# Association Between HIF-1 Alpha Gene Polymorphisms and Response in Patients Undergoing Neoadjuvant Chemotherapy for Locally Advanced Cervical Cancer

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Background: The aim of the study was to assess whether HIF-1α polymorphisms have an effect on the response to chemotherapy of locally advanced cervical cancer (LACC) patients treated with platinum-based neoadjuvant chemotherapy (NACT) and radical surgery.

Material/Methods: We conducted a retrospective study in 162 LACC patients. Hypoxia-inducible factor 1α C1772T and G1790A genetic polymorphisms were ascertained using direct sequencing methods.

Results: The C1772T polymorphism was significantly related to response to chemotherapy (P=0.002), and there was an increased chance of treatment response in patients with the C/C genotype (OR=4.7; 95% CI: 1.67–13.49; P=0.004). The C1772T polymorphism was also associated with poor tumor grade (adjusted OR, 2.98; 95% CI: 1.08–8.13; P=0.037). However, The G1790A polymorphism was not associated with response (P>0.05).

Conclusions: The C1772T polymorphism was significantly related to response to chemotherapy and poor tumor grade. Our results may help to better manage individual patients and to improve clinical decision making regarding use of NACT.

MeSH Keywords: Biomarkers, Pharmacological • Hypoxia-Inducible Factor 1 • Neoadjuvant Therapy • Uterine Cervical Neoplasms

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## Background

Although the incidence of cervical cancer has decreased with the implementation of screening programs, survival of women with locally advanced cervical cancer (LACC), which includes tumors at stage IB2 to IVA according to the International Federation of Gynecology and Obstetrics (FIGO), has remained substantially unchanged using conventional treatments.

Concomitant chemoradiation (CT/RT) has been regarded as the standard therapy for LACC patients since 1999 based on results from randomized phase III studies [1,2]. Neoadjuvant chemotherapy (NACT) followed by radical hysterectomy has also been used in Europe, East Asia, and Latin American countries as an alternative therapeutic option [3,4].

There are several reasons for the use of NACT. Decreased tumor size may facilitate subsequent surgery, and NACT has been suggested to increase radiosensitivity and decrease hypoxic cell component [5]. However, despite the theoretical benefits of preoperative NACT, its current clinical value in LACC remains controversial. In patients who were resistant to chemotherapy, the administration of other treatments may be delayed [6]; therefore, it is necessary to establish some markers that can identify patients who are relatively chemoresistant.

Tumor hypoxia is known to be involved in resistance to chemotherapy and radiotherapy, as well as the malignant tumor phenotype, involving increased tumor growth rate, invasiveness, and metastasis [7]. In the hypoxic microenvironment, a signaling pathway involving a key regulator of cellular response to hypoxia, termed the hypoxia inducible factor (HIF), is activated. HIF-1 consists of a constitutively expressed HIF-1β subunit and an oxygen-regulated HIF-1α subunit [8]. HIF-1α regulates the cellular adaptation to hypoxia and trans-activates many genes that are associated with many cellular processes, such as energy metabolism, angiogenesis, proliferation, differentiation, and viability [9]. Many studies, including our previous study, have reported that HIF-1α protein is highly expressed in cervical cancer and has a significant influence on prognosis [10,11]. It has been thought that, in addition to intratumoral hypoxia, some genetic and epigenetic alterations are also part of a mechanism underlying the increased levels of HIF-1α in cancer and stromal cells [12].

Interestingly, 2 single-nucleotide polymorphism (SNP) sites (C1772T and G1790A) located in the oxygen-dependent degradation (ODD)/pVHL binding domain in exon 12 of the HIF-1α gene cause substantially higher transcriptional activity than the wild-type by substitution of amino acids from proline to serine and from alanine to threonine, respectively [13]. Several studies and meta-analyses have reported associations between HIF-1α gene polymorphisms and several types of cancer, including cervical cancer [14–16]. However, to date there have been no published studies regarding the relationship between HIF-1α polymorphisms and cervical cancer treated with preoperative chemotherapy and radical surgery.

In the present study we investigated the association between the C1772T and G1790A polymorphism of the HIF-1α gene and chemotherapeutic response of LACC patients who received NACT and radical surgery. To the best of our knowledge, this is the first report to explore on the clinical significance of HIF-1a polymorphisms in patients with LACC.

## Material and Methods

### Study design

Retrospective data collection was performed using medical records of patients with uterine cervical carcinoma FIGO stage IB2 to IIB who underwent platinum-based NACT followed by radical hysterectomy and pelvic lymphadenectomy from January 2006 through January 2012 at the Department of Obstetrics and Gynecology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University. The proposed treatment and the possible alternative approaches were explained to the patients, and informed consent was obtained. The institutional review board approved the research protocol for this study.

### Patient selection

The patients were identified by using the gynecologic oncologic database of the institution. The inclusion criteria were: (1) cervical cancer, confirmed by pathological examination; (2) received 2–3 course of platinum-based NACT followed by radical hysterectomy; (3) FIGO stage IB2-IIB; (4) complete basic clinical data. The exclusion criteria were: (1) previous treatments for cervical cancer; (2) previous or concomitant cancer; (3) insufficient data to evaluate survival and clinical response; and (4) insufficient tissue to analyze genotype.

### Evaluation and treatment of patients

FIGO stage of cervical cancer in all patients was evaluated using pelvic examination and magnetic resonance imaging (MRI) or computed tomography (CT) scanning before therapy. All included patients received platinum-based chemotherapy intravenous infusion for 2–3 cycles. The chemotherapeutic regimens consisted of paclitaxel/platinum agent (TP). Each cycle consisted of cisplatin (60 mg/m$^2$/day) and paclitaxel (135 mg/m$^2$/day). Chemotherapy was withdrawn in cases of tumor progression or in patients who did not respond after 2 cycles.

Two to three weeks after the final course, the response to chemotherapy was clinically evaluated by the same imaging method and by directly measuring the diameters of resected tumor blocks. After NACT, the patients underwent a type III radical hysterectomy and bilateral pelvic lymphadenectomy with or without para-aortic lymphadenectomy. Postoperative radiotherapy was administered according to pathological results in the presence of histopathologic risk factors.

### Evaluation of response

The tumor response was evaluated according to the response evaluation criteria for solid tumors (RECST, version 1.1) [17] as follows: complete response (CR) was defined as the absence of any residual tumor after treatment at any site level; partial response (PR) was defined as at least a 30% reduction in the sum of the largest diameter of the measurable lesions; stable disease (SD) was defined as neither reduction that qualified as PR nor sufficient accrual that qualified as progressive disease (PD); and PD was defined as at least a 20% increase in the sum of the largest diameter of the measurable lesions. The overall response was defined as CR plus PR.

### DNA extraction and genotyping

Samples were recruited from the formalin-fixed, paraffin-embedded surgical specimens. DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions.

Polymerase chain-reaction (PCR) was performed using specific primers described by Fransen et al. [18]. The sequence of primers was: 5'-TGTGGCCATTGTAAAAACTCA-3' (forward) and 5'-CTTGCGGAACTGCCTTCTAA-3' (reverse). The amplicon containing both HIF-1a C1772T and G1790A site was 147bp in length. The PCR conditions used were: 1 cycle of 94°C for 4 min, followed by 40 cycles of 94°C for 1 min, 55°C for 1 min, and 1 min at 72°C with final extension at 72°C for 10 min. For each assay, a negative control (without DNA template) was added to monitor PCR contamination. After confirming the integrity of the amplicons, all PCR products were further purified using a MiniElute PCR purification kit (Qiagen) for commercial sequencing. The sequencing primer was the same as the forward primer used for the PCR reaction. ChromasPro software (Technelysium Pty Ltd., Tewantin, QLD, Australia) was used for the reading of sequences on chromatograms.

### Statistical analysis

Genetic polymorphisms and clinicopathologic parameters were assessed using the Pearson chi-square test or the Fisher exact test. Logistic regression models were used to account for potential confounding factors associated with tumor progression.

**Table 1.** Patient characteristics.

| Variable | | |
|---|---|---|
| Age, year (median) | 47 | 24–70 (range) |
| Histological types | | |
|     Squamous | 143 | 88.30% |
|     Adenocarcinoma | 14 | 8.64% |
|     Adenosquamous | 3 | 1.90% |
|     Others | 2 | 1.23% |
| FIGO stage | | |
|     IB2 | 53 | 32.71% |
|     IIA | 65 | 40.12% |
|     IIB | 44 | 27.16% |
| Tumor grade | | |
|     1 | 20 | 12.35% |
|     2 | 65 | 40.12% |
|     3 | 77 | 47.50% |
| Tumor size | | |
|     <4 cm | 48 | 29.63% |
|     ≥4 cm | 114 | 70.37% |
| DOI | | |
|     ≤1/2 | 54 | 33.33% |
|     >1/2 | 108 | 66.67% |
| LVI | | |
|     Yes | 49 | 30.24% |
|     No | 113 | 69.75% |
| LNM | | |
|     Yes | 59 | 36.42% |
|     No | 103 | 63.58% |

FIGO – International Federation of Gynecology and Obstetrics; DOI – depth of cervical invasion; LVI – lymphovascular space invasion; LNM – lymph node metastasis.

For all tests, a 2-tailed P≤0.05 was regarded as significance. All these statistical analyses were performed using the SPSS program (SPSS 15.0; SPSS Inc., Chicago, IL).

## Results

### Patient characteristics

A total of 162 patients were enrolled in this study according to the inclusion criteria. The median age of the patients was 47 years (range, 24–70 years). The characteristics are presented in Table 1.

**Table 2.** Genotype distribution of HIF-1a polymorphisms in cervical cancer patients.

| Nucleotide | Amino acid | Genotype or allelotype | Patients N (%) |
|---|---|---|---|
| C1772T | P582S | | N=162 |
| | | CC | 139  (85.8%) |
| | | CT | 23  (14.2%) |
| | | TT | 0  (0.0%) |
| | | T allele | 0.071 |
| G1790A | A588T | | N=162 |
| | | GG | 151  (93.2%) |
| | | GA | 11  (6.8%) |
| | | AA | 0  (0.0%) |
| | | A allele | 0.034 |
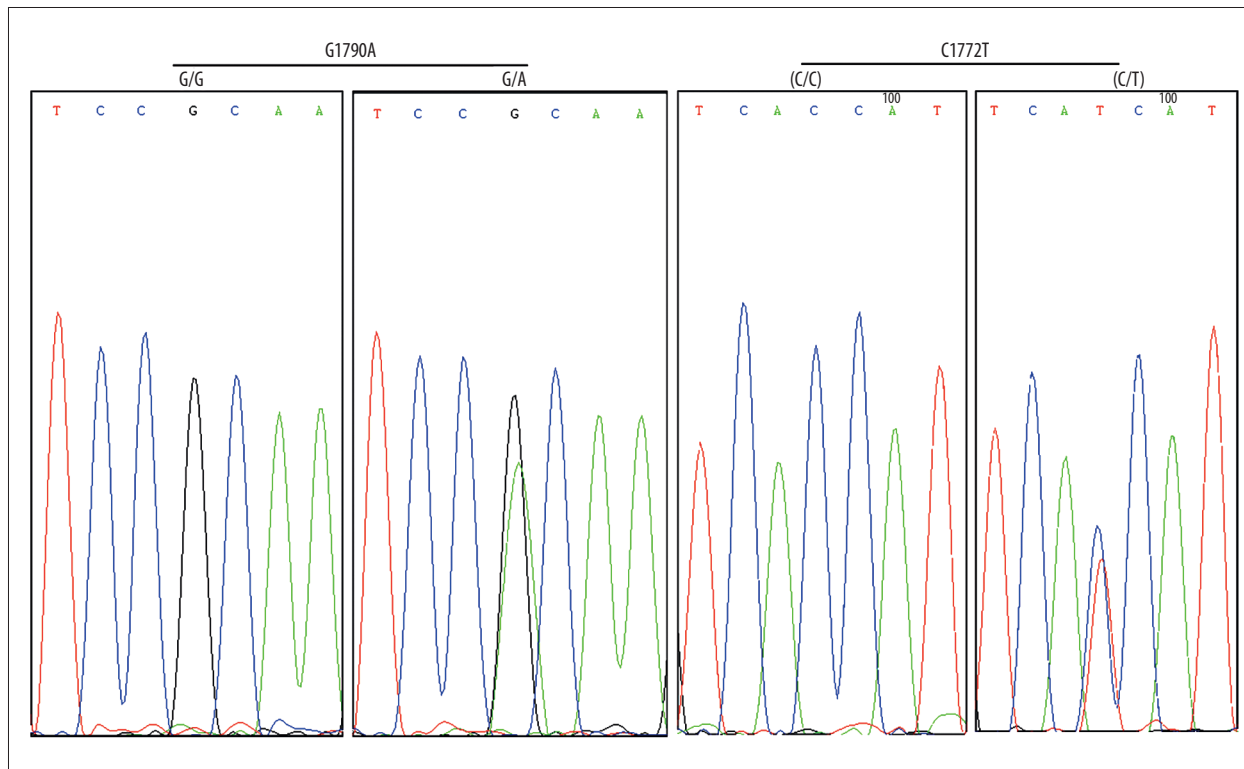


**Figure 1.** Polymorphisms in the HIF-1a gene. Chromatograms of DNA sequence analysis of HIF-1a showing the allelic variations at position 1772 and 1790. Homozygous wild-type SNP C1772T (C/C) and G1790A (G/G) as well as heterozygous SNP C1772T (C/T) and G1790A (G/A).

## Mutation analysis

The genotype frequencies of the C1772T polymorphism and the G1790A polymorphism in the HIF-1α gene of the 162 cervical cancer patients are displayed in Table 2 and Figure 1. The C1772T genotype distributions were 139 CC (85.8%), 23 CT (14.2%), and 0 TT (0.0%). The G1790A genotype distributions were 151 GG (93.2%), 11 GA (6.8%), and 0 AA (0.0%). Neither site had variant homozygote.

## Genotype distribution and clinicopathologic characteristics

For analysis, variant homozygous and heterozygous subjects were grouped as T or A carriers. Association analysis was

**Table 3.** Genotype related to clinicopathological parameters.

| Characteristics | C1772T polymorphism (162 cases) (%) | | | Adjusted OR (95% CI)* | Adjusted P* | G1790A polymorphism (162 cases) (%) | | |
|---|---|---|---|---|---|---|---|---|
| | CC | T carrier | P value | | | GG | A carrier | P value |
| Median age | | | 0.1 | | | | | 0.19 |
| ≤47 y | 71 | 16 | | | | 79 | 8 | |
| >47 y | 68 | 7 | | | | 72 | 3 | |
| Histology type | | | 0.983§ | | | | | 0.240** |
| Squamous | 123 | 20 | | | | 135 | 8 | |
| Other | 16 | 3 | | | | 16 | 3 | |
| FIGO stage | | | 0.464 | | | | | 0.95** |
| IB2 | 47 | 6 | | | | 50 | 3 | |
| IIA+IIB | 92 | 17 | | | | 101 | 8 | |
| Tumor grade | | | **0.026** | **2.98 (1.08–8.13)** | **0.037** | | | 0.89 |
| 1+2 | 68 | 17 | | | | 79 | 6 | |
| 3 | 71 | 6 | | | | 72 | 5 | |
| Tumor size | | | 0.927 | | | | | 0.99** |
| <4 cm | 41 | 7 | | | | 45 | 3 | |
| ≥4 cm | 98 | 16 | | | | 106 | 8 | |
| DOI | | | 0.75 | | | | | 0.44** |
| ≤1/2 | 47 | 7 | | | | 52 | 2 | |
| >1/2 | 92 | 16 | | | | 99 | 9 | |
| LVI | | | 0.136 | | | | | 0.91** |
| Yes | 39 | 10 | | | | 45 | 4 | |
| No | 100 | 13 | | | | 106 | 7 | |
| LNM | | | 0.229 | | | | | 0.99** |
| Yes | 48 | 11 | | | | 55 | 4 | |
| No | 90 | 12 | | | | 96 | 6 | |

* Adjusted for histology type, FIGO stage, tumor size, DOI, LVI, and LNM; ** Calculated by Fisher Exact test, other P values refer to the Pearson chi-square test.

performed between HIF-1α polymorphism and clinicopathologic characteristics. Most clinicopathologic results failed to show a significant relationship with HIF-1α polymorphism except for tumor grade. Tumors had significantly worse differentiation in patients who had C1772T variant after adjustment for histology type, FIGO stage, tumor size, DOI, LVI, and LNM (adjusted OR, 2.98; 95% CI: 1.08–8.13; P=0.037) (Table 3).

**Genetic polymorphism and Response to chemotherapy**

A statistically significant association was found between polymorphisms of C1772T and favorable response to platinum-based chemotherapy (P=0.002). Logistic regression analysis showed a significantly increased chance of treatment response in patients with the C/C genotype versus the C/T genotype (odds ratio 4.7; 95% CI: 1.67-13.49; P=0.004). No significant differences were found between G1790A and chemotherapeutic response. The data are displayed in Table 4.

**Table 4.** Genotype and tumor response to preoperative chemotherapy.

| Genotype | CR | PR | SD | PD | p value* |
|---|---|---|---|---|---|
| All case | 12 | 72 | 67 | 11 | |
| C1772T polymorphism | | | | | 0.002** |
| CC | 10 | 69 | 55 | 5 | |
| CT/TT | 2 | 3 | 12 | 6 | |
| G1790A polymorphism | | | | | |
| GG | 12 | 67 | 62 | 10 | 0.45** |
| GA/AA | 0 | 5 | 5 | 1 | |

CR – complete response; PR – partial response; SD – stable disease; PD – progressive disease. * CR/PR *vs*. SD/PD; ** Calculated by Pearson Chi-Square.

## Discussion

The value of NACT followed by surgery in cervical cancer is still controversial. Despite its unclear role, it has been widely accepted that NACT has a beneficial effect on cervical cancer patients who are sensitive to chemotherapy.

This study aimed to evaluate the relationship between HIF-1α polymorphisms and chemotherapeutic response in patients with LACC who received NACT followed by radical surgery. The results provide evidence that C1772T polymorphism was associated with response to chemotherapy. We also explored the relationship between HIF-1α polymorphisms and the disease progression. The results suggest that C1772T polymorphism was also associated with poorly differentiated tumors.

SNPs of genes coding enzymes are involved in drug metabolism and transportation, and the apoptosis cascade was associated with the response to chemotherapy in various cancers. Some genotypes related to DNA repair have been reported to affect the response to chemotherapy [19].

In the hypoxic microenvironment, hypoxic cancer cells acquire invasive and metastatic properties and resistance to chemotherapy in the process of adapting to hypoxia; these properties are mediated by HIFs [20]. Genetic and epigenetic alterations may also be a mechanism underlying the increased levels of HIFs in cancer cells [12].

In the present study, only C1772T polymorphism was associated with poorly differentiated grade. A meta-analysis published recently also concluded that HIF-1α C1772T polymorphism was associated with histological grade of cancer, especially in Asian cancer patients [21]. The underlying mechanism remains unclear. Further studies are necessary to validate the result and to elucidate the mechanism.

Some limitations to the present study should be acknowledged. Firstly, due to limitations imposed by retrospective chart review, there were insufficient data to explore the relationship between HPV infection and HIF-1α polymorphism, and some studies have reported there is an interaction between HPV and HIF-1α gene [22,23]. Secondly, the sample size of our study was relatively small, and variant homozygotes were not found among those samples; therefore, we could not analyze the relationship between variant homozygote and chemotherapeutic response.

## Conclusions

This is the first study to explore the relationship between HIF-1α polymorphisms and response in patients undergoing neoadjuvant chemotherapy for locally advanced cervical cancer. The C1772T polymorphism has an effect on cervical cancer patients' response to chemotherapy and may be related to cervical cancer progression. Our findings may help to improve management of individual patients and to enhance clinical decision making regarding NACT.

Further studies may be necessary to explore the mechanism of C1772T polymorphism, which affects the response of cervical cancer patients undergoing neoadjuvant chemotherapy.

### conflicts of interest

The authors declare no conflicts of interest and received no writing assistance.

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