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Genetic variations in the PI3K/AKT pathway predict platinum-based neoadjuvant chemotherapeutic sensitivity in squamous cervical cancer

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ABSTRACT

Aims: Cervical cancer is one of the most frequent malignant tumours in women. The PI3K/Akt pathway plays a role in chemoresistance to platinum-based neoadjuvant chemotherapy (NAC). The objective of this study was to evaluate the association between genetic polymorphisms in the PI3K/Akt pathway and chemotherapeutic outcomes following platinum-based NAC in Northwestern Chinese Han patients with squamous cervical cancer (SCC).

Main methods: In total, 17 tagging single nucleotide polymorphisms (tSNPs) in four genes (PIK3CA, Akt1, Akt2, PTEN) were identified as being associated with chemotherapeutic response in 259 patients with stage IB_2-IIB SCC. Each of these patients received more than two cycles of NAC. These tSNPs were genotyped by the Sequenom MassArray system.

Key findings: The heterozygous genotype of two loci in the PIK3CA gene (rs3729679: uncorrected P = 0.022 and rs12494623: uncorrected P = 0.018) was associated with an increased risk of chemoresistance in SCC patients. The stratified analysis also showed that these same SNP polymorphisms were associated with a poor response to NAC in the cisplatin-based subgroup. Furthermore, NAC non-responders had a higher frequency of the rs10416620 and rs62107593 G alleles in the Akt2 gene (rs10416620 and rs62107593: uncorrected P = 0.037). The rs34716810 A allele was associated with a poor response to chemotherapy (uncorrected P = 0.037). Moreover, rs2498786 (uncorrected P = 0.036) and the GGCC haplotype of polymorphisms in Akt1 showed a high risk for non-response to NAC (uncorrected P = 0.018).

Significance: The findings from this study demonstrate that genetic polymorphisms in the PI3K/Akt pathway are associated with sensitivity to platinum-based chemotherapy in SCC patients.

1. Introduction

Cervical cancer continues to be a major public health problem in developing countries [1]. Reports estimate that there are approximately 500,000 new cases of cervical cancer diagnosed each year. Most of these new cases occur in developing countries, and 70% are diagnosed at an advanced stage [2]. According to the Clinical International Federation of Gynecology and Obstetrics (FIGO) staging system, local or bulky tumours measuring larger than 4 cm are defined as advanced cervical cancer [3]. Advanced cervical cancer has long been recognized as a high-risk disease due to frequent recurrence and poor prognosis [4]; however, a consensus in standard clinical care has yet to be reached. Recently, neoadjuvant chemotherapy (NAC) prior to surgery or radiotherapy has been investigated as a new therapeutic strategy for bulky or locally advanced disease. The potential advantages of NAC have been reinforced due to its effectiveness in shrinking tumor size and controlling micrometastasis, thereby improving survival and quality of life in patients with this disease [5].

Currently, there is no standardized regimen for NAC. The combination of paclitaxel and platinum-based drugs is considered the most effective regimen in chemotherapy for cervical cancer [6]. Although this treatment regimen is effective as a first-line treatment, most patients develop resistance to these drugs. Multiple mechanisms have been proposed in platinum-based resistance, including reduced accumulation of the drug, increased levels of glutathione and metallothionein, and enhanced DNA repair [7]. Nevertheless, platinum-based resistance cannot be fully explained by the above-mentioned mechanisms. In the present

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research, signalling through the PI3K/Akt pathway may be involved in driving platinum resistance. The PI3K/AKT pathway activates cellular growth and survival by binding to tyrosine kinase receptors, including the epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and fibroblast growth factor receptor (FGFR) [8,9]. These receptors then activate PI3K resulting in cell survival, growth and angiogenesis signals. PTEN negatively regulates the PI3K/Akt pathway by catalysing the dephosphorylation of PI (3, 4, 5) and P3 (3–5). Loss of PTEN activity leads to continuous activation of the PI3K/AKT signalling pathway [10].

Recently, it was reported that genetic variations within the PI3K/Akt pathway are associated with different responses to certain therapeutic approaches in a variety of cancers [11–16]. Specifically, genetic aberrations, such as PIK3CA mutations and/or PTEN loss/mutations, were suggested to influence therapeutic sensitivity in cervical cancer patients [17–20]. Because of the varied response to platinum-based chemotherapy in patients with squamous cervical cancer (SCC), there is a need for efficient biomarkers to predict who will benefit from the chemotherapy while avoiding the development of adverse events. In this study, we determined the association of genetic polymorphisms in Akt1, Akt2, PIK3CA (catalytic subunit of PI3K), and PTEN with clinical outcomes in SCC patients treated with platinum-based NAC.

2. Material and methods

2.1. Subjects

In total, 259 patients were diagnosed with SCC by experienced gynecologists, and histologically confirmed SCC cases were treated with primary platinum-based (carboplatin or cisplatin) combination chemotherapy. All of the patients were recruited from the First and Second Hospital of Lanzhou University and the People's Hospital of Gansu Province between November 2010 and July 2012. All of the subjects were ethnically classified as Han Chinese from the Gansu Province and its surrounding region. Peripheral blood specimens for genetic analysis were collected from each patient at the time of diagnosis prior to chemotherapy. NAC was applied to these patients, including FIGO stage IB2–IIB tumours [21], and each of the patients received more than two cycles of NAC. The NAC regimens were 175 mg/m^2 paclitaxel followed by either 70 mg/m^2 cisplatin or carboplatin at an area under the curve (AUC) of 5 on day 1 of one cycle. The pathological diagnosis following the operations was assessed as high and/or moderately differentiated SCC. All participants were informed about the methods and aims of this study and provided written consent to participate. The study was approved by the Committee for Ethical Affairs of Lanzhou University.

2.2. Evaluation of the response to NAC

The short-term (~15 days of treatment) response to NAC was estimated by the change in tumor size, which was measured by physical examinations at the beginning and end of each preoperative chemotherapy cycle. Using Response Evaluation Criteria in Solid Tumours (RECIST) criteria [22], the response was classified 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 NAC responders, and patients with stable or progressive disease were considered NAC non-responders.

2.3. DNA preparation

Whole blood samples were collected using ethylenediamine tetraacetic acid-K (EDTA-K)-treated tubes. Genomic DNA was extracted using the Human Whole Blood Genomic DNA Extraction Kit (Tian Gen Ltd., Beijing, China) according to the manufacturer's protocol. After extraction, the genomic DNA was diluted to a final concentration of 20 ng/μl for the genotyping assays.

2.4. SNP selection and genotyping

Candidate tag single nucleotide polymorphisms (tSNPs) were selected as target SNPs from four genes: PTEN, Akt1, Akt2 and PIK3CA. Tag SNPs were screened using Haploview software v4.2 (Mark Daly's lab of Broad Institute, Cambridge, MA, USA) according to the Han Chinese in Beijing (CHB) data set (URL: http://www.hapmap.org/index.html.en) with an r^2 threshold of 0.8 and a minor allele frequency of >0.1. Functional SNP selection was determined using the web portal (http://snpinfo.niehs.nih.gov) and regions of the gene that were considered included the promoter, 3′UTR, 5′UTR and exonic regions. Sequenom's MassArray Designer (Sequenom, San Diego, CA, USA) was used to design PCR and extension primers for each SNP. Finally, nineteen tSNPs were selected to represent genetic variation in the pathway. Primer information for the selected tSNPs is listed in Supplementary Table 1. Genotyping was performed at Bomiao Tech (Beijing, China) using iPlex Gold Genotyping Assay and Sequenom MassArray. Laboratory personnel were blinded to the identity and source of the DNA sample.

2.5. Statistical analysis

The allele and genotype frequencies of the tSNPs were obtained by direct counting. The association between the SNP genotypes and NAC response was assessed by the Pearson's Chi-squared test. The Hardy–Weinberg equilibrium (HWE) values were calculated for the SNPs. Odds ratios (ORs) and corresponding 95% confidence intervals (95% CI) were used to evaluate the associations between genetic polymorphisms and risk of chemoresistance. Statistical analysis was performed with SPSS for Windows (version 18.0; SPSS Inc., Chicago, Illinois, USA). All P values were two-tailed, and P values less than 0.05 were considered statistically significant. Moreover, P values were corrected with the Bonferroni correction by multiplying with N (the number of tSNPs in one gene). Haplotype constructions were analyzed using SHEsis, a

Table 1
Patient characteristics.

Characteristic	SCC patients	%
Total number	259	
Age		
Range	28–75	
Mean	48.89	
SD	9.18	
FIGO stage		
Ib2	53	20.5
IIA+ IIB	206	79.5
NAC regimen		
Cisplatin + paclitaxel	149	57.5
Carboplatin + paclitaxel	110	42.5
Response to NAC		
NAC responders (CR + PR)	168	64.9
NAC non-responders (SD + PD)	91	35.1

SCC, squamous cervical cancer; FIGO, International Federation of Gynecology and Obstetrics; CR, complete response; PR, partial response; SD, stable disease; and PD, progressive disease.