Research Paper

Genetic Variations of Melatonin Receptor Type 1A are Associated with the Clinicopathologic Development of Urothelial Cell Carcinoma

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Abstract

Melatonin counteracts tumor occurrence and tumor cell progression in several cancer types in vitro and in vivo. It acts predominantly through its melatonin receptor type 1A (MTNR1A), and genetic variations of MTNR1A affect the susceptibility several diseases and cancer. The purpose of this study was to explore the effect of MTNR1A gene polymorphisms on the susceptibility to and clinicopathological characteristics of urothelial cell carcinoma (UCC). We recruited 272 patients with UCC and 272 normal controls to analyze three common single-nucleotide polymorphisms (SNPs) (rs2119882, rs13140012, and rs6553010) of MTNR1A related to cancer risk and clinicopathological relevance according to a TaqMan-based real-time polymerase chain reaction (PCR). We found that these three SNPs of MTNR1A were not associated with UCC susceptibility. However, patients with UCC who had at least one G allele of MTNR1A rs6553010 (in intron 1) were at higher risk (1.768-fold, 95% confidence interval: 1.068~1.849) of developing an invasive stage (p < 0.026), compared to those patients with AA homozygotes. In conclusion, polymorphic genotypes of rs6553010 of MTNR1A might contribute to the ability to predict aggressive phenotypes of UCC. This is the first study to provide insights into risk factors associated with intronic MTNR1A variants in the clinicopathologic development of UCC in Taiwan.

Key words: Melatonin receptor type 1A; Single-nucleotide polymorphisms; Urothelial cell carcinoma; Clinicopathologic development.

Introduction

Urothelial cell carcinoma (UCC) arises from the epithelial lining of the entire urinary tract, including the urinary bladder, ureter, and kidneys and has histologic features similar to those of cell carcinoma and is considered to have an analogous etiology [1]. UCC comprises more than 90% of bladder cancers in both genders. The worldwide age-standardized incidence rates (per 100,000 person/year) of bladder cancer are 9.0 for men and 2.2 for women [2]. In Taiwanese, bladder cancer is the ninth leading malignancy among men and the sixteenth leading malignancy among women with respective incidences.
of about 8.82 and 3.11. Mortality rates of bladder cancer ranked 12th among all cancer deaths for men and 13th among women [3]. The best-known risk factors are tobacco use and aromatic amine exposure, but in Taiwan, arsenic exposure in potable water and traditional herbs containing aristolochic acid are unique risk factors [4-7]. In addition to environmental and dietary factors, recent articles emphasized the importance of genetic factors in the development of UCC [8-10].

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone mainly produced by the pineal gland and other organs [11]. It has many protective roles in several physiological areas such as circadian rhythm control, seasonal reproduction, effective endogenous free radical scavengers, and anti-apoptosis in normal cells [12-16]. On the contrary, melatonin also plays a vital oncostatic role in different cancers through antiproliferative, anti-invasive, anti-metastatic and proapoptotic actions, stimulation of anticancer immunity, modulation of oncogene expression, and its anti-inflammatory, antioxidant, and antiangiogenic effects [17-23]. Melatonin has to bind to its membranous G protein-coupled receptors to execute its cellular functions. Melatonin receptors are divided into type Ia (MTNR1A or MT1) and Ib (MTNR1B or MT2) with high binding affinity, and are largely responsible for mediating the downstream effects of melatonin [24]. It is widely accepted that melatonin mostly binds to MTNR1A to exhibit its anticancer effects [18, 25]. Higher MTNR1A expression was reported to be correlated with a less-malignant histologic subtype of breast cancer and a higher survival rate of breast cancer patients [26]. A similar correlation was also found in oral squamous cell carcinoma [27]. To the present, little research has been conducted into melatonin and its anticancer activity in urothelial cancer.

Among DNA sequence variations, single-nucleotide polymorphisms (SNPs) are the most common event. The variant frequency occurs in more than 1% of the population, and correlates with disease susceptibility [28]. Previous studies demonstrated that SNPs of MTNR1A were linked to several kinds of disease, including coronary artery disease, calcium nephrolithiasis, and polycystic ovary syndrome [29-31]. A recent study further disclosed that MTNR1A polymorphisms interact with environment factors to possibly raise oral-cancer susceptibility and even development of an advanced clinical stage and metastatic status [32]. Although associations of genetic polymorphisms of MTNR1A with several diseases and cancer were disclosed, knowledge of potential roles of MTNR1A genetic polymorphisms in susceptibility to UCC is still lacking. In this study, we intended to explore associations of polymorphisms within the MTNR1A gene with UCC risk and the clinicopathologic development of UCC in Taiwanese patients.

Materials and methods

Study subjects and specimen collection

In 2010–2013, we recruited 272 patients with urothelial carcinoma, diagnosed at Taichung Veteran General Hospital in Taichung, Taiwan. There were 179 male and 93 female patients. For the control group, 272 participants with a similar male-to-female ratio and age distribution were enrolled in the study. This control group had no self-reported history of cancer at any site and was included from among those undergoing a physical examination at the hospital. Both case and control groups were reviewed for exposure history to tobacco consumption. The staging of urothelial carcinoma was according to the American Joint Committee on Cancer (AJCC) system, including the primary tumor extent, lymph node involvement, and distant organ metastasis status at the moment of disease diagnosis. Cancer cell differentiation was determined by histopathologic grading and examination by a pathologist. Tumors were classified as superficial tumors (pT0~1, n = 165) or invasive tumors (pT2~4, n = 107). Metastasis into lymph nodes was detected in 28 cases (10.3%), and four patients (1.5%) had distal metastasis. The study was approved by the Institutional Review Board (IRB) of Taichung Veteran General Hospital (IRB no. CF11094), and informed written consent was obtained from each participant. Whole-blood specimens collected from controls and UCC patients were placed in tubes containing ethylenediaminetetraacetic acid (EDTA), immediately centrifuged, and then stored at -80 °C.

Genomic DNA extraction and MTNR1A polymorphism selection

Genomic DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) based on the manufacturer’s instructions as previously described [33]. In this study, we selected three SNPs of the MTNR1A gene from data of the International HapMap Project as previously described [32]. We included -184T/C (rs2119882) in the promoter region. Rs13140012 and rs6553010, which are located in intron 1 of MTNR1A, were selected in this study since these two SNPs were found to modify the binding affinities of several transcription factors [30].

Real-time polymerase chain reaction (PCR)

Allelic discrimination for the MTNR1A SNPs,
rs2119882 (Assay ID: C_16100974_10), rs13140012 (Assay ID: C_31861431_10), and rs6553010 (Assay ID: C_11782809_10), were assessed using a TaqMan assay with an ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and further analyzed with SDS vers. 3.0 software (Applied Biosystems, Foster City, CA, USA). The final volume for each reaction was 5 µL, containing 2.5 µL TaqMan Genotyping Master Mix, 0.125 µL TaqMan probe mix, and 10 ng genomic DNA. The real-time PCR consisted of initial denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and finally at 60 °C for 1 min.

Statistical analysis

We compared differences in demographic characteristics between urothelial carcinoma patients and the controls using the Mann-Whitney U-test and Fisher’s exact test. A goodness-of-fit X²-test was used to assess Hardy-Weinberg equilibrium (HWE) for biallelic markers. The odds ratios (ORs) and 95% confidence intervals (CIs) of the risk association of genotype distributions with clinical and histopathological characteristics were evaluated using multiple logistic regression models. A p value of <0.05 was interpreted as being statistically significant. Data were analyzed with SAS statistical software (SAS Institute, Cary, NC, USA).

Results

Statistical analyses of demographic characteristics of both the case and control groups are shown in Table 1. We found no significant differences in distributions of age, gender, or tobacco use between control participants and UCC patients. Our study population was predominantly male (62.9%) with a low proportion of smokers (25.3% in the control and 28.5% in the UCC group). Most patients (60.7%) were suffering from superficial tumors (stage pTa~pT1).

In our recruited control group, the genotype distributions of MTNR1A SNPs rs2119882 and rs13140012 met the Hardy-Weinberg equilibrium in the normal controls (p=0.449, χ² value: 0.574 and p=0.560, χ² value: 0.340, respectively). Reconstructed linkage disequilibrium (LD) plots for the three SNPs were previously shown [32]. The genotype distributions and associations between UCC and gene polymorphisms of MTNR1A are shown in Table 2. The distribution of MTNR1A genotypes revealed that the most frequent alleles were heterozygous T/C and A/T for the rs2119882 and rs13140012 loci, respectively, and homozygous A/A for the rs6553010 locus. There were no significant differences in genotype distributions or associations between urothelial carcinoma patients and the controls for the rs2119882, rs13140012, or rs6553010 SNPs (Table 2).

| Variable | Controls (N=272) | Patients (N=272) | p value |
|----------|----------------|----------------|---------|
| Age (yrs) | Mean ± S.D. | Mean ± S.D. | p=0.349 |
| Gender | Male | 181 (66.5%) | 179 (65.8%) | 0.856 |
| | Female | 91 (33.5%) | 93 (34.2%) | |
| Tobacco consumption | No | 201 (73.9%) | 196 (72.1%) | 0.629 |
| | Yes | 71 (26.1%) | 76 (27.9%) | |
| Stage | Superficial tumor (pTa-pT1) | 165 (60.7%) | 107 (39.3%) | |
| | Invasive tumor (pT2-pT4) | 107 (39.3%) | 165 (60.7%) | |

Table 1. The distributions of demographical characteristics in 272 controls and 272 urothelial cell carcinoma patients.

| Variable | Controls (N=544) | Patients (N=544) | OR (95% CI) |
|----------|----------------|----------------|-------------|
| rs2119882 | n (%) | n (%) | |
| TT | 113 (41.5%) | 108 (39.7%) | 1.00 |
| TC | 129 (47.4%) | 119 (43.8%) | 0.965 (0.672~1.387) |
| CC | 30 (11.1%) | 45 (16.5%) | 1.569 (0.922~2.672) |
| TC+CC | 159 (58.5%) | 164 (60.3%) | 1.079 (0.766~1.520) |
| rs13140012 | n (%) | n (%) | |
| AA | 111 (40.8%) | 103 (37.9%) | 1.00 |
| AT | 122 (44.9%) | 130 (47.8%) | 1.148 (0.797~1.654) |
| TT | 39 (14.3%) | 39 (14.3%) | 1.078 (0.642~1.810) |
| AT+TT | 161 (59.2%) | 169 (62.1%) | 1.131 (0.802~1.596) |
| rs6553010 | n (%) | n (%) | |
| A | 344 (63.2%) | 336 (61.8%) | 1.00 |
| T | 200 (36.8%) | 208 (38.2%) | 1.065 (0.833~1.361) |

Table 2. Distribution frequencies of MTNR1A genotypes in 272 controls and 272 urothelial cell carcinoma patients

The odds ratios (ORs) and with their 95% confidence intervals (CIs) were estimated by logistic regression models.
As shown in Tables 3–5, we further analyzed genotype frequencies of individual polymorphisms with relevance to the clinicopathological status, including the cancer stage, tumor status, lymph node involvement, metastatic status, and histopathologic grading, in UCC patients. We classified UCC patients into two subgroups. In the first subgroup, patients had homozygous wild-type (WT) alleles; in the other subgroup, they had at least one polymorphic allele. No significant associations of the rs2119882 or rs13140012 gene polymorphisms with the clinicopathologic status were observed (Tables 3, 4). However, subjects with at least one G allele of rs6553010 (AG+GG) exhibited a significantly (p = 0.026) higher risk of 1.768-fold (95% CI: 1.041–2.063) of having the invasive type of UCC compared to their corresponding WT homozygotes (Table 5).

Discussion

Recently, many studies have shown that polymorphisms of MTNR genes (MTNRIA and MTNRIB) may affect susceptibility to several diseases, such as adolescent idiopathic scoliosis [34], coronary artery disease [29], type 2 diabetes mellitus [35], calcium nephrolithiasis [30], and polycystic ovary syndrome [31], and also influence the clinicopathologic development of oral cancer [32]. In the present study, we investigated associations of variants of the MTNRIA gene with the UCC risk and its clinicopathologic development in a Taiwanese population.

Table 3. Distribution frequencies of the clinical status and MTNRIA rs2119882 genotype frequencies of 272 patients with urothelial cell carcinoma

| Variable             | MTNRIA (rs2119882) |
|----------------------|--------------------|
|                      | TT (%) | TC+CC (%) | OR (95% CI) | p value |
| Stage                |         |          |            |         |
| Superficial tumor (pTa~pT1) | 72 (66.7%) | 93 (56.7%) | 1.00 |
| Invasive tumor (pT2~pT4) | 36 (33.3%) | 71 (43.3%) | 1.527 (0.921–2.531) | 0.100 |
| Tumor T status       |         |          |            |         |
| T0                   | 33 (30.6%) | 41 (25.0%) | 1.00 |
| T1–T4                | 75 (69.4%) | 123 (75.0%) | 1.320 (0.769–2.267) | 0.314 |
| Lymph node status    |         |          |            |         |
| N0                   | 99 (91.7%) | 145 (88.4%) | 1.00 |
| N1+N2                | 9 (8.3%) | 19 (11.6%) | 1.441 (0.626–3.316) | 0.388 |
| Metastasis           |         |          |            |         |
| M0                   | 107 (99.1%) | 161 (98.2%) | 1.00 |
| M1                   | 1 (0.9%) | 3 (1.8%) | 1.994 (0.205–19.421) | 0.545 |
| Histopathologic grading |     |          |            |         |
| Low grade            | 11 (10.2%) | 28 (17.1%) | 1.00 |
| High grade           | 97 (89.8%) | 136 (82.9%) | 0.551 (0.262–1.160) | 0.113 |

OR, odds ratio; CI, confidence interval.

Table 4. Distribution frequencies of the clinical status and MTNRIA rs13140012 genotype frequencies in 272 patients with urothelial cell carcinoma

| Variable             | MTNRIA (rs13140012) |
|----------------------|---------------------|
|                      | AA (%) | AT+TT (%) | OR (95% CI) | p value |
| Stage                |         |          |            |         |
| Superficial tumor (pTa~pT1) | 69 (67.0%) | 96 (56.8%) | 1.00 |
| Invasive tumor (pT2~pT4) | 34 (33.0%) | 73 (43.2%) | 1.543 (0.926–2.573) | 0.095 |
| Tumor T status       |         |          |            |         |
| T0                   | 29 (28.2%) | 45 (26.6%) | 1.00 |
| T1–T4                | 74 (71.8%) | 124 (73.4%) | 1.080 (0.624–1.869) | 0.784 |
| Lymph node status    |         |          |            |         |
| N0                   | 94 (91.3%) | 150 (88.8%) | 1.00 |
| N1+N2                | 9 (8.7%) | 19 (11.2%) | 1.323 (0.575–3.046) | 0.510 |
| Metastasis           |         |          |            |         |
| M0                   | 102 (99.0%) | 166 (98.2%) | 1.00 |
| M1                   | 1 (1.0%) | 3 (1.8%) | 1.843 (0.189–17.960) | 0.593 |
| Histopathologic grading |     |          |            |         |
| Low grade            | 10 (9.7%) | 29 (17.2%) | 1.00 |
| High grade           | 93 (90.3%) | 140 (82.8%) | 0.519 (0.242–1.116) | 0.089 |

OR, odds ratio; CI, confidence interval.

Table 5. Distribution of the clinical status and MTNRIA rs6553010 genotype frequencies in 272 patients with urothelial cell carcinoma

| Variable             | MTNRIA (rs6553010) |
|----------------------|---------------------|
|                      | AA (%) | AG+GG (%) | OR (95% CI) | p value |
| Stage                |         |          |            |         |
| Superficial tumor (pTa~pT1) | 78 (68.4%) | 87 (55.1%) | 1.00 |
| Invasive tumor (pT2~pT4) | 36 (31.6%) | 71 (44.9%) | 1.768 (1.068–2.928) | 0.0026* |
| Tumor T status       |         |          |            |         |
| T0                   | 32 (28.1%) | 42 (26.6%) | 1.00 |
| T1–T4                | 82 (71.9%) | 116 (73.4%) | 1.078 (0.628–1.849) | 0.786 |
| Lymph node status    |         |          |            |         |
| N0                   | 101 (88.6%) | 143 (90.5%) | 1.00 |
| N1+N2                | 13 (11.4%) | 15 (9.5%) | 0.815 (0.372–1.787) | 0.609 |
| Metastasis           |         |          |            |         |
| M0                   | 112 (98.2%) | 156 (98.7%) | 1.00 |
| M1                   | 2 (1.8%) | 2 (1.3%) | 0.718 (0.100–5.174) | 0.741 |
| Histopathologic grading |     |          |            |         |
| Low grade            | 13 (11.4%) | 26 (16.5%) | 1.00 |
| High grade           | 101 (88.6%) | 132 (83.5%) | 0.653 (0.320–1.335) | 0.241 |

OR, odds ratio; CI, confidence interval.

Although MTNRIA gene SNPs (rs2119882, rs13140012, and rs6553010) alone did not contribute to UCC susceptibility in our study, a major finding of this study was the significant association between rs6553010 MTNRIA genotypes and the clinicopathological development of UCC. We observed that the frequency of the A/G and G/G combined genotypes was greater in patients with invasive UCC (44.9%) than in the controls (31.6%). This finding is similar to findings from our previous study regarding genetic polymorphisms of MTNRIA which alone were unable to predict the risk of oral
cancer. However, after being combined with information on carcinogen exposure, a significant effect for predicting oral-cancer susceptibility was observed [32]. It is well-known that tobacco smoking is also the leading risk factor for developing bladder cancer. From a meta-analysis of smoking's effects on bladder cancer, there was an association of increased risks (odds ratios) of about 4.23 for male smokers and 1.35 for female smokers [36]. However, after being combined with information on carcinogen exposure, genetic polymorphisms of MTNR1A still could not predict UCC susceptibility in our recruited populations. This might have been due to a bias in the ratio of individuals with a smoking habit among our recruited UCC patients. In previous SNP-related studies of UCC, significantly higher ratios of individuals with a smoking habit among UCC patients than in the controls were observed [37]. However, we found no significantly different distributions of tobacco use between control participants and UCC patients in this study. In our future work, more UCC patients with a smoking habit should be recruited to further explore the combined effect of MTNR1A genetic variants and exposure to tobacco carcinogens on the risk of UCC.

Melatonin was demonstrated to exert oncostatic effects including antimetastatic activity both in vivo and in vitro in various types of malignancies via the MTNR1A receptor [17-19, 38-40]. Expression of MTNR1A in cancer cells seems to increase the efficacy of melatonin's oncostatic activity. The expression level of MTNR1A was inversely correlated with the invasive abilities of breast cancer cell lines [38]. In clinical specimens, MTNR1A messenger (m)RNA expression was negatively correlated with the malignancy grade of invasive ductal breast carcinomas (IDC). Moreover, higher MTNR1A expression was associated with patients' longer overall survival (OS) in patients with estrogen receptor positive (ER+) breast cancers who were treated with tamoxifen. MTNR1A was recognized as an independent prognostic factor in ER+ tumors for OS and disease-free survival in ER+ tumors [26]. Those results indicated that the MTNR1A expression level might affect the invasive ability of breast cancer. In our study, we found that patients with one G allele of MTNR1A rs6553010 had higher risks of developing advanced invasive UCC than those with the WT. Although we still have no evidence that MTNR1A expression can affect the invasive ability of UCC, the intrinsic rs6553010 SNP is itself a functional polymorphism that exerts a direct effect on MTNR1A gene expression in patients with UCC. Several reports previously indicated that an intrinsic SNP can affect gene expressions in different diseases and also affect the susceptibility or metastasis in different cancers including UCC [41-45]. Previous reports indicated that some intrinsic polymorphic variants can induce either alternative or aberrant splicing of mRNA and further affect gene expressions [46]. Moreover, Esposito et al. indicated that another SNP, rs13140012 (A>T mutation), in intron 1 of the MTNR1A gene can affect the binding affinity of several transcriptional factors [30]. The promoter activity assay in oral cancer showed that a fragment containing exon 1 and intron 1 within the MTNR1A gene showed remarkable transcriptional activity [27]. We assumed that intrinsic the A/G SNP rs6553010 may act alone or in combination with other yet unidentified functional variants in the gene to influence MTNR1A expression.

Despite our best efforts, a significant proportion of patients suffering from UCC will develop advanced disease, and we do not currently have sufficiently reliable tools to predict who these patients are. In this study, we found a significant association between the invasive UCC type and the rs6553010 A/G and G/G combined genotypes. The rs6553010 G allele may act as a risk factor. In order to precisely evaluate MTNR1A polymorphisms and clinicopathological development of UCC, a much-larger sample size is needed. Ultimately, we suggest that future studies of the functional activities of these polymorphisms and their effects on tumor invasion would help us understand the underlying mechanisms in UCC development.

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Competing Interests

The authors have declared that no competing interest exists.

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