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The impact of *GGH -401C>T* polymorphism on cisplatin-based chemoradiotherapy response and survival in cervical cancer

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ABSTRACT

Aims: Cervical cancer is the third most frequent cancer in women worldwide, mostly treated with cisplatin-based chemoradiotherapy. Since it is known that folate metabolism might interfere with cisplatin effectiveness, we intended to study the influence of the *Gamma Glutamyl Hydrolase -401C>T* polymorphism in treatment response in cervical cancer.
Methods: We retrospectively reviewed the clinical data of 167 patients with bulky cervical cancer submitted to cisplatin-based chemoradiotherapy. The genotypes of *GGH -401C>T* SNP were determined by real-time PCR and statistical analysis was performed by χ^2 test and survival analysis.
Results: The genotypes of *GGH-401C>T* were significantly associated with the response to platinum-based chemoradiotherapy. Treatment response was higher in patients carrying the CC genotype, who presented a significant increased chance of treatment response (survival time in months/genotype: 91 for CC Vs 72 for CT/TT; p = 0.035, log rank test). A Cox regression analysis accordingly showed that the presence of the T allele was significantly linked to a worse treatment response (HR = 3.036; CI 95% 1.032-8.934, p = 0.044).
Conclusions: The results of our study suggested the potential interest of *GGH -401C>T* as a predictive factor of the outcome of cervical carcinoma treated with cisplatin-based chemoradiotherapy.

1. Introduction

Cervical carcinoma was the third most common cancer in women in 2008. Since it is a platinum-sensitive disease, cisplatin-based chemoradiotherapy is the standard of care for advanced cervical cancer stages (IB2-IVA FIGO stages) (Candelaria et al., 2006; Ferlay et al., 2010; Tewari and Monk, 2010). Although weekly cisplatin at 40 mg/m^2 for six weeks is the standard of care for locally advanced cervical carcinoma in many cancer centers as ours, its optimal scheduling and dosing have yet to be established due to the frequent development of therapy resistance (Candelaria et al., 2006). Different mechanisms have been proposed to reduce cisplatin response, including altered drug accumulation, enhanced drug detoxification and DNA repair, or upregulation of specific biochemical pathways (Ottone et al., 1997; Siddik, 2003). Thus, the identification of molecular predictors of response urges (Siddik, 2003). As patients' genetic background might change the response, metabolism and toxicity of cytotoxic agents as cisplatin (Le et al., 2005; Siddik, 2003), polymorphisms of genes coding enzymes involved in drug or cell metabolism as well as the DNA synthesis and repair have been studied (Kim et al., 2008).

Due to the synergism between alkylating radiosensitive agents and radiotherapy, it is possible to get good local and systemic control rates. However, what is the impact of molecular modulators on each treatment modality on cervical cancer is still controversial.

GGH is a lysossomal enzyme that regulates intracellular folate pools and folate metabolism homeostasis (Odin et al., 2003; Organista-Navaa et al., 2010; Schneider and Ryan, 2006; Yin et al., 2003). The *GGH −401 C>T* SNP, which is one of its most common polymorphisms, is a promoter polymorphism that causes the loss of an inhibitory transcription-factor binding-site. Due to its influence on one-carbon metabolism and cell survival, its role in cervical carcinogenesis and treatment response is biologically plausible (Odin et al., 2003).

Abbreviations: GGH, Gamma Glutamyl Hydrolase; C, cytosine; T, thymine; SNP, single-nucleotide polymorphism; FIGO, International Federation of Gynecology and Obstetrics; Gy, Gray; RECIST, response evaluation criteria in solid tumors; PCR, polymerase chain reaction; A, Adenine; G, Guanine; SPSS, statistical packages for the social sciences; χ^2, Chi-Square; OS, Overall survival; SD, standard deviation; HR, hazard ratio; CI, confidence interval; dTMP, thymidilate synthase.

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With this study, we intended to investigate the influence of the *GGH −401 C>T* polymorphism in determining chemoradiotherapy response in cervical cancer.

2. Subjects and Methods

2.1. Subjects

We conducted an hospital-based retrospective study analyzing 167 Caucasian women from Northern Portugal with histologically confirmed cervical cancer IB2-IVA FIGO stages, admitted in the Portuguese Institute of Oncology, Porto, Portugal. All women were treated with first line cisplatin-based chemoradiotherapy in the Portuguese Institute of Oncology from Porto. Assessment of tumor stage was based on the FIGO system. All samples were obtained with the informed consent of the participants prior to their inclusion in the study, according to the declaration of Helsinki.

2.2. Concurrent chemoradiotherapy treatment

Chemotherapy regimen consisted of cisplatin (40 mg/m^2, iv) administered weekly in a total of six weeks. Concurrent radiotherapy consisted of pelvic external beam radiotherapy (for a total dose of 45–50 Gy of pelvic irradiation) and one to three intracavitary brachytherapy applications after the completion of external pelvic radiotherapy (cumulative dose at point A: 75 Gy; cumulative dose to point B: 55 Gy). For patients with lymph node metastasis, the treatment field was set to extend beyond the known extent of disease. From 167 patients evaluated for *GGH -401C>T* genotypes, 101 completed 6 cycles of the chemotherapy treatment. Hematological toxicity was the main factor causing treatment interruption.

2.3. Evaluation of chemotherapy response

Patients were followed on average for a period of 32 months. The response to cisplatin was estimated by the change in tumor size, which was measured by physical and CT exams performed before and after completing the prescribed treatment. The longest diameter of the lesion was measured. Using RECIST criteria, the response was graded as complete response (cervical lesion eradication), 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.

2.4. Sample collection and genotyping

Genomic DNA was extracted from blood samples by using QIAamp® DNA Blood Mini Kit (QIAGEN®). For the detection of the *GGH -401C>T* polymorphism, we used real-time polymerase chain reaction (PCR) by TaqMan allelic discrimination assay according to manufacture instructions. Probes used were flagged with VIC®/FAM™ dyers, respectively linked to the wild-type and the variant allele: CTGGCCAACCCAGGTCCTCGAGAGG[A/G]GAGGTTGGGTGTCCCCGGCC GAGTT. Results were analyzed on a sequence detection system ABI 7300, version 1.2.3 (Applied Biosystems, USA) Negative controls were included in each run and 10% of the samples' genotyping was repeated for quality control. Samples were tested in a blind fashion.

2.5. Statistical analyses

Analysis of data was performed using the computer software Statistical Package for Social Sciences (SPSS) for Windows (version 18.0). Difference in frequencies of the *GGH* genotypes between different chemotherapy response groups were evaluated by χ^2 test, considering $p<0.05$ statistically significant. Overall survival was defined as the time, in months, between diagnosis and either death or time of the last clinical evaluation. OS curves were plotted using the Kaplan–Meier method and were compared with the log-rank test. Multivariate analysis (Cox regression) was performed with variables considered important prognostic factors in cervical cancer, which were the tumor stage, histology, the presence of lymph node metastasis and the genotype *GGH -401CC*.

3. Results

3.1. Patient characteristics

The median age of patients at diagnosis was 47 years with a mean age of 48.58 years (SD = 12.78). The FIGO stages of the enrolled patients were as follow: 13 patients with IB2, 4 patients with IIA, 43 patients with IIB, 24 patients with III and IV stages. Most patients (80%) presented squamous cell carcinoma and no lymph node metastasis (94%). All patients received cisplatin-based chemoradiotherapy. From those, 71 patients carrying the CC genotype were good responders and 5 were poor responders. From patients carrying the CT/TT genotypes, 81 were good responders and 10 were poor responders (OR = 0.741, 95% IC 0.487-1.129; p = 0.140). Sample characteristics are reported in Table 1.

3.2. SNP genotyping

We analyzed *GGH -401C>T* polymorphism genotypes using the real-time PCR methodology. The genotype frequencies observed were 45.1% (78 cases) for CC genotype, 42.2% (73 cases) for CT heterozygous genotype and 12.7% (22 cases) for TT genotype. The genotypic frequencies were in genetic equilibrium according to the Hardy–Weinberg law

Table 1
Sample characterization.

Variables	N	(%)
Smoking habits		
Yes	23	15.5
No	115	77.7
Unknown	10	6.8
Oral contraceptives		
Yes	70	45.5
No	70	45.5
Unknown	14	9
Tumor FIGO stage		
IB2	13	7.6
IIA	4	2.4
IIB	43	32.6
IIIA	2	1.2
IIIB	20	11.6
IVA	2	1.2
Histology		
Squamous cell carcinoma	139	80.3
Adenocarcinoma	22	12.7
Adenosquamous	7	4.0
Others	5	2.9
Lymph node metastasis		
Yes	7	5.9
No	112	94.1
Number of chemotherapy cycles completed		
1-3	6	3.7
4-6	151	83.5
Treatment response		
CR	123	71.9
PR	29	17.0
SD	9	5.3
DP	6	3.5

CR: complete response; PR: partial response; SD: stable disease; DP: disease progression.

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($\chi^2 = 0.32$). Genotypic differences between cancer subtypes were additionally tested but no statistically significant differences were observed (data not shown).

3.3. Response to chemotherapy in relation to GGH -401C>T polymorphism

Regarding mean survival time, we used the Kaplan–Meier methodology to calculate the differences between the survival times of women carrying the CC genotype and the CT/TT genotypes. A statistically significant association between the *GGH -401C>T* polymorphism and overall survival was observed in the univariate analysis [survival time in months/genotype: 91 for CC (95% CI 82,77 - 99,33) Vs 72 for CT/TT (95% CI 60,80 - 82,68); p = 0.035, log rank test]. Accordingly, the multivariate analysis (Cox regression) adjusted to tumor stage, histology and the presence of lymph node metastasis showed that *GGH* genotypes were significantly linked to treatment response. Accordingly, individuals carrying the T allele had 3 times more risk of death relatively to women carrying the CC genotype (HR = 3.036; CI 95% 1.032-8.934, p = 0.044) (Fig. 1).

4. Discussion

Although evidence suggests that folate deprivation acts synergistically with alkylating agents (Courtemanche et al., 2004; Novakovic et al., 2006; Whiteside et al., 2006), the activation of compensatory mechanisms leading to cell survival have been described. According to (Hayashi et al., 2007), who studied folate depletion in colon cancer cells, folate deprivation and disrupted one-carbon metabolism could be compensated by adaptive mechanisms that enable cells to maintain critical one-carbon metabolism reactions (Hayashi et al., 2007). The development of a survival advantage was also observed in Chinese Hamster Ovary cells resistant to the growth-limiting effects of folate depletion, enabling them to better withstand cisplatin cytotoxicity (Branda et al., 1998). (Cole et al., 2001) also described compensatory changes susceptible of affecting drug sensitivity after GGH overexpression in MCF7 cells (Cole et al., 2001).

Since the *GGH -401C>T* polymorphism leads to GGH overexpression, we thought that an impairment in folate metabolism might activate compensatory pathways involved in DNA repair and maintenance of cell survival. According to our main hypothesis, the *GGH -401C>T* SNP might be involved in the development of cisplatin-based chemoradiotherapy resistance. The fact cisplatin causes an increase in the intracellular levels of 5,10-methylene-tetrahydrofolate and tetrahydrofolate, and enhances the gene expression of enzymes involved in dTMP synthase cycle supports our suggestion (Lu et al., 1988; Scanlon and Kashani-Sabet, 1988; Whiteside et al., 2006).

According to our results, the CC carriers had a significantly higher overall survival than the T allele carriers [survival time in months/genotype: 91 for CC (95% CI 82,77 - 99,33) Vs 72 for CT/TT (95% CI 60,80 - 82,68); p = 0.035, log rank test]. A multivariate analysis supported this observation, showing that patients carrying the T allele had 3 times more risk of death relatively to women carrying the CC genotype (HR = 3.036; CI 95% 1.032-8.934, p = 0.044) (Fig. 1). However, it is important to note that folate depletion poses different metabolic stresses in cells depending on the cell type, which will result in different adaptive regulation of folate metabolism enzymes (Hayashi et al., 2007; Novakovic et al., 2006). A cDNA microarray analysis of a human squamous cell carcinoma cell line treated with cisplatin for 5 days accordingly revealed a 2.55 signal ratio for GGH, suggesting its involvement in cisplatin cytotoxicity (Yatomi et al., 2007).

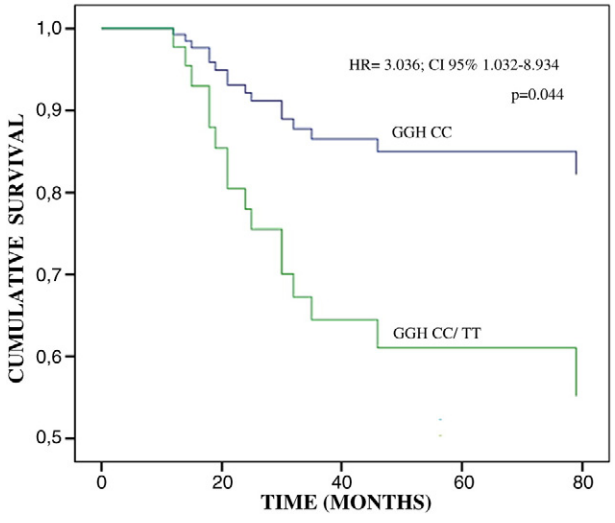

Fig. 1. Cox regression analysis of cervical cancer patients adjusted with FIGO stage, tumor histology and the presence of lymph node metastasis as covariates, according to *GGH -401C>T* genotypes (HR = 3.036; CI 95% 1.032-8.934, p = 0.044).

5. Conclusions

On the light of these results we might suggest that this polymorphism might be a predictive factor of the outcome of cervical carcinoma treated with cisplatin-based chemoradiotherapy, though future and larger studies would be necessary to confirm it. As GGH is a non-specific enzyme, whose expression is dependent on the analyzed tissue, it might not be appropriate to draw a generalized conclusion regarding other cancer models, whose folate requirements for growth are different. The fact we could not study other genes involved in folate metabolism, DNA repair or treatment response is one drawback of this study.

Although an increase in neoadjuvant cisplatin-based chemotherapy response for cervical cancer patients carrying the CC genotype has already been observed by (Chung et al., 2006), to the best of our knowledge there are currently no published studies on the relationship between *GGH -401C>T* polymorphism and cervical cancer chemoradiotherapy response.

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