Both AA genotype of rs165599 and Val/Val genotype of rs4680 in COMT are associated with an increased risk of recurrence of prostate cancer

**Type**
Research paper

**Keywords**
prostate cancer, miRNA, COMT, rs165599, rs4680, Recurrence in prostate cancer

**Abstract**

**Introduction**
COMT expression was reported to be associated with the recurrence of prostate cancer. Furthermore, it has been shown that rs165599 SNP in the 3'UTR of COMT affects the interaction between miR-138 and COMT, while another polymorphism (rs4680) located in the gene of COMT also affects its expression. Here, we explored the combined effect of rs165599 and rs4680.

**Material and methods**
Kaplan-Meier analysis was performed to analyze the PSA-progression-free survival of prostate cancer patients carrying different genotypes of rs165599 and rs4680 SNPs. IHC and Western blot were used to evaluate the expression of COMT. Real-time PCR was carried out to examine the expression of miR-138 and COMT mRNA. Luciferase assay was performed to explore the inhibitory role of miR-138 in the expression of COMT carrying different genotypes.

**Results**
The PSA-progression-free survival was significantly increased in prostate cancer patients carrying the rs165599-AA + rs4680-Val/Val genotype. In concordance with this, the expression of COMT was remarkably elevated in the tissue samples collected from prostate cancer patients carrying the rs165599-AA + rs4680-Val/Val genotype. Luciferase assay demonstrated that COMT-G at rs165599 was effectively suppressed by miR-138 through binding to the 3' UTR of COMT-G. Furthermore, COMT expression was notably suppressed by miR-138 precursors in primary prostate cancer cells carrying GG genotype at rs165599.

**Conclusions**
The findings of this study demonstrated that the carriers of AA genotype of rs165599 and Val/Val genotype of rs4680 showed an increased risk of prostate cancer recurrence, and these two polymorphisms may jointly function as a novel biomarker for the prognosis of prostate cancer recurrence.
Both AA genotype of rs165599 and Val/Val genotype of rs4680 in COMT are associated with an increased risk of recurrence of prostate cancer

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Abstract

Background COMT expression was reported to be associated with the recurrence of prostate cancer. Furthermore, it has been shown that rs165599 SNP in the 3’UTR of COMT affects the interaction between miR-138 and COMT, while another polymorphism (rs4680) located in the gene of COMT also affects its expression. Here, we explored the combined effect of rs165599 and rs4680 on the recurrence of prostate cancer. Methods Kaplan-Meier analysis was performed to analyze the PSA-progression-free survival of prostate cancer patients carrying different genotypes of rs165599 and rs4680 SNPs. IHC and Western blot were used to evaluate the expression of COMT. Real-time PCR was carried out to examine the expression of miR-138 and COMT mRNA. Luciferase assay was performed to explore the inhibitory role of miR-138 in
the expression of COMT carrying different genotypes. **Results** The PSA-progression-free survival was significantly increased in prostate cancer patients carrying the rs165599-AA + rs4680-Val/Val genotype. In concordance with this, the expression of COMT was remarkably elevated in the tissue samples collected from prostate cancer patients carrying the rs165599-AA + rs4680-Val/Val genotype. Luciferase assay demonstrated that COMT-G at rs165599 was effectively suppressed by miR-138 through binding to the 3’ UTR of COMT-G. Furthermore, COMT expression was notably suppressed by miR-138 precursors in primary prostate cancer cells carrying GG genotype at rs165599. **Conclusion** The findings of this study demonstrated that the carriers of AA genotype of rs165599 and Val/Val genotype of rs4680 showed an increased risk of prostate cancer recurrence, and these two polymorphisms may jointly function as a novel biomarker for the prognosis of prostate cancer recurrence.

**Running Title:** Two SNPs of the Catechol-O-Methyltransferase gene reduce the risk of prostate cancer recurrence

**Key words:** Prostate Cancer, COMT, miRNA, rs165599, rs4680, Recurrence in prostate cancer.

**Introduction**

Pancreatic cancer (PC) is the 7th leading contributor for cancer fatality in China as well as the 4th leading contributor for cancer fatality in the USA [1, 2]. In the USA alone, greater than 250,000 PC patients die annually. As a result of the problem that little improvement has actually been made in the diagnosis as well as surgical procedures in the treatment of PC in recent years, the prognosis of PC patients is worst among all kinds of cancers [3, 4]. It is actually of significant significance to find unique approaches to address the conditions of individual PC patients.

The gene of catechol-O-methyltransferase (COMT) is found on chromosome 22q11.2 [5]. There are actually 2 forms of COMT proteins translated from COMT, one is soluble and the other is bound on cell membrane [6]. COMT is an enzyme associated with the inactivation of catecholamines as well as catechol estrogens, which have actually been demonstrated to possess carcinogenetic capacities along with the capacity of DNA damage and induction of PC in rats [7]. Meanwhile, COMT can catalyze the O-methylation of catechol estrogen 2-
hydroxyestradiol to produce 2-methoxyestradiol, which has anti-angiogenic as well as anti-proliferative properties [8]. A typical polymorphism in COMT is a G to A mutation that takes place at codon 158, which is also called Val158 Met or rs4680. The replacement of G for A causes the substitute of valine by methionine as well as 3 to 4 fold reduction in COMT activity. The Val/Val phenotype (or G/G genotype) of COMT has actually been shown to be more active and stable as compared with the Met/Met phenotype (or A/A genotype). Many research performed genome wide screening without pinpointing the genres of COMT with considerable prospect for suicidal behaviors, except a few exceptions [9-12].

MicroRNA (miRNA) makes up a family of non-coding RNAs that are less than 23 nucleotides in size and can control the endogenous expression of genes through target mRNA degradation and translational inhibition [13]. In the last few years, miRNAs have actually been demonstrated to regulate several biological activities, such as cells growth, neuron distinction as well as apoptosis [14-16]. Significantly, expanding evidence signifies that miRNA expression might be related to cancer cell growth in PC patients [14, 17, 18]. Amongst many miRNAs, miR-138 was lately shown as a significant tumor suppressor in various cancers, including osteosarcoma, cervical cancer, ovarian cancer, non-small cell lung cancer, as well as gallbladder cancer [19-21]. Primarily, miR-138 prevents the spread of cancer cells and causes their apoptosis while boosting their chemo sensitivity [22-24]. It was actually discovered that down-regulation of miR-138-5p was linked to autophagy in PC cells. Nevertheless, elevated miR-138-5p expression prevented the activation of autophagy dependent on the SIRT1/FoxO1/Rab7 signaling. Also, SIRT1 might repress the growth of PC cells, making miR-138-5p a possible target in PC treatment [25].

It has been shown that COMT expression is associated with prostate cancer recurrence [26]. Furthermore, it has been shown that rs165599 in the 3’ untranslated region (3’UTR) of COMT affects the interaction between miR-138 and COMT, and another polymorphism (rs4680) located in the gene of COMT also affects the expression level of COMT [27, 28, 29]. Moreover, polymorphisms located in estrogen metabolism-related genes, such as the AA genotype and the A allele of rs4680 in COMT, have been proved to be inversely associated with the risk of prostate cancer in Afro-Caribbean and native African men [31]. In this study, we hypothesized
that genotype difference of rs165599 and rs4680 are associated with the risk of prostate cancer recurrence and PSA progression free survival period. To study the effect of genotype difference upon prostate cancer recurrence, we studied the combined effect of both rs165599 and rs4680 single nucleotide polymorphisms (SNPs) on the recurrence of prostate cancer.

**Materials as well as Methods**

**Human subjects as well as sample collection**

In this work, to study the combined effect of rs165599 as well as rs4680 SNPs on prostate cancer, we recruited 224 prostate cancer patients and then partitioned them into four groups according to their genotypes of rs165599 as well as rs4680 SNPs, i.e., 1. rs165599- AA + rs4680- Val/Val group (N=62); 2. rs165599- AA + rs4680- (Val/Met + Met/Met) group (N=55); 3. rs165599- (GG+GA) + rs4680-Val/Val group (N=58), and 4. rs165599-(GG+GA) + rs4680-(Val/Met + Met/Met) group (N=49). The information of all participants, including their age, tumor status as well as Gleason sum score, was collected and compared among the four groups. Then, one-way analysis of variance (ANOVA) was utilized to perform the statistical comparison among the above four groups. In the next step, tissue as well as peripheral blood samples were collected from all subjects for the analyses described below. Institutional ethical committee has approved the protocol of this study.

**Genotyping by direct sequencing**

Collected tissue as well as peripheral blood samples were treated with a GoldMag Mini Purification assay kit (GoldMag Co. Ltd., Xi'an, China) in accordance with the specific assay protocol provided by the assay kit manufacturer to extract Genomic DNA content. Then, the quality as well as concentration of each isolated DNA sample were assessed by using a Nanodrop 3000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) in accordance with the specific operating protocol provided by the instrument manufacturer. In the next step, the genotypes of rs165599 as well as rs4680 SNPs were measured by using a multiplexed SNP Mass EXTEND assay designed with the Agena Mass ARRAY version 3.0 software (Agena Bioscience, San Diego, CA) in accordance with the specific instructions provided by the software vendor. The multiplexed SNP Mass EXTEND assay was carried out by making use of an Agena
Mass ARRAY RS1000 system in accordance with the specific operating protocol provided by the
instrument manufacturer.

**RNA isolation and real-time PCR**

In order to explore the effect of rs165599 and rs4680 polymorphisms on the expression of their host gene COMT, we carried out qPCR and ELISA to analyze the expression of COMT in tissue samples of prostate cancer as well as peripheral blood samples collected from all prostate cancer patients carrying different genotypes. We also performed qPCR to examine the expression of miR-138 in these tissue and peripheral blood samples. To carry out the measurements, the total RNA content was first isolated from each sample by utilizing an RNAiso Plus RNA extraction reagent (Takara, Tokyo, Japan) in accordance with the specific protocol provided by the reagent manufacturer. Then, the isolated RNA from each sample was reverse transcribed into cDNA templates by making use of a Taqman Advanced cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA) along with a Reverse Transcription kit (Applied Biosystems, Foster City, CA) in accordance with the specific assay protocols provided by the assay kit manufacturers. Then, quantitative real time PCR was performed by making use of a Fast Start Universal SYBR Green Master Mix assay kit (Roche, Basel, Switzerland) in accordance with the specific assay protocol provided by the assay kit manufacturer. The real time PCR reaction was done on an ABI Prism 7900HT real time PCR machine (Applied Biosystems, Foster City, CA) using U6 as well as GAPDH as the internal control for the normalization of measured relative expression of target genes, i.e., COMT mRNA and miR-138.

**Cell culture and transfection**

Primary prostate cancer cells were bought from the Shanghai Cell Bank at the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in a RPMI 1640 medium added with 10% of fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA) and suitable antibiotics. The culture conditions were saturated humidity, 37 °C along with 5% carbon dioxide. To further explore the regulatory network of rs165599 polymorphisms, miR-138 and COMT, we cultured primary prostate cancer cells carrying the GG and AA genotypes, and then transfected them with COMT siRNA and miR-138 precursors, respectively. In brief, the primary prostate cancer
cells carrying the GG genotype of the rs165599 SNP were divided into 3 groups, i.e., 1. Scramble control group (primary prostate cancer cells treated with 50 nM of a scramble control siRNA); 2. COMT siRNA group (primary prostate cancer cells treated with 50 nM of COMT siRNA); and 3. miR-138 precursor group (primary prostate cancer cells treated with 50 nM of miR-138 precursors). Similarly, the primary prostate cancer cells carrying the AA genotype of the rs165599 SNP were also divided into 3 groups, i.e., 1. Scramble control group (primary prostate cancer cells treated with 50 nM of a scramble control siRNA); 2. COMT siRNA group (primary prostate cancer cells treated with 50 nM of COMT siRNA); and 3. miR-138 precursor group (primary prostate cancer cells treated with 50 nM of miR-138 precursors). In the next step, the cells were transfected with corresponding siRNA/miRNA by using Lipofectamine 3000 (Invitrogen, Carlsbad, CA) in accordance with the specific transfection protocol provided by the manufacturer of the transfection reagent. At 48 hours post transfection, the cells were harvested for the analysis of target gene expression.

Vector construction, mutagenesis and luciferase assay

Our sequence analysis showed that miR-138 could potentially bind to the 3' UTR of COMT. In order to examine whether rs165599 polymorphisms affect the expression of COMT, the 3' UTR of COMT carrying the A and G allele of the rs165599 polymorphism was cloned into pcDNA luciferase vectors (Promega, Madison, WI), respectively, to establish COMT-G and COMT-A vectors, which were then transfected into PC3 cells along with miR-138 mimics by using Lipofectamine 3000 in accordance with the specific transfection protocol provided by the manufacturer of the transfection reagent. At 48 hours post transfection, the luciferase activity of transfected cells was assayed by using a Dual Luciferase reporter gene assay kit (Promega, Madison, WI) in accordance with the specific assay protocol provided by the manufacturer of the assay kit.

Western blot analysis

The total protein content was extracted from each sample by making use of a radio immunoprecipitation assay kit (Solarbio Science & Technology Co., Ltd., Beijing, China) in accordance with the specific assay protocol provided by the assay kit manufacturer. Then, 50 μg
of each protein sample were resolved by using SDS-PAGE and blotted onto polyvinylidene fluoride (PVDF) membranes, which were then blocked by utilizing 5% skimmed milk at ambient temperature for 1 h before they were incubated for 24 h at 4 °C with anti-COMT primary antibody (Abcam, Cambridge, MA) followed by incubation with suitable HRP-conjugated secondary antibodies for 1 h at room temperature in accordance with the specific instructions provided by the antibody manufacturer. After color development by utilizing an enhanced chemiluminescence (ECL) assay kit (Amersham Pharmacia, Piscataway, NJ) in accordance with the specific assay protocol provided by the assay kit manufacturer, the relative protein expression of COMT in each sample was determined by means of a Bio-Rad imaging system (Bio-Rad Laboratories, Hercules, CA) in accordance with the instructions provided by the machine manufacturer.

Immunohistochemistry (IHC)

In order to explore the effect of rs165599 and rs4680 polymorphisms on the expression of their host gene COMT, we collected prostate cancer tissue samples from all patients and performed immunohistochemistry to evaluate COMT expression in each sample. In brief, collected prostate cancer tissue samples were embedded in paraffin, fixed, deparaffinized, made transparent, and blocked with 5% goat serum before they were treated with anti-COMT primary antibodies and matching Alexa Fluor 488 labeled secondary antibodies in sequence (Santa Cruz Biotechnology, Santa Cruz, CA) in accordance with the instructions provided by the antibody manufacturer. After counter staining with DAPI, the positive expression of COMT in each sample was evaluated by using a confocal microscope.

ELISA

The COMT activity in collected peripheral blood samples was measured by using a commercial ELISA assay kit (Thermo Fisher Scientific, Waltham, MA) in accordance with the specific assay protocol provided by the assay kit manufacturer.

Statistical analysis
Data analysis was carried out by using SPSS 19.0 software (SPSS, Chicago, IL). The Kaplan-Meier method was used to compare the prostate-specific antigen (PSA)-progression-free survival of patients in the four groups. Comparisons between 2 groups was done by using Student’s t tests. Results were presented as mean ± standard deviation. One-way ANOVA was used to compare multiple groups. A P value of <0.05 was deemed statistically significant.

**Results**

Patients with rs165599-AA + rs4680-Val/Val genotypes had highest PSA-progression-free survival.

In this work, we recruited 224 prostate cancer patients and partitioned them into four groups according to their genotypes at rs165599 and rs4680 SNP. The information of participants including their age, tumor status and Gleason sum, was collected and listed in Table 1. ANOVA was utilized to perform the statistical comparison among the above four groups, and the results revealed that there is no obvious difference in all characters among the above four groups. The Kaplan-Meier method was used to compare the PSA-progression-free survival of patients in the four groups. The PSA-progression-free survival was the highest in patients carrying the rs165599-AA + rs4680-Val/Val genotype was and the lowest in patients carrying the rs165599-(GG+GA) + rs4680-(Val/Met + Met/Met) genotype. The PSA-progression-free survival of patients carrying the rs165599-AA + rs4680-(Val/Met + Met/Met) and rs165599-(GG+GA) + rs4680-Val/Val genotypes were moderate (Fig.1).

The expression of COMT in tissue samples and peripheral blood were remarkably elevated in patients carrying the rs165599-AA + rs4680-Val/Val genotype.

We performed immunohistochemistry to evaluate COMT expression in various samples. The expression of COMT was significantly higher in tissue samples collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype, and was remarkably lower in tissue samples collected from patients carrying the rs165599-(GG+GA) + rs4680-(Val/Met + Met/Met) genotype (Fig.2). Moreover, we carried out qPCR and ELISA to analyze the expression of COMT in tissue samples and peripheral blood. The expression of COMT mRNA in tissue samples (Fig.3A) and peripheral blood (Fig.3B) was in good agreement with that revealed by IHC analysis.
MiR-138 inhibited the expression of COMT carrying the rs165599-G genotype by binding to the 3’ UTR of COMT.

No obvious difference was observed for the expression of miR-138 in either tissue samples (Fig.4A) or peripheral blood (Fig.4B). Sequence analysis showed that miR-138 could potentially bind to the 3’ UTR of COMT (Fig.5A). In order to examine whether rs165599 polymorphisms affect the expression of COMT, luciferase vectors carrying COMT-G and COMT-A were established and transfected into PC3 cells along with miR-138 mimics. The luciferase activity of COMT-G vector was significantly suppressed by miR-138 mimics, whereas no apparent suppression was observed for COMT-A vector (Fig.5B).

**MiR-138 precursors effectively suppressed the expression of COMT in primary prostate cancer cells carrying the GG genotype.**

To further explore the regulatory network of rs165599 polymorphisms, miR-138 and COMT, we cultured primary prostate cancer cells carrying the GG and AA genotypes, and then transfected them with COMT siRNA and miR-138 precursors, respectively. The dramatically elevated expression of miR-138 in primary prostate cancer cells carrying the GG (Fig.6A) and AA (Fig.7A) genotypes indicated successful transfection of miR-138 precursors. QPCR and Western blot analysis showed that the expression of COMT mRNA (Fig.6B) and protein (Fig.6C) was notably decreased in primary prostate cancer cells carrying the GG genotype after transfection with miR-138 precursors. The inhibitory effect of miR-138 precursors on the expression of COMT in primary prostate cancer cells carrying the GG genotype was even stronger than that of COMT siRNA. In addition, the expression of COMT mRNA (Fig.7B) and protein (Fig.7C) was also decreased to a lesser extent in primary prostate cancer cells carrying the AA genotype after transfection with miR-138 precursors.

**Discussion**

The findings of this study supported our hypothesis that the genotype of rs165599 and rs4680 are associated with the risk of prostate cancer recurrence and PSA progression free survival period. In this study, we found that the PSA-progression-free rate was the highest in patients carrying the rs165599-AA + rs4680-Val/Val genotype and the lowest in patients carrying the
rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype. In addition, the expression and activity of COMT were significantly higher in samples collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype, and remarkably lower in samples collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype.

Yu et al. found out that miR-138-5p displayed a suppressive impact on the growth of PC, suppressing the formation of tumors [31]. Tian et al. showed that miR-138-5p inhibited autography and PC growth, suggesting that miR-138-5p could function as a vital factor in PC [25, 32]. One SNP, rs165599, demonstrated the highest correlation with mental illness [33]. While SNP rs165599 is actually located downstream of the site of COMT polyadenylation and hence cannot be found in a lot of EST sequences recorded in the GenBank database, this SNP could be amplified from brain cDNA free of genomic contamination, which validates that SNP rs165599 is actually expressed in the brain [34]. As a result, the site of COMT with additional 3 polyadenylation should also be present in human brain. When SNP rs165599 was utilized as an expression tag for COMT transcripts containing the 3 sequence, notable variations were noted in allelic expression, indicating that the SNP is actually linked to larger modifications in the transcripts containing the 3’ UTR. In this study, we carried out luciferase assays to explore the effect of rs165599 polymorphisms on the expression of COMT. The luciferase activity of COMT-G vector was remarkably suppressed by miR-138 mimics. Furthermore, we cultured primary prostate cancer cells carrying GG and AA genotypes to explore the effect of miR-138 precursors on the expression of COMT. MiR-138 precursors showed a stronger effect on suppressing the expression of COMT mRNA and protein than COMT siRNA in primary prostate cancer cells carrying the GG genotype, but not in primary prostate cancer cells carrying the AA genotype.

However, there are limitations of our study. The number of patients included in our study for genetic analysis is relatively small to be convincible and supportive. And the according genetic analysis was not comprehensive. In our future study, it is necessary to include a more comprehensive genetic analysis.

Conclusion
The findings of this study demonstrated that the carriers of AA genotype of rs165599 and Val/Val genotype of rs4680 SNPs showed an increased risk of prostate cancer recurrence and shorter of PSA progression free survival period, both of which indicated a worse prognosis. Therefore, these two polymorphisms may jointly function as a novel biomarker for the prognosis of prostate cancer recurrence.

Conflict of interest

None

Funding information:

This study was supported by Hubei Provincial Natural Science Foundation (No. 2018CFB786).

Figure legends

Table 1

Basic information of participant grouped according to their genotypes

Fig.1

The PSA-progression-free survival was significantly higher in patients carrying the rs165599-AA + rs4680-Val/Val genotype and lower in patients carrying the rs165599-(GG+GA) + rs4680-(Val/Met + Met/Met) genotype.

Fig.2

Immunohistochemistry analysis showed that the expression of COMT protein was increased in the tissue samples collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in tissue samples collected from patients carrying the rs165599-(GG+GA) + rs4680-(Val/Met + Met/Met) genotype.

Fig.3

The expression and activity of COMT were increased in patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in patients carrying the rs165599-(GG+GA) + rs4680-(Val/Met + Met/Met) genotype.
A: QPCR analysis showed that the expression of COMT mRNA was increased in the tissue samples collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in the tissue samples collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype.

B: ELISA analysis showed that the activity of COMT was increased in the serum collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in the serum collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype.

Fig.4

No obvious difference was observed for the expression of miR-138 in the tissue samples and serum collected from patients carrying different genotypes.

A: No apparent difference was observed for the expression of miR-138 in the tissue samples collected from patients carrying different genotypes.

B: No apparent difference was observed for the expression of miR-138 in the serum collected from patients carrying different genotypes.

Fig.5

The luciferase activity of COMT-G was inhibited by miR-138.

A: Sequence analysis showed that miR-138 could potentially bind to COMT carrying the G allele of rs165599 SNP.

B: The luciferase activity of COMT-G was suppressed by miR-138 in PC3 cells (* P value < 0.05 vs. miRNA controls).

Fig.6

MiR-138 precursors effectively suppressed the expression of COMT in primary prostate cancer cells carrying the GG genotype.
A: Dramatic elevation of miR-138 expression indicated efficient transfection of miR-138 precursors into primary prostate cancer cells (* P value < 0.05 vs. scramble control).

B: The inhibition of COMT mRNA expression by miR-138 precursors was stronger than that by COMT siRNA in primary prostate cancer cells carrying the GG genotype (* P value < 0.05 vs. scramble control).

C: The inhibition of COMT protein expression by miR-138 precursors was stronger than that by COMT siRNA in primary prostate cancer cells carrying the GG genotype (* P value < 0.05 vs. scramble control).

Fig. 7

MiR-138 precursors slightly suppressed the expression of COMT in primary prostate cancer cells carrying the AA genotype.

A: Dramatic elevation of miR-138 expression indicated efficient transfection of miR-138 precursors into primary prostate cancer cells carrying the AA genotype (* P value < 0.05 vs. scramble control).

B: The inhibition of COMT mRNA expression by COMT siRNA was stronger than that by miR-138 precursors in primary prostate cancer cells carrying the AA genotype (* P value < 0.05 vs. scramble control).

C: The inhibition of COMT protein expression by COMT siRNA was stronger than that by miR-138 precursors in primary prostate cancer cells carrying the AA genotype (* P value < 0.05 vs. scramble control).

References


Table 1. Basic information of participant grouped according to their genotypes

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The PSA-progression-free survival was significantly higher in patients carrying the rs165599-AA + rs4680-Val/Val genotype and lower in patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype.
Fig. 2
Immunohistochemistry analysis showed that the expression of COMT protein was increased in the tissue samples collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in tissue samples collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype.
The expression and activity of COMT were increased in patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype.

A: QPCR analysis showed that the expression of COMT mRNA was increased in the tissue samples collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in the tissue samples collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype.

B: ELISA analysis showed that the activity of COMT was increased in the serum collected from patients carrying the rs165599-AA + rs4680-Val/Val genotype and decreased in the
serum collected from patients carrying the rs165599-(GG+GA) + rs4680- (Val/Met + Met/Met) genotype.
No obvious difference was observed for the expression of miR-138 in the tissue samples and serum collected from patients carrying different genotypes.

A: No apparent difference was observed for the expression of miR-138 in the tissue samples collected from patients carrying different genotypes.

B: No apparent difference was observed for the expression of miR-138 in the serum collected from patients carrying different genotypes.
The luciferase activity of COMT-G was inhibited by miR-138.
A: Sequence analysis showed that miR-138 could potentially bind to COMT carrying the G allele of rs165599 SNP.
B: The luciferase activity of COMT-G was suppressed by miR-138 in PC3 cells (* P value < 0.05 vs. miRNA controls).
MiR-138 precursors effectively suppressed the expression of COMT in primary prostate cancer cells carrying the GG genotype.

A: Dramatic elevation of miR-138 expression indicated efficient transfection of miR-138 precursors into primary prostate cancer cells (* P value < 0.05 vs. scramble control).

B: The inhibition of COMT mRNA expression by miR-138 precursors was stronger than that by COMT siRNA in primary prostate cancer cells carrying the GG genotype (* P value < 0.05 vs. scramble control).

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MiR-138 precursors slightly suppressed the expression of COMT in primary prostate cancer cells carrying the AA genotype.

A: Dramatic elevation of miR-138 expression indicated efficient transfection of miR-138 precursors into primary prostate cancer cells carrying the AA genotype (* P value < 0.05 vs. scramble control).

B: The inhibition of COMT mRNA expression by COMT siRNA was stronger than that by miR-138 precursors in primary prostate cancer cells carrying the AA genotype (* P value < 0.05 vs. scramble control).

C: The inhibition of COMT protein expression by COMT siRNA was stronger than that by miR-138 precursors in primary prostate cancer cells carrying the AA genotype (* P value < 0.05 vs. scramble control).