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XRCC1 Arginine194Tryptophan and GGH-401Cytosine/Thymine polymorphisms are associated with response to platinum-based neoadjuvant chemotherapy in cervical cancer

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Abstract

Objective. The objective of this study was to evaluate the association of single nucleotide polymorphisms (SNPs) of genes which are involved in DNA synthesis and repair with the response to platinum-based neoadjuvant chemotherapy (NAC) and disease-free survival (DFS) in patients with cervical cancer who were treated with NAC followed by radical hysterectomy.

Methods. A retrospective review was performed on 66 patients with cervical cancer who were treated with NAC followed by radical hysterectomy in our institute between January 1999 and February 2007. DNA was extracted from the paraffin-embedded, formalin-fixed tissue blocks of hysterectomy specimens. The genotypes of SNPs (MTHFR 677Cytosine/Thymine, XRCC1 Arginine194Tryptophan, GGH-401Cytosine/Thymine, and GSTP1 Isoleucine105Valine) were determined using a single base primer extension assay. The association of SNP genotypes with the response to NAC, which was measured by physical and colposcopic examinations, was evaluated. In addition, DFS based on SNP genotypes was examined.

Results. The genotypes of XRCC1 Arginine194Tryptophan and GGH-401Cytosine/Thymine were significantly associated with the response to NAC ($P = 0.023$ for XRCC1 Arginine194Tryptophan; $P = 0.046$ for GGH-401Cytosine/Thymine). However, the genotypes of MTHFR 677Cytosine/Thymine and GSTP1 Isoleucine105Valine were not associated with the response to NAC. In subgroup analysis with 39 patients who were treated with regimens containing 5-fluorouracil (5-FU), the genotypes of GGH-401Cytosine/Thymine were significantly associated with the response to NAC ($P = 0.039$). In multifactor dimensionality reduction (MDR) analysis, the combination of XRCC1 Arginine194Tryptophan and GGH-401Cytosine/Thymine genotypes was associated with the response to NAC ($P < 0.001$). However, no SNP genotypes were associated with DFS, but the cisplatin dose intensity of NAC was associated with DFS.

Conclusions. The genotypes of XRCC1 Arginine194Tryptophan and GGH-401Cytosine/Thymine were associated with the response to NAC in patients with cervical cancer. However, no SNP genotypes were associated with DFS.

Keywords: Polymorphism; Single nucleotide; Uterine cervical neoplasms; Neoadjuvant therapy; Drug therapy; Dose-response relationship; Drug

Introduction

Although it is still an experimental treatment, there is a trend to use neoadjuvant chemotherapy (NAC) prior to surgery for an improvement in respectability rate and survival in bulky cervical cancer [1]. Several studies have been conducted to explore the feasibility of NAC followed by hysterectomy in stage 1B cervical cancer [2–5]. According to a recent GOG trial comparing NAC followed by surgery with surgery alone, the progression-free and overall survivals were not different between two groups [3]. However, another trial demonstrated

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a survival advantage of NAC in stage 1B cervical cancer [5]. Although we do not have an explanation for these conflicting results, some studies have suggested that the proper selection of patients for NAC could be important [6,7].

As a way to select patients who will respond well to NAC, we have been focusing on the single nucleotide polymorphisms (SNPs) of genes which are involved in DNA synthesis and repair. In our previous study, we reported that the genotypes of XRCC1 Arginine399Glutamine and SLC19A1 6318Cytosine/Thymine were associated with a response to platinum-based NAC in patients with bulky cervical cancer [8]. However, there were other SNPs which were not associated with a response to NAC in our previous study. We hypothesized that some SNPs did not reach statistical significance due to the small sample size. Therefore, we re-evaluated the association of SNPs from our previous study with the response to NAC in larger samples. Two folate metabolism-related SNPs (GGH-401Cytosine/Thymine and MTHFR 677Cytosine/Thymine), which were weakly associated with the response to NAC in our previous study, were chosen for this study. In addition, the XRCC1 Arginine194Tryptophan SNP, which was associated with the response to platinum agent in non-small cell lung cancer [9], and the GSTP1 Isoleucine105Valine SNP, which was correlated with disease-free survival (DFS) in ovarian cancer [10], were selected for this study.

The objective of this study was to evaluate the association of SNPs of genes which are involved in DNA synthesis and repair with the response to platinum-based NAC. In addition, we examined the association of SNP genotypes with DFS in patients with cervical cancer who were treated with NAC followed by radical hysterectomy.

Materials and methods

Subjects

We performed a retrospective review on patients with cervical cancer who were treated with NAC followed by radical hysterectomy in our institute between January 1999 and February 2007. Among 70 patients who were identified, two patients with a small, endophytically-growing tumor were excluded because the tumor size was not measurable by physical and colposcopic examinations. In addition, another two patients were excluded because the records on tumor size in preoperative examination were unavailable. For the remaining 66 patients, the associations of SNP genotypes with the response to NAC and DFS were evaluated.

Evaluation of the response to NAC

The response to NAC was estimated by the change in tumor size, which was measured by physical and colposcopic examinations at the initial and preoperative visits. Specifically, before each cycle of NAC and surgery were performed, the largest diameter of tumor was measured by physical and colposcopic examinations, respectively. When the diameter measured by physical examination was different from that measured by colposcopic examination, the larger one was chosen as the tumor size. Using RECIST criteria, the response was graded as follows: complete response (eradication of the cervical lesion), partial response (at least a 30% decrease in the longest diameter of the cervical lesion), stable disease (neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease), and progressive disease (at least a 20% increase in the longest diameter of the cervical lesion). Patients with complete or partial responses were classified as good responders and patients with stable or progressive disease were regarded as poor responders.

Sample collection and genotyping

DNA was extracted from the paraffin-embedded tissue blocks of hysterectomy specimens.

The genotyping was analyzed by a single base primer extension assay using the SNaPShot assay kit (ABI, Foster City, CA, USA), according to the manufacturer's recommendation. The genomic DNA region containing the SNP was amplified with a PCR reaction. Each PCR reaction contained the following: 10.0 ng of DNA, 1× PCR buffer, 0.125 U of *AmpliTaq Gold* DNA polymerase (ABI, Foster City, CA, USA), 3.0 mM $MgCl_2$, 0.25 mM of each dNTP, and 0.5 pmol of each primer in 10 μl reaction volume. Reactions were incubated at 95 °C for 10 min, then cycled 30 times at 95 °C for 30 s, 60 °C for 1 min, and 72 °C for 1 min, followed by 72 °C for 5 min. The characteristics of the SNPs and primers are shown in Table 1.

Table 1
Characteristics of SNP and PCR primers

SNP	rs #	Strand	Primer	Sequence (5′–3′)
MTHFR 677Cytosine/Thymine	1801133	Reverse	Forward primer	AAGCAGGGAGCTTTGAGGC
			Reverse primer	CAAGTGATGCCCATGTCG
			Genotyping primer	GAAAAGCTGCGTGATGATGAAATCG
XRCC1 Arginine194Tryptophan	1799782	Forward	Forward primer	AGGATGAGAGCGCCAACT
			Reverse primer	TACTCACTCAGGACCCACGT
			Genotyping primer	GAGGCCGGGGGCTCTCTTCTTCAGC
GGH-401Cytosine/Thymine	3758149	Reverse	Forward primer	GAATCCCCTGCCAGCCT
			Reverse primer	TCAACTGTTACGTCGATGTGG
			Genotyping primer	CCCAGGTCCTCGAGAGG
GSTP1 Isoleucine105Valine	1695	Forward	Forward primer	GGTGGACATGGTGAATGACG
			Reverse primer	GGCACAAGAAGCCCCTTTC
			Genotyping primer	GTGGAGGACCTCCGCTGCAAATAC