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Original article

Analysis of *hOGG1* genotype as a prognostic marker for muscle invasive bladder cancer: A novel approach using peptide nucleic acid-mediated, real-time PCR clamping

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Abstract

Objective: DNA damage repair mechanisms are a source of genetic mutation and are believed to play an important role in human cancer. Human 8-oxoguanine DNA glycosylase 1 (hOGG1) is involved in the recognition and repair of DNA damage. The value of the *hOGG1* genotype as a prognostic indicator for bladder cancer (BC) was assessed using a novel technological approach.

Materials and methods: The association between genetic polymorphisms of *hOGG1* codon 326 and clinicopathologic characteristics of 337 patients with BC was analyzed using peptide nucleic acid (PNA)-mediated real-time PCR clamping.

Results: Tumor grade and size were significantly associated with the *hOGG1* codon 326 genotype in non-muscle-invasive bladder cancer (NMIBC). The Cys326Cys polymorphism was significantly associated with progression and cancer specific survival in patients with muscle-invasive bladder cancer (MIBC). Multivariate Cox regression analysis indicated that the *hOGG1* Cys326Cys polymorphism is associated with a protective effect on progression and a more dominant survival benefit than the Ser326Ser polymorphism in MIBC (hazard ratio 0.284 and 0.305, respectively).

Conclusions: Analysis of genotypes and clinical data for 337 BC patients indicates that the *hOGG1* genotype may be a useful prognostic genetic marker for MIBC.

Keywords: Urinary bladder neoplasms; DNA damage repair mechanisms; *hOGG1*; Biological tumor markers; Prognosis; PNA

1. Introduction

Bladder cancer (BC) can be of two types, non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC), depending on pathological findings. Recurrence and progression are the most serious risks following treatment of NMIBC, whereas local invasion and distant metastasis are life-threatening issues in patients with MIBC. Numerous factors are involved in cancer recurrence, progression, and patient survival, including both environmental and hereditary factors [1]. Many agents can attack cellular DNA, thereby initiating tumorigenesis and promoting disease recurrence, progression, and metastasis [2]. Humans have acquired DNA repair mechanisms to repair DNA that is damaged by these agents [3,4].

DNA lesions that contain the mutagenic base 8-oxoguanine are repaired by base excision repair [5]. Human 8-oxoguanine DNA glycosylase 1 (*hOGG1*) encodes a DNA glycosylase that catalyzes the excision of 8-oxoguanine from oxidatively damaged DNA [6,7]. A Ser326Cys polymorphism of *hOGG1* has been identified in bladder, lung, and esophageal cancers [8–10].

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The use of peptide-nucleic-acid (PNA) oligomers was introduced as a way to detect trace amounts of mutant DNA [11]. PNAs are non-extended oligonucleotides in which the ribose-phosphate backbone is replaced by 2-aminoethyl glycine units linked through amide bonds. In PNA-mediated PCR clamping, PNA oligomers suppress the amplification of the complementary strand of the sequence amplified by a pair of DNA oligonucleotide primers because PNAs are not substrates of DNA polymerase. The PNA-clamped probe assay is more sensitive than direct sequencing, with the ability to detect mutations in samples containing less than 1% mutant alleles [12,13]. This novel method has been used most successfully to detect K-*ras* mutations in various cancers [14,15].

We previously reported that in patients with NMIBC, the *hOGG1* genotype is related to recurrence. The *hOGG1* Ser326Ser and Ser326Cys polymorphisms were identified as risk factors for recurrence in NMIBC compared with Cys326Cys [8]. We carried out a long-term follow-up study of patients with BC to assess whether *hOOG1* genotype influenced the clinical course of disease. Data were drawn from a previous study population as well as new cases, and the genotype was analyzed using a novel PNA-mediated, real-time PCR clamping method.

2. Materials and methods

2.1. Study population

A total of 377 patients with transitional cell carcinoma of the urinary bladder were recruited from Chungbuk National University Hospital. To delineate a more homogenous study population, patients with concomitant carcinoma in situ (CIS), short-term follow-up periods (less than 6 months), and incomplete data were excluded. Within the study population, 264 patients had primary NMIBC and 113 had MIBC. A blood sample (5 ml) was collected from each patient into 0.1 ml of EDTA, and then frozen in liquid nitrogen and stored at −80°C until use. The collection and analysis of samples was approved by the local institutional review board and informed consent was obtained from each subject.

Tumors were staged according to the 2002 TNM classification and the 1973 WHO grading systems [16,17]. For cases of NMIBC, transurethral resection (TUR) of the tumor was performed. A second TUR was performed 2 to 4 weeks after the initial resection when a BC specimen did not include proper muscle or when a high-grade tumor was detected [16]. Patients who had multiple tumors, large tumors (≥3 cm in diameter), or high grade NMIBC received 1 cycle of intravesical treatment (bacillus Calmette-Guérin or mitomycin-C) [16,18]. Response to treatment was assessed by cystoscopy and urinary cytology. Patients who were free of disease at 3 months after commencement of treatment were assessed every 3 months for the first 2 years and every 6 months thereafter [16,18]. Recurrence was defined as relapse of primary NMIBC of a lower or equivalent pathologic stage, and progression was defined as disease of a higher TNM stage upon relapse.

Patients with MIBC and good performance status (ECOG 0 or 1), underwent radical cystectomy and complete pelvic lymph node dissection. On the other hand, patients who were not eligible for radical cystectomy such as metastatic stage, poor life expectancy, or poor performance status (ECOG more than 2) underwent TUR or biopsy for histopathologic diagnosis. Patients with pT3, pT4, or node-positive disease based on the analysis of radical cystectomy specimens, or with metastatic stage with good performance status, received at least 4 cycles of cisplatin-based chemotherapy. However, patients with poor general condition, old age, and reluctant to chemotherapy could not receive chemotherapy. MIBC patients with radiation therapy for any reason were excluded in this study. Each patient was followed and managed according to standard recommendations [19].

2.2. Genomic DNA extraction

Genomic DNA was extracted from human whole blood using a genomic DNA purification kit (Promega, Madison, WI). DNA was precipitated using isopropanol and then washed with 70% ethanol. The quality of DNA was assessed by agarose gel electrophoresis. Genomic DNA samples were stored at −20°C until use.

2.3. PNA-mediated real-time PCR clamping method

All PNA oligomers were synthesized and purified using HPLC (Panagene, Daejeon, Korea). A specific 120 base pair genomic fragment from *hOGG1* exon 7 was amplified using the set of primers listed in Table 1. A Rotor-Gene 6000 (Corbett Research, Mortlake, Australia) was used for real-time PCR. PCR reactions were performed in a final volume of 20 μl containing 10 μl of 2 × SYBR GREEN master mix (Takara Bio Inc., Otsu, Japan), 1 μl of each of the forward and reverse primers (10 pmol/μl), and 8 μl of genomic DNA (2.5 ng/μl). To identify the codon 326 genotype, samples were analyzed without PNA probe, and with 0.1 μM of Ser- or Cys-specific PNA probe (3 different reactions for each sample). PCR thermal cycling parameters were as

Table 1
DNA sequences of the PCR primers and PNA probes

Name	Sequence
hOGG1-F	TCAGTGCCGACCTGCGCCAA
hOGG1-R	AGGTGCTTGGGGAATTTCTT
Ser PNA probe	TGCGCCAATCCCGCCATG
Cys PNA probe	TGCGCCAATGCCGCCATGC

hOGG1 = human 8-oxoguanine DNA glycosylase 1; F = forward; R = reverse; PNA = peptide-nucleic-acid.