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Laboratory-Bladder cancer

Impact of endothelial nitric oxide synthase polymorphisms on urothelial cell carcinoma development

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Abstract

Objectives: Urothelial cell carcinoma (UCC), a major malignancy of the genitourinary tract, is induced through carcinogenic etiological factors. Endothelial nitric oxide synthase (eNOS) is one of the major isoforms of nitric oxide synthase and is involved in various pathophysiologic and physiologic processes. In this study, eNOS single-nucleotide polymorphisms were investigated to evaluate UCC susceptibility and clinicopathological characteristics.

Materials and methods: Two single-nucleotide polymorphisms of eNOS in 431 patients with UCC and 862 controls without cancer were analyzed using real-time polymerase chain reaction.

Results: The results showed that 272 men with UCC having eNOS 894 G > T rs1799983 "GT + TT" variants had a high risk of developing a large tumor (T1−T4, $P = 0.038$). Furthermore, a correlation was observed between the expressions of eNOS and invasive tumor, metastasis and poor survival in urothelial carcinoma in The Cancer Genome Atlas data set.

Conclusion: Our results indicated that male patients with UCC carrying eNOS 894 G > T rs1799983 "GT + TT" genetic variants have a high risk of developing a large tumor, and eNOS polymorphisms may serve as a marker or therapeutic target in UCC treatment.

Keywords: Transitional cell carcinoma; eNOS; Polymorphism

1. Introduction

Urothelial cell carcinoma (UCC) of the urinary bladder is a major malignancy with considerable morbidity and mortality. It causes approximately 150,000 deaths per year worldwide and is the seventh and 17th most common cancer in men and women, respectively [1,2]. In Taiwan, it is the eighth most common cause of male malignancy and the second most common malignancy of the genitourinary tract [3,4]. Etiological risk factors such as genetic susceptibility, tobacco smoking, occupational exposure to carcinogenic aromatic amines, diet, and environmental pollution have been suggested to be correlated with urothelial bladder cancer carcinogenesis [1,4].

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Nitric oxide (NO) is a highly reactive free radical and multifunctional gaseous molecule [5,6]. NO is synthesized from NADPH, L-arginine, and oxygen through nitric oxide synthase (NOS) [5,7]. Endothelial nitric oxide synthase (eNOS) is one of the major isoforms of NOS encoded by nitric oxide synthase 3 (NOS3) [8,9]. eNOS is constitutively expressed, and hence is also termed as constitutive NOS [10,11]. Furthermore, NO is involved in various pathophysiologic and physiologic processes including carcinogenesis, immunity, and neurotransmission [11,12]. Studies have suggested that NO potentially prevents cancer progression and tumor cell proliferation [13,14]. However, NO has been indicated to be correlated with increased blood flow, induction of angiogenesis and metastasis, and inhibition of tumor-suppressive p53 functions, which increase tumor progression [14−16]. Therefore, although the exact role of NO in tumor biology is controversial, it indeed plays an essential role in mediating carcinogenesis and tumor progression.

The eNOS gene is located on chromosome 7q36 [17,18]. The 3 clinically most relevant polymorphisms of the NOS3 gene have been noted, which could modify the transcription and endogenous production of NO: rs1799983 in exon 7 (encoding Glu298Asp), rs2070744 (T-786C) in the promoter region, and a variable number tandem repeat in intron 4 [19−21]. Studies have suggested that eNOS polymorphisms are associated with risk of cancers, such as breast cancer, lung adenocarcinoma, and oral cancer [11,16,22−25]. However, correlations between eNOS polymorphisms and UCC remained poorly investigated. In this study, we investigated the association between eNOS 894 G > T rs1799983 and eNOS −786 T > C rs2070744 and investigated their correlations with UCC clinicopathological characteristics.

2. Materials and methods

2.1. Study subjects

In this study, for the study group, we recruited 431 patients with UCC (272 men and 159 women; mean age = 68.6 ± 11.8 years) between 2010 and 2015 at the Taichung Veterans General Hospital in Taichung, Taiwan. For the control group, 862 gender matched healthy controls (566 men and 296 women; mean age = 57.2 ± 10 years) who entered the hospital for physical examination were enrolled. Patients with UCC were clinically staged during diagnosis according to the tumor/node/metastasis staging system of the American Joint Committee on Cancer [26]. Individuals of the control group enrolled in this study had no self-reported cancer history of any site. We obtained information on personal characteristics of the study participants through interviewer-administered questionnaires on demographic characteristics. Informed consent forms were obtained from all individuals before initiating the study. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital (IRB no. CF11094).

2.2. DNA extraction and eNOS genotyping

For DNA extraction, EDTA-anticoagulated venous blood from both patients with UCC and participants of the control group were obtained using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. The final eluted DNA was dissolved in Tris ethylene buffer (10 mmol/l Tris and 1 mmol/l EDTA; pH 7.8) and then quantified through measurement of OD260 nm using NanoDrop 2000 spectrophotometers. Extracted DNA was preserved at −20°C and used as a template in polymerase chain reactions (PCRs). Allelic discrimination assessment for eNOS 894 G > T rs1799983 (assay IDs: C_3219460_20) and eNOS −786 T > C rs2070744 (assay IDs: C_15903863_10) was performed using TaqMan assay with an ABI StepOnePlus Software v2.3 real-time PCR system and further evaluated with SDS 7000 series software (Applied Biosystems, Foster City, CA).

2.3. Expression analysis of The Cancer Genome Atlas data

We used The Cancer Genome Atlas (TCGA; URL: https://tcga-data.nci.nih.gov/tcga/) to obtain the NOS3 (eNOS) normalized expression data and associated clinical data, which corresponds to the bladder urothelial carcinoma dataset ($n = 408$). Box plots for NOS3 (eNOS) expression values were generated with respect to the TNM status including tumor size, lymph node status, and overall survival. The "Low" and "High" expression of NOS3 (eNOS) was determined by median for Kaplan Meier plots.

2.4. Statistical analysis

The age, gender differences, and demographic characteristic distributions were compared between the study and control groups using Fisher's exact test and the Mann −Whitney U test. The odds ratio and 95% confidence interval of the association between genotype frequencies and UCC risk and the clinical pathological characteristics were estimated using multiple logistic regression models. A P value < 0.05 was considered statistically significant. Data were analyzed using SAS statistical software (Version 9.1, 2005; SAS Institute, Cary, NC).

3. Results

Demographical characteristics distribution is presented in Table 1. Analysis of demographical characteristics of study participants revealed that 34.8% (300/862) of controls and 30.4% (131/431) of patients with UCC were smokers. Significant distributional differences were observed in terms of age ($P < 0.001$) between controls and patients with UCC, whereas no significant differences were

Table 1
The distributions of demographical characteristics in 862 controls and 431 patients with UCC.

Variable	Controls ($N = 862$) n (%)	Patients ($N = 431$) n (%)	P value
Age (y)			<0.001
≤65	697 (80.9%)	166 (38.5%)	
>65	165 (19.1%)	265 (61.5%)	
Mean ± S.D.	57.2 ± 10	68.6 ± 11.8	<0.001
Gender			0.365
Female	296 (34.3%)	159 (36.9%)	
Male	566 (65.7%)	272 (63.1%)	
Tobacco consumption			0.113
No	562 (65.2%)	300 (69.6%)	
Yes	300 (34.8%)	131 (30.4%)	
Stage			
Nonmuscle invasive tumor (pTa−pT1)		235 (54.5%)	
Muscle invasive tumor (pT2−pT4)		196 (45.5%)	
Tumor T status			
Ta		90 (20.9%)	
T1−T4		341 (79.1%)	
Lymph node status			
N0		380 (88.2%)	
N1 + N2		51 (11.8%)	
Metastasis			
M0		417 (96.8%)	
M1		14 (3.2%)	
Histopathologic grading			
Low grade		53 (12.3%)	
High grade		378 (87.7%)	

Student's t test or chi-squared test was used between controls and patients with UCC.

observed between them in terms of tobacco consumption ($P = 0.113$).

The genotype distributions and associations between UCC and *eNOS* genetic polymorphisms are presented in Table 2. The *eNOS* rs1799983 and rs2070744 genetic polymorphisms exhibited the highest distribution frequency in both controls and patients with UCC homozygous for GG and TT, respectively. After adjustment for several variables such as age, gender, and tobacco consumption, no significant differences were observed between patients with UCC having rs1799983 and rs2070744 polymorphisms of the *eNOS* gene and those with the wild-type gene.

Table 2
Genotype distributions of eNOS gene polymorphisms in 862 controls and 431 patients with UCC.

Variable	Controls ($N = 862$) n (%)	Patients ($N = 431$) n (%)	OR (95% CI)	AOR (95% CI)
rs1799983				
GG	676 (78.4%)	343 (79.6%)	1.000 (reference)	1.000 (reference)
GT	176 (20.4%)	80 (18.6%)	0.896 (0.667−1.203)	0.927 (0.665−1.293)
TT	10 (1.2%)	8 (1.9%)	1.577 (0.617−4.031)	1.576 (0.547−4.541)
GT+TT	186 (21.6%)	88 (20.4%)	0.932 (0.701−1.240)	0.963 (0.698−1.329)
rs2070744				
TT	682 (79.1%)	348 (80.7%)	1.000 (reference)	1.000 (reference)
TC	168 (19.5%)	82 (19%)	0.957 (0.713−1.283)	0.970 (0.695−1.355)
CC	12 (1.4%)	1 (0.2%)	0.163 (0.021−1.261)	0.169 (0.020−1.424)
TC + CC	180 (20.9%)	83 (19.3%)	0.904 (0.676−1.208)	0.916 (0.659−1.273)

Bold font indicates statistical significance ($P < 0.05$).

The odds ratio (OR) with their 95% confidence intervals were estimated by logistic regression models.

The adjusted odds ratio (AOR) with their 95% confidence intervals were estimated by multiple logistic regression models after controlling for age, gender, and tobacco consumption.

CI = confidence interval; eNOS = endothelial nitric oxide synthase.

Table 3
Distribution frequency of the clinical status and eNOS rs1799983 genotype frequencies in 272 male UCC patients.

Variable	eNOS (rs1799983)			
	GG (%) ($n = 207$)	GT + TT (%) ($n = 65$)	OR (95% CI)	P value
Stage				
Nonmuscle invasive tumor (pTa−pT1)	117 (56.5%)	34 (52.3%)	1.000 (reference)	
Muscle invasive tumor (pT2−pT4)	90 (43.5%)	31 (47.7%)	1.043 (0.907-1.2)	0.551
Tumor T status				
Ta	55 (26.6%)	9 (13.8%)	1.000 (reference)	
T1−T4	152 (73.4%)	56 (86.2%)	**1.225 (1.011−1.484)**	**0.038**
Lymph node status				
N0	182 (87.9%)	57 (87.7%)	1.000 (reference)	
N1 + N2	25 (12.1%)	8 (12.3%)	1.011 (0.661−1.546)	0.960
Metastasis				
M0	198 (95.7%)	63 (96.9%)	1.000 (reference)	
M1	9 (4.3%)	2 (3.1%)	0.698 (0.147−3.318)	0.652
Histopathologic grading				
Low grade	33 (15.9%)	6 (9.2%)	1.000 (reference)	
High grade	174 (84.1%)	59 (90.8%)	1.865 (0.744−4.673)	0.184

Bold font indicates statistical significance ($P < 0.05$).
The odds ratio (OR) with their 95% confidence intervals were estimated by logistic regression models.
CI = confidence interval; eNOS = endothelial nitric oxide synthase.

To clarify the role of *eNOS* genetic polymorphisms in UCC statuses, such as clinical stage, tumor size, LN metastasis, distant metastasis, and histopathological grading, the distribution frequency of clinical statuses and *eNOS* genotype frequencies in patients with UCC were estimated. The rs2070744 genetic polymorphism showed no significant association with clinicopathological statuses. However, 272 male patients with UCC carrying the polymorphic rs1799983 gene had a higher risk of developing tumors of greater size (odds ratio = 1.225, 95% confidence interval = 1.011−1.484, $P = 0.038$) than those carrying the rs1799983 wild-type gene, but no significant difference was observed in terms of clinical stage, lymph node status, metastasis, or histopathological grading (Table 3).

We further used TCGA data set (bladder urothelial carcinoma; $n = 408$) to analyze and clarify the findings of our study (Table 4). eNOS is encoded by NOS3 [9]. The NOS3 (eNOS) mRNA levels, pathological stage, tumor size, and lymph node status of bladder urothelial carcinoma were assessed (Fig. 1 and 2). The NOS3 expression levels in bladder urothelial carcinoma tissues and normal tissues are exhibited in Fig. 1A and B. A higher level of NOS3 was detected in normal tissues than in bladder urothelial carcinoma tissues ($P = 0.0125$; Fig. 1A). Moreover, the adjacent normal tissues revealed a higher NOS3 mRNA level than the tumor tissues ($P = 0.0007$; Fig. 1B). Among patients with bladder urothelial carcinoma, the relative levels of NOS3 mRNA were significantly higher in Stage III + IV patients than in Stage I + II patients ($P = 0.0429$; Fig. 1C). The relative levels of NOS3 mRNA were not significantly associated with the tumor size and lymph node status (Fig. 1D and E). However, the relative levels of NOS3 mRNA were significantly higher in M1 patients than in M0

Table 4
The distributions of demographical and clinical characteristics in TCGA database.

Variable	Patients ($N = 408$)
Age (y)	
<55	43 (10.5%)
≥55	365 (89.5%)
Tobacco smoking history (y)	
<3	198 (48.5%)
≥3	197 (48.3%)
N/A	13 (3.2%)
Gender	
Male	301 (73.8%)
Female	107 (26.2%)
Stage	
I + II	132 (32.3%)
III + IV	274 (67.2%)
N/A	2 (0.5%)
Tumor T status	
T0	1 (0.2%)
T1 + T2	122 (29.9%)
T3 + T4	252 (61.8%)
Tx	1 (0.2%)
N/A	32 (7.9%)
Lymph node status	
N0	237 (58.1%)
N1 + N2 + N3	129 (31.6%)
Nx	36 (8.8%)
N/A	6 (1.5%)
Metastasis	
M0	196 (48.1%)
M1	11 (2.7%)
Mx	198 (48.5%)
N/A	3 (0.7%)

N/A = not available; TCGA = The Cancer Genome Atlas.

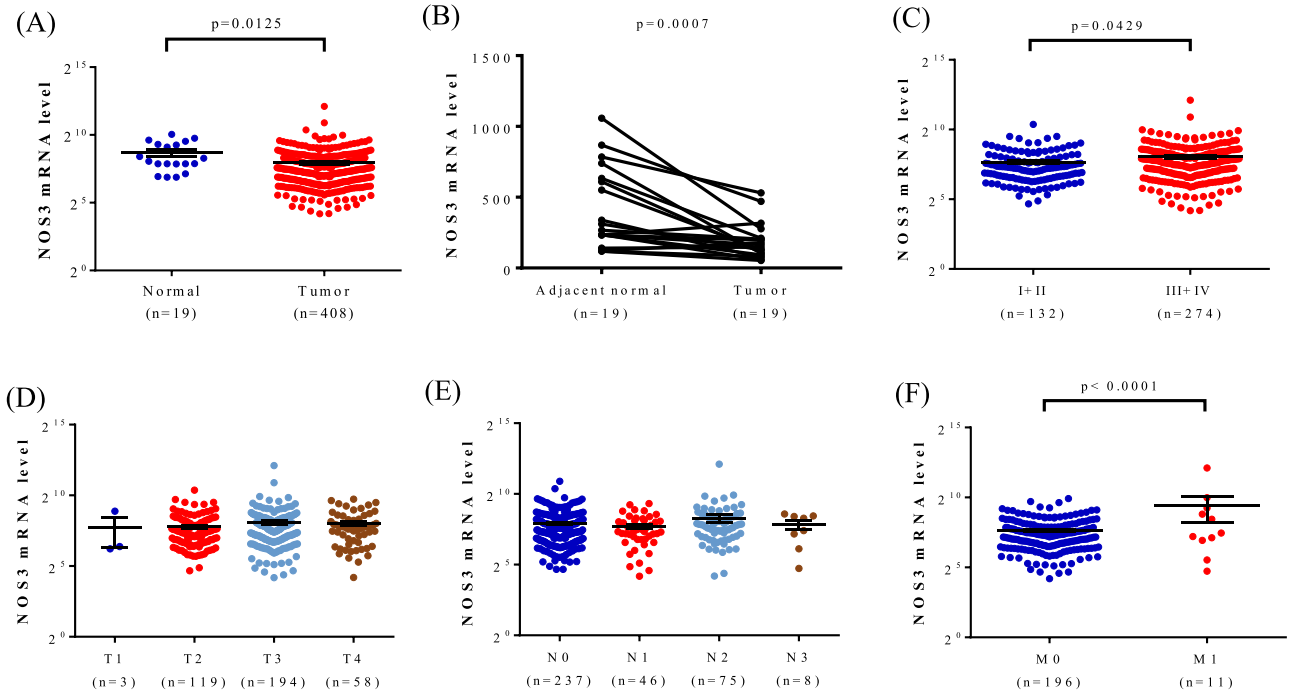

Fig. 1. NOS3 (eNOS) mRNA level of bladder urothelial carcinoma patients from TCGA database. (A) NOS3 (eNOS) levels were compared according to normal tissue and bladder urothelial carcinoma tumor tissues. (B). Relative expression of NOS3 (eNOS) in 19 pairs of bladder urothelial carcinoma tumor tissues and their corresponding adjacent noncancerous tissues. (C) NOS3 (eNOS) levels were compared according to stage. (D) NOS3 (eNOS) levels were compared according to tumor size status. (E) NOS3 (eNOS) levels were compared according to lymph node status. (F) NOS3 (eNOS) levels were compared according to distant metastasis status.

eNOS = endothelial nitric oxide synthase; NOS3 = nitric oxide synthase 3; TCGA = The Cancer Genome Atlas.

patients (Fig. 1F). In addition, we also analyzed overall survival. As shown in Fig. 2, TCGA data set of overall patients (Fig. 2A) and female patients (Fig. 2C) with bladder urothelial carcinoma revealed that a high NOS3 expression was not associated with a poor overall survival. However, results from TCGA data set of male patients with bladder urothelial carcinoma revealed that a higher NOS3 expression was associated with a poor overall survival ($P = 0.021$; Fig. 2B). These data suggest that eNOS expression may be involved in UCC regulation.

4. Discussion

This study revealed the correlation of *eNOS* genetic polymorphisms with UCC. Tobacco consumption has been considered one of the major risk factors for UCC

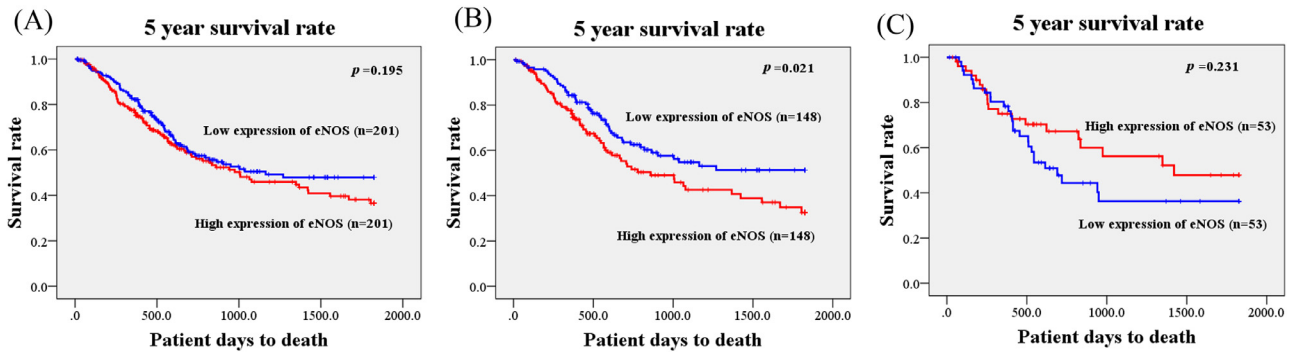

Figure 2. Analysis of NOS3 (eNOS) mRNA expression and overall survival in bladder urothelial carcinoma patients from TCGA database. Effect of NOS3 (eNOS) mRNA expression on the overall survival of patients with bladder urothelial carcinoma evaluated using the Kaplan–Meier method. An overall survival curve was produced for (A) overall patients ($n = 402$), (B) male patients ($n = 296$), and (C) female patients ($n = 106$) with high (red lines) and low (blue lines) NOS3 (eNOS) mRNA expression levels. The P values were determined using a log-rank test. (Color version of figure is available online.)

eNOS = endothelial nitric oxide synthase; NOS3 = nitric oxide synthase 3; TCGA = The Cancer Genome Atlas.

carcinogenesis [1,27,28], and the history of tobacco smoking exposure was associated with UCC grade [29]. In a study of secondhand smoking and vascular inflammation, the expression of eNOS and P-eNOS was similarly decreased in passive and active smokers compared with control subjects, suggesting that smoking plays a role in eNOS impairment [30]. However, our study found no significant association between tobacco smoking and UCC (Table 1). Bladder cancer is predominantly seen in elderly patients [31], and advancing age is correlated with endothelial dysfunction [32]. The augmented production from intracellular enzymes NADPH oxidase and uncoupled eNOS is responsible for greater endothelial oxidative stress with aging [32]. Therefore, although we did not directly detect plasma nitrite levels, patients with UCC may sustain endothelial dysfunction and uncoupled eNOS, although the association of carcinogenic impairment with eNOS from smoking is statistically nonsignificant.

We further analyzed the genotype distributions of *eNOS* gene polymorphisms in 862 controls and 431 patients with UCC. In a study of the association of *eNOS* polymorphism with age onset of menarche in female patients with sickle cell disease (SCD) in India, the SCD late menarche group had a significantly low level of plasma nitrite concentration for all 3 *eNOS* gene polymorphisms, *eNOS* 4a/b, *eNOS* 894 G > T (rs1799983), and *eNOS* −786 T > C (rs2070744) when compared with the controls and the SCD early menarche group, suggesting a role of *eNOS* gene polymorphisms in age onset of disease and downregulation of plasma nitrite concentration [33]. However, no significant association was found between *eNOS* 894 G > T and *eNOS* −786 T > C genetic polymorphisms and controls or patients with UCC in our study (Table 2). This result implied that the direct impact of *eNOS* polymorphism on UCC carcinogenesis may be limited to Taiwanese patients, where most of these patients were aged ≥65 years (61.5%; Table 1). A study of *eNOS* polymorphisms in a Turkish population revealed that the *eNOS* G894T GT and TT polymorphic genotypes significantly varied among the control group and patients with bladder cancer, and the genetic frequency of the 894T allele was significantly higher in patients with bladder cancer [34]. However, in a study between Swedish urinary-bladder cancer patients and polymorphisms in the NOS3 gene [35], a significantly increased association with bladder cancer for CC-homozygous carriers of the −786 T > C polymorphism (rs2070744) was found. No association was found between the Glu298Asp polymorphism (rs1799983) and increased cancer risk, but there was an association between tumor grade and Glu298Asp [35]. Conversely, our study demonstrated that both *eNOS* 894 G > T (rs1799983) and *eNOS* −786 T > C (rs2070744) were not significantly associated with the control or study groups. Since bladder cancer is a heterogeneous disorder depending on both environmental exposures and genetic susceptibilities for the risk of disease [35,36]. Therefore, different ethnicities and genetic susceptibility or other epigenetic factors that may influence *NOS3* gene functions might be responsible for this discrepancy.

We further analyzed the association between UCC clinical status and *eNOS* polymorphisms. A previous study suggested that the *eNOS* −786 T > C (rs2070744) polymorphism is associated with significantly decreased eNOS promoter activity and endothelial NO production [37]. However, no significant association was observed between *eNOS* −786 T > C and our study participants (data not shown). Intriguingly, in 272 male patients with UCC but not in female patients, we found that the *eNOS* 894 G > T (rs1799983) polymorphism was associated with a greater tumor size (T1−T4, $P = 0.038$; Table 3). A previous study that focused on breast cancer suggested that the *eNOS* 894 G > T polymorphism is associated with breast cancer risk [25]. Consistent with this result, our study demonstrated that the *eNOS* genetic polymorphism 894 G > T (rs1799983) may be involved in UCC cell proliferation and tumor progression in male patients with UCC (Table 3). In Taiwan, smoking is more prevalent among men than women [38], and smoking was suggested to be correlated with decreased eNOS expression [30]. Because tobacco smoking revealed no significant differences between the study and control groups in our study (Table 1), it is possible that the *eNOS* 894 G > T (rs1799983) polymorphism may play a more crucial role in elderly male individuals (age >65 years) with UCC, because smoking is a well-known environmental carcinogenic risk factor [39] and was not significantly associated with UCC in our study. However, the exact mechanism and detailed regulation of the *eNOS* genetic polymorphism 894 G > T (rs1799983) to UCC remained unclear.

Besides, results of the NOS3 (eNOS) TCGA data set in bladder urothelial carcinoma analysis revealed some intriguing and controversial phenomenon. NO has known to act as tumor progressor or suppressor according to its concentration [40]. Generally, low concentrations of NO lead to tumor progression, while higher concentrations of NO was suggested to have antitumor effects [40−43]. Although NO plays a major role including the control of vascular tone and angiogenesis [44−46] and eNOS was suggested to contribute to angiogenic endothelial signaling [5,47−49], it was hypothesized that NO may have beneficial effects on tumor progression through attenuating the tumor-progressing effects of matrix and myofibroblasts derived from these cells [49−51]. Of note, the tumor microenvironment and eNOS interaction was also suggested to influence eNOS expression and the effects of NO in tumor progression. A previous study has demonstrated that NO gradient within the liver tumor microenvironment coordinate molecular interactions between tumor cells and stroma to influence tumor progression [49]. Moreover, the catalytic eNOS activity is regulated by intracellular localization through trafficking to caveolae and the interaction with some regulatory molecules includes the inhibitor caveolin-1 [5]. Caveolin-1 is the structural protein of caveolae, and

the physical interaction of eNOS with caveolin-1 scaffolding domain maintains the enzyme in an inactivated stage and keeps eNOS in basal, unstimulated conditions to prevent eNOS activation [46,52]. Decreased caveolin-1 expression was reported in endothelial cells lining tumor blood vessels [46,53,54], and exacerbated angiogenesis was associated with deficit in the expression of caveolin in tumor blood vessels, suggesting the role of NO-dependent angiogenesis under hypoxic tumor microenvironment [46,53]. Taken together, it can be assumed that the down-regulated NOS3 (eNOS) mRNA level in early stage of bladder urothelial carcinoma (compared with the normal tissues) symbolize the initiation of UCC carcinogenesis (Fig. 1A, B). As the UCC progress, a shifting balance of NO result from NO-dependent angiogenesis under hypoxic tumor microenvironment occurred, leading to caveolin-1 deficit, and ultimately result in elevated NOS3 (mRNA) levels (Fig. 1C−F), implying the advancing of UCC progression and poor prognosis.

The 894 G > T polymorphism (rs1799983) located in exon 7 leads to an amino acid substitution at position 298 (Glu298Asp), which was suggested to induce proteolytic cleavage of the eNOS protein and down-regulate nitric oxide bioavalibility rather than affect NO production, in subjects with the polymorphic T variant compared with the GG wild-type carriers in a dose-dependent manner [55−58]. This result may provide a possible link of eNOS rs1799983 G > T polymorphisms in Taiwanese male UCC patients to a greater risk of developing a larger tumor size regardless the concentration of NO. In this aspect, it is not the concentrations of NO but the NO bioavalibility that leads to tumor progression and poor prognosis, suggesting a vulnerability of monitoring the eNOS mRNA expressing levels alone in evaluating UCC progression and the application of NO donors in cancer treatment. However, the expressions of eNOS −786 T > C (rs2070744) and 894 G > T (rs1799983) polymorphisms and their correlations with clinical relevance in different ethnicities is not consistent [34,35]. Besides, since the bladder cancer is a disease with sex differences [59,60]. Perhaps our findings of eNOS 894 G > T rs1799983 polymorphic variant may provide a possible mechanism to explain the sex differences in bladder cancer (Table 3). However, the reason why the effects of eNOS 894 G > T rs1799983 polymorphic variant and the correlations of 5-year survival rate with NOS3 (eNOS) mRNA expressing levels is not consistent between male and female UCC patients remained mysterious (Table 3; Fig. 2).

Limitations to our study are that we lack of the data of 5 year survival rate and the information of eNOS mRNA levels to both of our study groups, so more detailed analysis and comparison of eNOS rs1799983 G > T polymorphisms to clinical relevance and TCGA data could not be performed. Besides, most of the UCC patients in our study are subjects with greater tumor size (79.1%) and are less with lymph node metastasis (11.8%) and distant metastasis (3.2%), whereas a great proportion of these patients were diagnosed as high histopathological grade (87.8%; Table 1). Perhaps these patients may be alerted with the major or irritative UCC symptoms such as hematuria, dysuria, and urgency [61,62], and then consult for treatment and partly benefit with early diagnosis. Therefore, sampling bias may exist, extended observation and follow-up period may reveal a different, more advancing and comprehensive clinical information between relevance of UCC progression and eNOS SNPs expression. In the future, well-designed studies are required to elucidate the mechanisms of *eNOS* polymorphisms in cancer progression, especially considering the controversial role of eNOS expression in various cancers and disease progression.

In conclusion, our study demonstrated the associations between *eNOS* polymorphisms and UCC. The male Taiwanese patients with UCC carrying the *eNOS* 894 G > T (rs1799983) “GT + TT” genetic variants had a high risk of developing a large tumor. Thus, *eNOS* polymorphisms may serve as a marker or a therapeutic target in UCC treatment.

Conflict of interest

The authors declared no conflict of interest.

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