1–4.0 ng/mL, the prostate cancer detection rates were 6.6, 10.1, 17.0, 23.9, and 26.9 %, respectively [8]. Using current recommended guidelines to determine the need for confirmatory biopsy, false positive rates of 55–75 % and false negative rates of at least 15 % have been reported which limit the sensitivity and specificity of serum total PSA test as an effective population-based cancer detection tool [9, 10]. More recent concepts to improve specificity include use of age-adjusted PSA, PSA velocity, volume-adjusted PSA, and percent-free PSA concentration [11, 12]. However, the diagnostic accuracy and predictive values for tests using quantitative serum PSA assays remain controversial. There is a need of more sensitive and specific biomarker to detect prostate cancer.

Glutathione S-Transferases

Glutathione S-transferases (GSTs) comprise of a multi-gene enzymes family of phase II detoxifying enzymes of the xenobiotic metabolism [13]. The members of this family of dimeric enzymes are identified on the basis of their substrate specificity and amino acid sequences. GSTs catalyze the reactions in which reduced glutathione is conjugated to toxic oxidizing compounds. These compounds are produced either due to normal cellular activity of the cell or due to exposure of cells to xenobiotics and environmental pollutants such as carcinogens, pesticides, drugs, and to endogenous molecules [14]. This conversion significantly detoxifies them by reducing their ability to react to cellular macromolecules. GSTs are ubiquitously present in every cell and in every living species examined, including both eukaryotes and in prokaryotes. Though most of these enzymes are composed of cytosolic proteins, a small family of microsomal and mitochondrial (kappa) GSTs is also characterized. GSTs are considered as cell housekeepers due to their ability to detoxify both endogenous as well as exogenous cell substances. In some mammalian and rodent organs, cytosolic GSTs can constitute as high as 4–10 % of cytosolic proteins. Soluble cytosolic GSTs exist as dimeric protein with an active site composed of two distinct functional groups including (i) hydrophilic, catalytically independent active G-site which binds to glutathione and (ii) physiological substrate of GSTs and an adjacent H-site which provides a hydrophobic environment for binding of electrophilic substrates with diverse structures [15, 16]. While G-site, which is in the amino terminal domain, is highly conserved among GSTs due to its high specificity for glutathione (GSH), the H-site, which is in the carboxy-terminal domain, can be very divergent among GSTs, exhibiting broad and variable specificity to substrate binding [17]. GSTs catalyze the conjugation of reduced GSH via a sulfhydryl group to electrophilic centers on substrates with variable binding specificity. This activity detoxifies several reactive, endogenously produced molecules such as α, β-unsaturated keto prostaglandins (i.e., PGA2), endogenous fatty acid oxidation products including 4-hydroxy-2-nonenal, peroxidized lipids, and xenobiotics [18].

The mammalian GST superfamily consists of seven classes of cytosolic GSTs which possess amino acid sequence similarity, substrate specificity, and immunological cross-reactivity [16, 17]. They are named as GSTα (alpha)/GSTA, GSTμ (mu)/GSTM, GSTπ (pi)/GSTP, GSTσ (sigma)/GSTS, GSTθ (theta)/GSTT, GSTω (omega)/GSTO, and GSTζ (zeta)/GSTZ [18–20]. As functional GST enzymes are dimeric and the GSTα (GSTA) and GSTμ (GSTM) can form heterodimers in addition to homodimers, the number of isoenzymes in each class is large. One of the highly conserved classes of cytoplasmic GST is glutathione S-transferase pi (GSTP1) that was found to be the predominant isoenzyme. It is mapped to chromosome 11q13, and the genes of this class are about 3 kb long and contained seven exons. GSTP1 protects cells from cytotoxic and carcinogenic agents [21]. It is expressed at variable levels in different cell types in normal tissues, and its altered activity and expression has been found to play an important role in determining susceptibility to different types of cancers, inflammatory disorders, asthma, and neurodegenerative disorders [22–24]. The role of pi class GST (GSTP1) is of particular interest in cancer biology. In humans, early loss of GSTP1 has been associated with cancer initiation and progression and suggested to possess tumor suppressor functions [25–27]. For example, $GSTP^{-/-}$ mice display a strong tendency to develop skin papillomas and lung cancer following carcinogen exposure, and loss of GSTP markedly enhances colon tumorigenesis in Apc(Min) mice [28–30]. On the contrary, the over-expression of pi-class GST has been associated with tumor progression and drug resistance [31, 32]. GSTP1 over-expression has been reported in many human tumors and has been shown to be correlated with advanced stage, disease aggressiveness, drug resistance, and poor survival [32]. A vast majority of human tumor cell lines over-expresses GSTP1, including cells selected in vitro for resistance to agents used for chemotherapy. In fact, in 58 of

the 60 human tumor cell lines used in the Drug Screen Program of the National Cancer Institute, GSTP1 was found to be the predominant isoenzyme (as high as 2.7 % of the total cytosolic protein). A significant quantitative correlation among enzyme activity, protein, and messenger RNA (mRNA) was shown particularly in those cell lines selected for resistance to alkylating agents [33]. Such comparable correlation was much less apparent for over-expression of GSTA and GSTPM.

Glutathione S-Transferase pi and Prostate Cancer

One of the most common epigenetic alterations described in human prostate cancer is the loss of expression of the GSTP1 which occurs in vast majority (>90 %) of prostate tumors regardless of grade or stage [34]. Studies in human prostate tumor specimens and cancer cell lines have shown that the GSTP1 gene is silenced due to epigenetic modifications [35]. Importantly, the loss of GSTP1 function appears to be the characteristic of prostatic intraepithelial neoplasia (PIN) and proliferative inflammatory atrophy (PIA) lesions, throughout to represent prostate cancer precursors [36]. It has been proposed that GSTP1 is a caretaker gene, protecting cells against genomic damage mediated by oxidants and electrophiles from inflammation or dietary exposures [37]. Reports suggest that loss of GSTP1 shifts the prooxidant–antioxidant balance toward an oxidative state, resulting in increased inflammation and oxidative stress to prostate epithelial cells [38••]. Studies have suggested age-related structural changes in the DNA of prostate tissue which is likely a result of oxidative damage induced by hydroxyl radicals [39]. Age-related oxidative DNA damage and increased accumulation of 8-oxo-2′-deoxyguanosine (8-OHdG) have been shown to be more pronounced in prostate neoplasms than in benign prostate tissue [40]. We have recently demonstrated that chronic intraprostatic inflammation causes premalignant and malignant changes in prostatic epithelium which may be due at least in part to accumulation of oxidative DNA products as a result of loss of GSTP1 expression in prostate epithelial cells [38••, 41]. Since GSTP1 is epigenetically silenced in early-stage prostate cancer, the elements of epigenetic GSTP1 regulation could serve as a better biomarker for detection and prognosis of prostate cancer.

Epigenetics and Gene Regulation

Gene expression is intricately regulated through the epigenetic modifications such as DNA methylation, post-translational modifications of histone proteins, and transcriptional regulation of gene expression by non-coding regulatory microRNA [42, 43]. Numerous studies have demonstrated that the regulatory sequences near the GSTP1 gene are commonly inactivated by DNA hypermethylation during the early stages of prostate carcinogenesis [44, 45]. Extensive methylation of deoxycytidine nucleotides distributed throughout the 5′CpG island region of GSTP1 is not detected in benign prostate tissue but has been detected in high-grade intraepithelial neoplasia (HGPIN) and prostate adenocarcinoma in the tissue and fluids including plasma, serum prostatic ejaculates, and urine specimens [46–48].

Histone modification is closely associated with DNA methylation in prostate cancer [49]. Studies have demonstrated that class I histone deacetylases (HDACs) are frequently over-expressed in prostate cancer [50]. Studies demonstrate that HDAC1 contributes to aggressive tumor behavior and poor prognosis, whereas HDAC2 expression is associated with shortened relapse-free survival time in prostate cancer patients [51]. HDAC1–3 are highly expressed in prostate cancer and in corresponding HGPIN lesions coincide with the loss of GSTP1 expression in tumor specimens [51, 52]. Li et al. demonstrated that the inhibition of HDAC1 by maspin, a tumor-suppressing serpin, increases GSTP1 expression in human prostate cancer cells, endorsing that HDAC1 plays a critical role in maspin-mediated GSTP1 re-expression [53].

Non-coding RNAs have emerged as a new class of key regulators of genes [54, 55]. MicroRNAs (miRNAs) are short (~20–24 nucleotides) non-coding RNAs that regulate gene expression mostly by facilitating the cleavage of target mRNA in plants [56]. Interestingly, miRNAs affect the expression of their target gene mostly by translational repression in animals. miRNAs target mRNAs by imperfect complimentary base-pairing to the 3′ untranslated region (3′UTR) to downregulate target's protein synthesis either by deadenylation of the targeted message or by repressing the translation at the actively translating ribosomes [57, 58]. Currently, miRBase has a compilation of 2,588 mature human miRNAs from human genome assembly (GRCh 38) to the GenBank [59]. These many miRNAs are predicted to target >45,000 sites that account for >60 % of human genes.

Numerous studies in various organismal systems show that miRNAs play important roles in cellular processes such as development, differentiation, proliferation, apoptosis, and metabolism [60]. Furthermore, strong evidence demonstrates that aberration in miRNAs' expression and their targeting activities have been implicated in human diseases, including cancers [61]. The aberrant and deregulated expression of miRNAs has been identified in the stages of carcinogenesis, development of resistance to therapeutics, and in metastasis [61–63]. In addition, the differential expression of miRNAs appears to play a significant role in the prognosis of various cancers including prostate cancer [61, 64, 65]. Studies of miRNAs show that over 50 % of the miRNA genes are coded

in the human genome at or near the sites of frequent deletion and amplification as well as at the CpG island methylation [66]. It is also becoming increasingly clear that miRNAs are transcriptionally silenced in various human cancers by epigenetic mechanisms including hypermethylation. These features of miRNA suggesting that deregulated expression of miRNAs play an important role in tumorigenesis in nearly all types of cancers and may be linked with specific clinical-pathological parameters, risk, aggressiveness, staging, and disease outcome [67, 68, 69••].

Potential Regulation of GSTP1 by miRNAs

Several computational and experimental approaches have been used to identify miRNAs and their targeting genes [70••, 71]. For this study we used computational approaches to identify miRNAs potentially targeting and regulating GSTP1 expression (Table 1). These miRNAs include miR-133a/b, miR-144/144*, miR-153-1/2, and miR-590-3p/5p. In this chapter a short description of these miRNAs and their involvement in cancer and GSTP1 regulation is highlighted.

miR-133a/b

A search for TargetScan Human database identified miR-133a/b as a candidate miRNA regulator of GSTP1 by potentially targeting nucleotides 2736–2750 in the 3′UTR (Fig. 1). The miR-133a1/a2-3p and miR-133b are transcribed from chromosome numbers 18, 20, and 6, respectively [72]. miR-133a and miR-133b differ only in one base at the 3′-end of the molecule (G→A). This position is furthest away from the seed region which is essential for miR:target interaction and its resultant effect on target mRNA translational repression. Therefore, it is likely that miR-133a and miR-133b will perform similar, if not, identical cellular function by regulating the expression of a common pool of target genes [73]. Interestingly, GENECODIS analysis revealed that miR-133a and miR-133b might supplement each other in many cancer pathways [74]. GSTP1 displays one single miR-133a and miR-133b binding site in its 3′UTR region (Fig. 1). The downregulation of miR133a/b has been reported in various human cancers including head and neck squamous cell carcinoma, esophageal squamous cell carcinoma, non-small cell lung cancer, and bladder, cervical, gastric, and colorectal cancers [75–79]. The over-expression of miR-133a/b has been shown to inhibit tumor cell proliferation and induce apoptosis in various human cancer cell types [78, 80]. Specifically, miR-133b has been found to be involved in the regulation of cell death through death receptor-mediated apoptosis in human prostate cancer PC-3 cells [81]. It is a target of androgen receptor (AR) and required for androgen's mediated stimulation in LNCaP cells and is also known to regulate CDC2L5, PTPRK, RB1CC1, and CPNE3 in prostate cancer cell lines [82]. Other putative targets of miR-133a/b are the Ras homolog gene family member A (RHOA), cell division cycle 42 (CDC42), FSCN1, LASP1, and c-MET oncogenic genes [83]. The association of miR-133b with prostate cancer progression and its potential as a diagnostic marker are also demonstrated by its downregulation observed in prostate secretion samples from patients; it has greater power (AUC 0.950) than PSA (AUC 0.463) to distinguish prostate cancer from benign prostate hyperplasia [84, 85].

miR-144/miR-144*

The gene encoding miR-144 is located on chromosome 17 and has a passenger strand (miR-144*). In silico analysis of miRNA-target mRNA prediction algorithm revealed single miR-144 and miR-144* binding sites in the GSTP1 3′UTR region with Watson-Crick match at miRNA positions 2736–2757 and 2759–2771, respectively. These sites raise the possibility that miR-144/144* are involved in the regulation of GSTP1 expression and possibly in detoxification aspect of the gene function. miR-144 is dysregulated and involved in the many human tumors including osteosarcoma, mesothelioma, and gastric and nasopharyngeal carcinomas [86–89]. Deregulation of miR-144 in colorectal cancer cells has been shown to activate mammalian target of rapamycin (mTOR) signaling, and its downregulation was associated with poor prognosis [90]. The miR-144 expression level has been shown to be significantly decreased in bladder cancer, and its downregulation increased bladder cancer cell proliferation by targeting histone-lysine *N*-methyltransferase EZH2 [91]. Similarly, gastric cancer stage IV patients also exhibit diminished expression of miR-144 where it promotes upregulation of ZFX proteins and subsequent cancer progression [88]. Notably, it has been demonstrated that the expression of miR-144 was reduced in thyroid cancer [92]. Recently, a comprehensive meta-analysis of miRNA expression microarray data sets revealed that miR-144 was downregulated in hepatocellular carcinoma, lung cancer, and prostate cancer [93, 94, 95••]. However, no information about the function or molecular mechanism(s) of miR-144 regulating GSTP1 has been reported. miR-144 was found to be over-expressed in high Gleason score (8 and 9) prostate tissue samples [94, 95••]. Moreover, miR-144* was significantly upregulated when compared to miR-144 in both metastatic and non-metastatic tumor xenograft models [95••].

miR153-1/2

miR-153-1 and miR-153-2 are encoded in the intron of protein tyrosine phosphatase, receptor type N (PTPRN) gene on the chromosome 2 [96]. On other hand, miR-153-2 was found to be encoded in the intron of PTPRN 2 on chromosome 7 where

Table 1 miRNAs' potential to target GSTP1

GSTP1 – 3′ UTR is 75 bases

```
GSTP1  5'      G               C   3'
                GGUUG GGGGGACU
                ||||| ||||||||
                CCAAC UCCCCUGG
miRNA  3' GUCGA     U        UUU 5' Another Site of miR-133-a/b (2-16)


GSTP1 5' G   UG GGGGAC    A  G G  3'
           GGU  G      UCUG GC G
           |||  |      |||| || |
           UCA  U      AGAU UG C
miRNA  3'     UG AGU       A  A AU 5' miR-144 (2-23)


GSTP1 5' U   A  GG       GCA      G 3'
           CUG GC       GAG   GAGUUU
           ||| ||       |||   ||||||
           GAC UG       CUU   UUCGAG
miRNA  3'     G  AAAAUA   A          5' miR-590-5P (14-34)


GSTP1 5' G   CUG   GGGAG          U 3'
           ACU   AGC     GCA  GAGUU
           |||   |||     |||  |||||
           UGA   UCG     UGU  UUUAA
miRNA  3'           AAUA    AU     U 5' miR-590-3P (11-34)


GSTP1 5'   G    A  U     CCU   U 3'
             GCAG GU    UG   UCC
             |||| |||   ||   |||
             UGUC UA    AC   AGG
miRNA  3' GAA    A  UACU  UAU     5' miR-144* (25-37)


GSTP1  5'      G   GGGG    CUGA    G 3'
                 UUG    GACU    GCGG
                 |||    ||||    ||||
                 AAC    CUGA    CGUU
miRNA  3' CUAGUGAA   A       UA        5' miR-153-1 and 2 (3-22)
```

the targeting site of miR-153-1/2 spans around nucleotides 2737–2756 in the GSTP1 3′UTR (Fig. 1). In general, miR-153 has been observed to be over-expressed in tumors compared to normal tissue at higher levels in metastatic compared with non-metastatic tumors [97]. Studies conducted using various human prostate cancer cell lines demonstrate that miR-153 represses phosphatase and tensin homolog (PTEN) expression to activate AKT kinase and downregulate the transcriptional activity of Forkhead box O (FOXO)1, leading to the upregulation of the G1/S transitional promoter cyclin D1 and downregulation of the cyclin-dependent kinase (CDK) inhibitor p21 [98]. In contrast, miR-153 levels are shown to be reduced in human glioblastoma multiforme [99]. miR-153 induces apoptosis in these tumors by targeting Bcl-2 and Mcl-1, suggesting that miR-153 functions as a tumor suppressor [100]. Studies have revealed a tendency toward downregulation of miR-153 in relation to lymph node metastasis in ovarian epithelial tumors [101] and downregulation of miR-153 in high-risk medulloblastomas [99]. Therefore, miR-153 may function as a tumor suppressor or an oncogene depending on the tissue. However, no information about the function or molecular mechanism of miR-153-1/2 regulating GSTP1 has been reported.

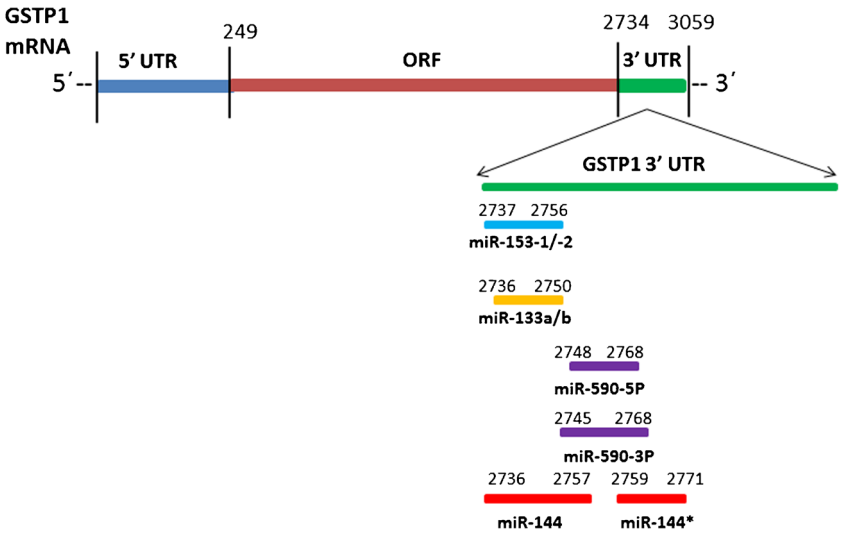

Fig. 1 Schematic representation of miR-133-a/b, miR-153-1/2, miR-590-3P/5P, and miR-144/144* target sites on GSTP1 3′ UTR

miR-590-3p/5p

microRNA target databases, such as TargetScan and miRanda, predicted that miR-590-3p/5p could regulate GSTP1 mRNA, and/or protein expression miR-590-3p forms an 7mer at positions 57–63 of GSTP1 3′UTR. An examination of the mRNA sequence of GSTP1 revealed that miR-590-3p/5p potential target sites are found at nucleotides 2748–2748 in the 3′UTR region (Fig. 1). miR-590-3p/5p has been shown to be upregulated in some human cancers including hepatocellular carcinoma, clear cell renal cell carcinoma, myeloid leukemia, and cervical cancer [102–105]. The upregulation of miR-590-5p has been shown to promote proliferation and invasion of clear cell renal cell carcinoma cells by downregulation of p21 (Waf1/Cip1) expression [103]. It is also shown to be differentially expressed in castrate-resistant prostate cancer compared to benign prostatic hyperplasia [106]. TargetScan predicted PTEN as the potential target genes of miR-590-3p/5p and was found to activate PI3K-AKT signaling pathway by downregulating PTEN to promote AKT1-S473 phosphorylation [107], which may play an important role in the regulation of GSTP1 in prostate cancer.

Conclusion, Limitations, and Future Direction

The measurement of serum PSA levels do not adequately detect prostate cancer or predict prognosis after definitive therapy. Therefore, there is a significant challenge to develop new non-invasive biomarker identification strategies to precisely detect and predict progression of prostate cancer. miRNAs are important regulators of oncogene and tumor suppressor genes that intercept various signaling pathways and pathological processes associated with tumorigenesis. Numerous publications have reported the potential of miRNA as a class of novel biomarkers in diagnosis and disease prognosis [63–68, 69••]. Our in silico analysis suggest that miRNAs can exert their functions by potentially regulating GSTP1 expression and play an important role in prostate carcinogenesis (Table 1; Fig. 1). With the identification of listed putative miRNAs that regulate GSTP1, a more detailed regulatory role may be deciphered which might be applicable in the early detection and prognosis of prostate cancer.

However, some limitations remain in their development and replacement to conventional biomarkers. This includes lack of established endogenous miRNA control to normalize for miRNA levels in body fluids. In this regard, U6 small nuclear RNA is frequently utilized to normalize miRNA levels; however, its unstable nature and degradation in serum do not qualify it as an ideal standard control. Another limitation is the release of miRNA into body fluids, and its functional role and consequences remain unknown and/or limited inconsistencies in the analysis of the function of miRNA in prostate cancers. Furthermore, the exact cause-effect mechanism(s) has not been established for many miRNAs in prostate cancer despite significant efforts into such research. Therefore, further studies are needed to understand the role and consequences of miRNA in prostate cancer through novel high-throughput techniques. Another caveat to consider is that the post-transcriptional gene regulation of miRNA as well as its target mRNA is further modulated by RNA-binding proteins (RBPs). Numerous RBP have been characterized recently and many play a role in the both miRNA and mRNA stabilities.

In conclusion, technical advancement in the detection of miRNAs regulating GSTP1 expression and function may have great promise as molecular biomarkers for prostate cancer. Further development and application of these miRNA-regulating GSTP1 assays to clinical specimens including blood, urine, ejaculate, and prostate biopsy for cancer

screening and early detection may have great promise as candidate clinical tests. A new series of studies critically assessing the predictive values of miRNAs in prostate cancer are needed.

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Compliance with Ethics Guidelines

Conflict of Interest Savita Singh, Girish C Shukla, and Sanjay Gupta declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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