Single Nucleotide Polymorphisms and Clinical Outcome in Patients with Biliary Tract Carcinoma Treated with Epirubicin, Cisplatin and Capecitabine

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Abstract. *Background: Biliary tract carcinoma (BTC) is a rare highly malignant neoplasia. Polymorphisms at the xeroderma pigmentosum group D (XPD), excision repair cross-complementing group-1 (ERCC1) and X-ray repair cross complementing group 1 (XRCC1) genes were evaluated and correlated with clinical outcome. Patients and Methods: Thirty-three patients with BTC were treated with intravenous or intra-arterial cisplatin and epirubicin and oral capecitabine. The ERCC1-C118T, XPD-Asp312Asn, XPD-Lys751Gln and XRCC1-Arg399Gln polymorphisms were studied. Results: A partial response (PR) occurred in 6 patients. The median progression-free (PFS) and overall survival (OS) were 4.8 and 18.9 months, respectively. No significant correlations were observed between response, PFS and OS in patients grouped according to all the studied polymorphisms. The analysis of survival starting from diagnosis resulted in a significant association of the XRCC1-Arg399Arg variant with a shorter survival. Conclusion: A role of the XRCC1-Arg399Gln polymorphism as a possible prognostic factor in patients affected by BTC is suggested.*

It is well known that a drug used for treatment of a disease often has differential effects on patients; it is now recognised that the way a person responds to a drug is a complex trait influenced by differing individual genetic constitutions. The main objective of pharmacogenetics is the identification of genotypes involved in clinically meaningful variations in drug responsiveness. Therefore, pharmacogenetics may reduce the variation in individual response to drugs by tailoring therapies according to genetic profile (1). In oncology a pharmacogenetic approach to customize the chemotherapy treatment according to individual genetic characteristics represents a modern and intriguing challenge. Biliary tract carcinoma (BTC) is a rare malignancy, although its incidence appears to be increasing worldwide (2) and the prognosis is poor. Surgical resection of the primary tumor is a potentially curative therapy, but less than 25% of patients have resectable tumors at presentation, and among those patients, relapse rates are high (3). No data are available concerning BTC prognostic factors, except for performance status (PS) and stage (4). The carbohydrate antigen 19.9 (CA 19.9) has been commonly used in the diagnosis of BTC and its role as a surrogate biomarker in BTC patients treated with different chemotherapeutic regimens should be further investigated (5).

Systemic chemotherapy has produced modest antitumor activity against BTC and currently no standard therapy has been established (6,7). The most commonly used single agent in BTC is 5-fluorouracil (5-FU), with response rates (RR) of 10-20% (8), while the ECF schedule (epirubicin, cisplatin and continuous infusion of 5-FU) offers the best RR, which is about 40% (9). Cisplatin and epirubicin administered through the hepatic artery, combined with systemic continuous infusion of 5-FU, according to the ECF regimen reported by our team (10), showed an overall RR of 40% , with an overall survival (OS) period of 13.2 months. A doublet with cisplatin and gemcitabine might also be used as a provisional standard of cure (11).

Cisplatin can be considered the backbone of polichemotherapy regimens used to treat BTC. Its activity is mediated through the formation of cisplatin-DNA adducts. The removal of these adducts, which leads to chemoresistance,

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is mainly carried out by the nucleotide excision repair (NER) and the base excision repair (BER) systems, including the excision repair cross-complementing group 1 (ERCC1), xeroderma pigmentosum group D (XPD) and the X-ray repair cross complementing group 1 (XRCC1) proteins, respectively (5). Previous studies in tumor cells treated with cisplatin have shown that the impaired function of NER/BER systems lead to greater induced DNA damage and thus to increased tumor cell death (12). Single nucleotide polymorphisms (SNPs) in any of the NER and BER genes might affect the repair function of the encoded proteins and contribute to individual variations in chemotherapy response (13).

To relate the ability of DNA-repair gene polymorphisms to response to chemotherapy and survival in patients with BTC, the distribution of the ERCC1-C118T, XPD-Asp312Asn, Lys751Gln, and XRCC1-Arg399Gln polymorphisms was assessed in a series of BTC patients treated with cisplatin, epirubicin and capecitabine, in our institution from February 2004 to June 2007.

Patients and Methods

Between June 2006 and June 2007 this pharmacogenetic study enrolled 33 patients treated and followed in our institution. All the patients were required to have histologically confirmed unresectable and measurable BTC and to be treated with cisplatin, epirubicin and capecitabine. The first patient began treatment in February 2004, the last one in June 2007. The study was approved by an internal review board and written informed consent was obtained from all the participating patients.

Sample collection and DNA isolation. Genomic DNA was extracted from peripheral blood (5 ml) using a QIAamp DNA mini Kit (Qiagen, Hilden, Germany). The DNA yields and integrity were checked by optical density at 260 nm with an Uvikon-940 spectrophotometer (Kontron, Milano, Italy), while testing for contamination by proteins was performed by measuring absorbance at 280 nm and calculating the 260/280 ratio.

SNP genotyping. The ERCC1-C118T, XPD-Asp312Asn, XPD-Lys751Gln and XRCC1-Arg399Gln polymorphisms were studied with Taqman® probes-based assays using an ABI PRISM 7900HT instrument equipped with Sequence Detection System version 2.0 software (Applied Biosystems, Foster City, CA, USA). Forward and reverse primers and probes (Applied Biosystems SNP Genotyping Assay products) were obtained using the File Builder version 1.0 software, on the basis of the GenBank database, and the sequences are available upon request. The PCR reactions were performed using 10-20 ng of genomic DNA diluted in 11.875 μl DNAse-RNAse free water, 12.5 μl of TaqMan Universal PCR Master Mix, with AmpliTaq Gold®, and 0.625 μl of the assay mix (forward and reverse specific primers and the specific probes), in concentrations optimized in preliminary reactions, in a total volume of 25 μl. After thermal cycling, the 7900HT instrument determined the allelic content of each sample in the plate by reading the generated fluorescence.

Treatment schedules. Twenty-six patients received epirubicin at 50 mg/m^2 and cisplatin at 60 mg/m^2 through the hepatic artery, while seven patients received epirubicin and cisplatin at the same dosages intravenously. The intra-arterial schedule was as follows: on day 1 the patients received epirubicin in 100 mL of normal saline and cisplatin in 120 mL of normal saline (both infused as a bolus into the hepatic artery by an angiographic catheter inserted into the femoral artery by the Seldinger method). The cisplatin infusion was preceded and followed by intravenous hydration and all the patients were given prophylactic antiemetic treatment with 5-hydroxytryptamine type 3 receptor antagonists and dexamethasone. Both groups received oral capecitabine at 1000 mg/m^2 twice a day, from day 2 to day 15. The cycles were repeated every three weeks. The response to treatment was evaluated every three cycles and the patients who responded or had stable disease (SD) received three additional cycles. In cases of disease progression, a further line of chemotherapy was permitted.

Assessment of response. The anticancer effects were evaluated by tumor size and CA 19.9 changes. All the patients had bidimensionally measurable lesions for response evaluation. The tumor size was evaluated by CT scan every three cycles. The response was assessed by the World Health Organization criteria. Bone metastases and pleural and peritoneal effusions were not considered to be evaluable, but they were taken into account for the determination of disease progression. PS and CA 19.9 serum levels were recorded at each cycle.

Statistical analysis. Demographic and clinical information was compared across CA 19-9, PS and genotype, using Pearson Chi-square and log-rank tests. Three groups of patients were defined: the patients achieving complete response (CR) or partial response (PR) as "responders"; the patients with progressive disease (PD) as "non-responders" and the patients with SD as "stable". Progression-free survival (PFS) was calculated from the first day of chemotherapy to the date of clinical and/or radiological evidence of progression or death, whichever occurred first, while OS was calculated from the day of treatment start to the end-point (death or censoring).

To further evaluate the prognostic role of the studied SNPs, a univariate analysis was also performed, with OS calculated from the day of diagnosis. The Kaplan-Meier method was used to plot the PFS and OS, and the log-rank test was used to compare the curves.

The data were analyzed using SPSS/PC+11.5 statistical software (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p<0.05$.

Results

Patient characteristics. The patient characteristics are summarized in Table I. The median ECOG performance status and median CA 19-9 level at diagnosis was 1 and 172, respectively.

Genotype information. The distribution of all the polymorphisms and the allelic frequencies are shown in Table II. All the polymorphisms followed Hardy-Weinberg equilibrium and the observed genotype frequencies were comparable with those reported in previous studies in Caucasian populations.

Table I. *Patient characteristics*.

Characteristics	No. of patients (%)
General	
Entered	33
Evaluable disease	33
Median age, years (range)	63.5 (37-80)
Gender	
Male	21
Female	12
ECOG performance status	
0	18 (55)
1	11 (33)
2	4 (12)
Tumor diagnosis	
Intrahepatic cholangiocarcinoma	25 (75)
Gallbladder carcinoma	5 (15)
Choledochus	3 (10)
Previous therapy	
None	25
Surgery	6
Radiotherapy	2
Other chemotherapy	11
Sites of metastases	
Liver	28
Involvement<50%	14
Involvement>50%	14
Lymph nodes	4
Peritoneum	3
Local disease recurrence	2
Others	2
Median CA 19-9 level, IU/mL (range)	172 (1-10480)

Table II. *ERCC1, XPD, and XRCC1 polymorphisms*.

Genotype	No. patients	%	Allelic frequencies
ERCC1 C118T			C 0.46
			T 0.54
C/C	4	12.1	
C/T	22	66.8	
T/T	7	21.1	
XPD Lys751Gln			A(Lys) 0.50
			C(Gln) 0.50
Lys/Lys	8	24.2	
Lys/Gln	17	51.6	
Gln/Gln	8	24.2	
XPD Asp312Asn			G(Asp) 0.53
			A(Asn) 0.47
Asp/Asp	10	30.3	
Asp/Asn	15	45.5	
Asn/Asn	8	24.2	
XRCC1 Arg399Gln			A(Arg) 0.71
			G(Gln) 0.29
Arg/Arg	17	51.5	
Arg/Gln	13	39.4	
Gln/Gln	3	9.1	

Table III. *Response according to ERCC1, XPD, and XRCC1 polymorphisms*.

Genotype	PR No. (%)	SD No. (%)	PD No. (%)	*P*
ERCC1 C118T:				
C/C	0	3	1	
C/T	9	10	3	0.14
T/T	0	6	1	
XPD Lys751Gln:				
Lys/Lys	1	5	2	
Lys/Gln	3	8	6	0.73
Gln/Gln	0	6	2	
XPD Asp312Asn:				
Asp/Asp	2	5	3	
Asp/Asn	1	8	6	0.60
Asn/Asn	1	6	1	
XRCC1 Arg399Gln				
Arg/Arg	9	7	1	
Arg/Gln	9	3	1	0.31
Gln/Gln	1	0	2	

PR: partial response; SD: stable disease; PD: progressive disease.

Response and survival. A PR was observed in 6 out of the 33 evaluable patients (18.2%). SD was observed in 17 patients (51.5%) and PD occurred in 10 patients (30.3%). After a median follow-up of 7.1 months (range 1-44.7), the median PFS was 4.8 months (range 0.7-21.7) and the median OS was 18.9 months. The 1-year survival rate was 56.2%.

Correlation between genetic polymorphisms and clinical outcome. The overall RR of the 33 patients enrolled in this study was 18.2% When grouping patients as those with response (PR), with SD and without response (PD), and as those with a genotype leading to different ERCC1, XPD, XRCC1 activity, no significant correlations were observed (Table III). Similarly, no significant correlations were observed between PFS and OS in the patients grouped according to all the studied polymorphisms (Table IV). However, the log-rank test suggested an association of the XRCC1-Arg/Arg variant with reduced OS (median OS, 8.3 months, 95% CI 3.4-13.2), which did not reach statistical significance (p=0.064). Furthermore, the analysis of survival starting from the date of diagnosis resulted in a significant association of the homozygous variant XRCC1-Arg/Arg with a shorter survival (11.0 *vs*. 45.6 months, p=0.01, Figure 1). In contrast, no significant correlations were observed between OS calculated from the date of diagnosis in the patients grouped according to all the other studied polymorphisms.

Table IV. *PFS and OS according to ERCC1, XPD, and XRCC1 polymorphisms.*

	Median OS			Median PFS		
Genotype	Mts	95% CI	*p*	Mts	95% CI	*p*
ERCC1 C118T:						
C/C	9.2	7.8-10.5	0.41	7.7	0.1-15.3	0.51
C/T	18.9	0.0-38.1		3.9	2.4-5.3	
T/T	Nr.	-		7.8	3.8-11.7	
XPD Lys751Gln:						
Lys/Lys	Nr.	-	0.90	7.8	0.0-16.1	0.57
Lys/Gln	27.2	3.9-50.5		4.4	2.5-6.2	
Gln/Gln	9.2	0.0-21.3		7.7	0.4-15.0	
XPD Asp312Asn:						
Asp/Asp	Nr.	-	0.59	7.8	0.0-17.1	0.39
Asp/Asn	6.3	0.0-24.1		4.4	1.9-6.8	
Asn/Asn	9.2	0.0-19.2		7.7	0.4-15.0	
XRCC1 Arg399Gln						
Arg/Arg	9.9	4.9-14.9	0.06	3.9	2.0-5.7	0.31
Arg/Gln	18.9	4.6-33.2		7.7	2.9-12.4	
Gln/Gln	Nr.	-		11.3	2.2-20.4	

PFS: progression-free survival; OS: overall survival; Mts: Months; Nr.: not reached.

Correlations between PS and basal CA 19-9 and clinical outcome. PS=0 was significantly associated with longer survival starting from diagnosis compared to PS=1 and PS=2 (p=0.001, Figure 2). Similarly, basal CA 19.9 <100 was significantly correlated with longer survival (p=0.02, Figure 3).

Discussion

Patients with BTC have a poor prognosis with a median survival period ≤6 months in those with gallbladder carcinoma and approximately 1 year in those patients with cholangiocarcinoma (14). In the current study cisplatin and epirubicin given intra-arterially or endovenously, combined with oral capecitabine showed a median OS of 18.9 months. These results confirmed the feasibility and efficacy of combined systemic and intra-arterial treatment in patients with unresectable BTC (15). In the present study the overall RR was 18.2% , and no CR was observed, as reported in our previous trial (10). Despite the lower RR, the OS was comparable and an interesting association between the XRCC1-Arg399Gln polymorphism significantly shorter and survival was found, suggesting a possible role of the XRCC1 polymorphism as a prognostic factor in patients affected by BTC.

XRCC1 is a multi-domain protein that interacts with at least three other proteins (poly-ADP-ribose polymerase, DNA ligase III, and DNA polymerase β) to repair single-strand breaks in DNA and the XRCC1-399Gln variant was associated with defective repair function in X-irradiated cells (16). The present data are in agreement with a previous

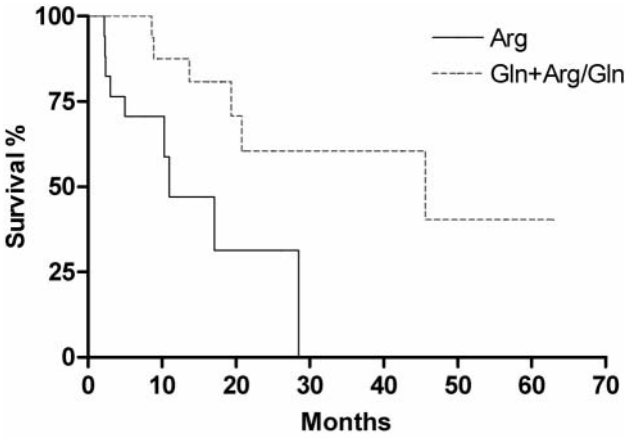

Figure 1. *OS according to XRCC1 polymorphisms; p=0.013.*

report showing an improved survival in colorectal cancer patients harbouring the XRCC1-399Gln allele receiving platinum-based chemotherapy (17). Similar results were observed in non-small cell lung cancer , head and neck, cervical carcinoma, breast, esophageal and gastric cancer (18-23). However, other studies reported a worse clinical outcome in NSCLC patients harbouring the XRCC1-399Gln allele (24, 25), while a recent study showed that the accumulation of polymorphic variants in DNA-repair genes increased by 2.94-fold the probability of achieving a CR to cisplatin treatment among patients affected by squamous cell carcinoma of the head and neck (13).

Several hypotheses may explain the distinct impact of this SNP. The DNA repair efficiency of the variants may be different for DNA damage caused by carboplatin, which produces d(GpNpG)Pt adducts and cisplatin, which produces d(GpG)Pt adducts. Furthermore, the defective function of the XRCC1 Gln/Gln genotype increases radiosensivity, which might also be involved in the clinical outcome in several of the previous studies, as suggested by Gurubhagavatula et al (24), while other biological or tissue-specific factors might account for different chemo-sensitivity/resistance in different neoplasms.

Several studies have focused on DNA repair genes as possible pharmacogenetic biomarkers in different tumor types. In a large retrospective trial a low ERCC1 tumor expression was related to better outcome to adjuvant cisplatin-based chemotherapy in patients with completely resected NSCLC (16). A significant association was also observed between the wild-type genotype (A/A) for XPD 751 and longer OS in metastatic colorectal cancer patients treated with 5-FU and oxaliplatin (26), while significant differences in OS according to the XPD-312 polymorphism were reported in a retrospective study on locally advanced NSCLC patients treated with platinum-based chemotherapy

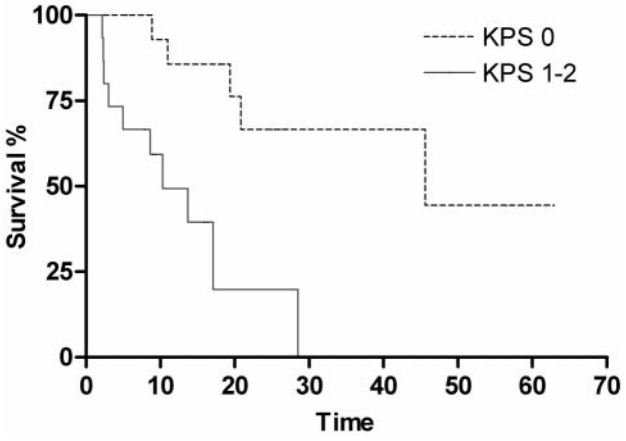

Figure 2. *Karnofsky performance status OS; p=0.0006.*

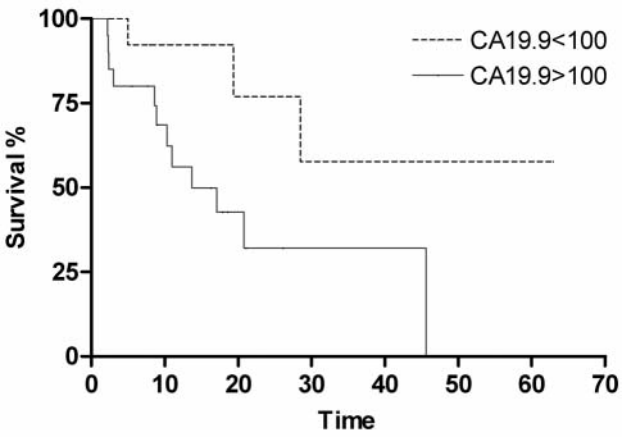

Figure 3. *Basal CA 19.9 OS; p=0.020.*

(24). Other studies showed no significant association between clinical outcome and the C/C genotype in codon 118 of ERCC1 (25) and with polymorphisms at codons 312 and 751 in XPD (27). Therefore, the use of genotypic analysis of NER genes as predictors of clinical outcome to platinum-based treatments is still controversial and further studies are warranted.

Multiple reasons may explain the different results obtained in the association between some SNPs and cancer OS. Most published studies are in different tumor types, with a variety of treatment regimens, and population differences.

Important limitations of the potential use of genotypic analysis as a predictor of clinical outcome must also be acknowledged. First of all, a genotype represents a static value unable to change in response to a new situation, such as response to chemotherapy and it may not reflect changes in tumor DNA, such as a loss of heterozygosity, thus limiting its predictive strength.

Most pharmacogenetic candidates for prediction of chemotherapy efficacy have been identified retrospectively in a small number of patients. A recent study by Marsh *et al*. explained how to assess appropriately the relationship between candidate polymorphisms and clinical outcomes (28). However, *in vitro* studies had already demonstrated the functional role of several polymorphisms, including the SNPs in DNA repair genes evaluated in this study, and the favorable effect of the XRCC1 399 Gln genotype on survival fitted the expectations of a suboptimal ability to remove DNA adducts.

Since BTC is an uncommon tumor, the present study was limited by its small size, but, to our knowledge, it is the first pharmacogenetic study in patients affected by this rare malignancy. A recent study showed that the T393C polymorphism in the gene which encodes the Gas subunit of the heterotrimeric G explanation protein *GNAS1* was associated with the clinical course in patients with intrahepatic cholangiocarcinoma (29). Yoshikawa *et al*. showed that epidermal growth factor receptor (EGFR) expression was associated with tumor progression and vascular endothelial growth factor (VEGF) expression may be involved in hematogenic metastasis in cholangiocarcinoma (30). Addionally a recent study of prognostic factors in patients submitted to surgical treatment suggested that the absence of mucobilia, non-papillary tumor type, advanced tumor staging, non-hepatectomy and lack of adjuvant chemotherapy were independent prognostic factors that adversely affected OS in BTC (4). The present study confirmed that both PS and basal CA 19-9 are prognostic factors in BTC patients.

The results observed in the present homogeneous population suggested that the XRCC1 genotype would be useful marker of clinical outcome in patients affected by BTC. If confirmed in further larger prospective trials, such information may help clinicians to predict the prognosis in patients affected by BTC.

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