Genetic polymorphisms of the XPG and XPD nucleotide excision repair genes in sarcoma patients.

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GENETIC POLYMORPHISMS OF THE XPG AND XPD NUCLEOTIDE EXCISION REPAIR GENES IN SARCOMA PATIENTS

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Short Title: *NER polymorphisms and sarcomas*

Keywords : NER, Polymorphism, Sarcoma, Chromosomal aberrations, Translocation

ABSTRACT

There are more than 50 subtypes of soft tissue sarcomas, among which 30% are associated with specific genetic alterations, including translocations. Several studies have reported associations between cancer risk and polymorphisms of DNA repair genes from the Nucleotide Excision Repair (NER) pathway. NER involves more than 20 proteins whose inactivation leads to Xeroderma pigmentosum (XP) or Cockayne syndrome (CS), among which XPD, a helicase allowing DNA strand excision by the endonuclease XPG. DNA from 93 patients with synovial sarcomas, myxoid liposarcomas, dermatofibrosarcomas protuberans (DFSP), malignant fibrous histiocytomas and leiomyosarcomas were genotyped for both XPD Lys751Gln and XPG Asp1104His polymorphisms. Departure from Hardy-Weinberg was highly significant for the XPG polymorphism with an excess of heterozygotes in synovial sarcomas ($p=1.5\times10^{-5}$), myxoid liposarcomas ($p=1.5\times10^{-4}$) and to a lesser extent in DFSP ($p=0.028$). In the case of XPD, a significant deviation was observed in synovial sarcomas ($p=3\times10^{-6}$) and DFSP ($p=0.0014$). When tumors were pooled according to their genetic alterations, the proportion of carriers of the variant XPG allele was significantly increased in sarcomas with specific translocations as compared to sarcomas with complex genetics ($p<10^{-9}$). No difference was found for XPD. Genotyping of the tumor samples in synovial sarcomas and myxoid liposarcomas revealed frequent loss of heterozygosity for XPG, mostly due to the loss of the frequent allele. For XPD, both alleles were lost with a similar frequency. Our results raise the potential implication of the XPG Asp1104His polymorphism in the occurrence of chromosomal translocations associated with specific subtypes of sarcomas.

INTRODUCTION

It is now well documented that deficiencies in DNA repair associated with high level of mutational events could participate to the early onset of tumor development [1]. The most striking example is probably Nucleotide Excision Repair (NER) that detects and removes UV-induced and a variety of bulky DNA damages.[1] NER involves more than 20 different proteins including XP (group A to F) and CS (A and B) factors. It is initiated by the recognition of the lesion by XPC or by CSA and CSB depending on whether the lesion is located in non-transcribed or transcribed genes, respectively.[1] The two helicases XPD and XPB are recruited to the site of the lesion in order to ensure the opening of the double-stranded DNA. The lesion is then accessible to both XPG and XPF/ERCC1 endonucleases responsible for the excision of the damaged strand. Patients defective for NER are suffering from Xeroderma pigmentosum (XP), an autosomal recessive disease characterized by an extreme sensitivity to sunlight and an increased risk to develop skin cancers.[1] Recently, a particular emphasis was brought on single nucleotide polymorphisms (SNPs) of NER genes that could result in altered mRNA stability and/or protein activity and their potential implication in increased cancer risk due to impaired NER efficiency[2]. 17 SNPs with an allele frequency above 0.1 have been identified, among which 6 were associated with an amino-acid substitution[3]. Numerous case-control studies have given evidence for correlations between some NER polymorphisms and enhanced risk of a variety of malignancies. Two XPD polymorphisms, Asp312Asn and Lys751Gln, both located in conserved regions of the protein, were shown to be associated with global DNA repair efficiency[4] and a higher risk of lung and skin cancers.[5,6] A study also reported that XPD Lys751Gln polymorphism appeared as a major factor influencing spontaneous chromosomal aberrations in lymphocytes.[7] In the case of XPG, a study showed that combined heterozygote and variant homozygote XPG Asp1104His genotype frequency was higher in breast cancer cases than controls.[8] Conversely to XPD, XPG Asp1104His polymorphism is not located in a conserved region of the protein. These studies suggested that the amino-acid changes linked to the presence of these polymorphisms could result in an alteration of the normal protein function, although no direct proofs of such alterations have yet been brought.

Soft tissue sarcomas represent a heterogeneous group of rare tumors, among which 30% are associated with simple genetic alterations including specific translocations.[9] These

chromosomal rearrangements are believed to play a role in the pathogenesis of these malignancies by a mechanism that is not fully understood. In this study, we genotyped normal and tumoral tissues of 93 patients with well-characterized sarcomas for the two “coding” polymorphisms, XPG exon 15 Asp1104His and XPD exon 23 Lys751Gln. Our results show a significant association between these two NER polymorphisms and some subtypes of sarcomas characterized by specific translocations, raising the question of a link between XPG/XPD constitutional genotype and the occurrence of chromosomal rearrangements. Genotyping of the tumor samples also revealed that loss of heterozygosity for XPG was always accompanied by a loss of the frequent allele which may have potential implications in terms of drug response prediction to NER-targeted drugs.

PATIENTS AND METHODS

Patients – 93 patients treated for sarcomas at the Bergonié Institute from 1989 to 2004 with tumors well characterized for histopathologic and genetic features were studied. These sarcomas included 34 synovial sarcomas, 15 myxoid/round cell liposarcomas, 15 dermatofibrosarcomas protuberans (Darier-Ferrand or DFSP), 15 malignant fibrous histiocytomas (MFH), and 14 leiomyosarcomas. The patients' characteristics are detailed in Table I. Diagnosis and classification of these sarcomas have been established based on WHO classification standards.[9] Normal and tumoral tissue samples were fixed in Holland Bouin and paraffin-embedded. A reference population of 53 Caucasian individuals was also used to validate the methodologies used for polymorphism determination. Their selection was issued from the routine activity of the molecular genetics laboratory and concerned non-related patients free of cancer, involved in pre-symptomatic test for familial mutations of either PTEN, BRCA1, BRCA2, MLH1, MSH2 or APC genes.

DNA extraction and genotyping – DNA extraction was performed as previously described[10] and purified using GFX columns (Amersham-Biosciences, UK). Single nucleotide polymorphisms in the XPG (Asp1104His, exon 15) and the XPD (Lys751Gln, exon 23) genes were determined by PCR-RFLP based method using previously published experimental conditions.[8,11] Polymorphisms were also verified by dHPLC method (conditions available upon request) and direct sequencing.

Statistical analysis – A χ^2 test was used to compare constitutional genotype distribution between the different sarcoma types, as well as to test for Hardy-Weinberg proportions within each group. When numbers were too small, the p-value was computed by using simulations.

When LOH was observed in the patients' tumors, random transmission of the lost allele was tested using a binomial distribution. When several tests were performed, a Bonferroni correction was performed.

RESULTS

Determinations of the two XPG Asp1104His (G>C) and XPD Lys751Gln (A>C) polymorphisms were performed using three different methodologies that were validated on a reference population of 53 healthy individuals and showed similar genotype proportions as compared to large-scale studies from the literature, with no departure from Hardy-Weinberg proportions (Table 2A). We then genotyped the constitutive DNA of 93 sarcoma patients in the same conditions. Table 2B displays the distribution of genotypes among the different groups of sarcomas with an apparent increase in heterozygotes for XPG in synovial sarcomas, myxoid liposarcomas and DFSP, and for XPD in synovial sarcomas, DFSP and leiomyosarcomas. We then tested for Hardy-Weinberg proportions in the different sarcoma groups. The results showed a highly significant departure from Hardy-Weinberg proportions with an excess of heterozygotes for the XPG polymorphism in synovial sarcomas and myxoid liposarcomas with p-values of 1.5×10^{-5} and 1.5×10^{-4}, respectively. A smaller difference was found for DFSP with a p-value of 0.028. We also found a significant deviation from Hardy-Weinberg proportions for XPD in synovial sarcomas and DFSP (p-values of 3×10^{-6} and 0.0014, respectively). It is interesting to note that all these deviations were due to an increase in heterozygotes.

Conversely to leiomyosarcomas and MFH that are characterized by complex genetic alterations, synovial sarcomas, myxoid liposarcomas and DFSP are three types of sarcomas with well-defined specific translocations; t(X;18), t(12;16), and t(17;22), respectively. We then tested whether the occurrence of a specific translocation could be linked to the presence of the variant allele of each polymorphism (Table 3). In the case of XPG, we found a significantly higher proportion of variant allele carriers (G/C + C/C genotypes) for sarcoma patients with specific translocations as compared to sarcomas with complex genetics ($p<10^{-9}$). The difference was not significant for XPD. Regarding sarcomas with complex genetics, the distribution of carriers of the variant allele was found to be not significantly different from that of the reference population for both XPG ($\chi^2 = 1.99$, 1 df) and XPD ($\chi^2 = 0.86$, 1 df). Thus, our results indicated a significant association between the variant allele carrier status and the occurrence of sarcomas with specific translocations for XPG. This was not the case for XPD.

We also genotyped XPG and XPD polymorphisms in the corresponding tumor samples. Table 4 shows the number of cases where LOH was observed and the allele that was lost in the

different tumors. Only in synovial sarcomas and myxoid liposarcomas did the allele loss at the XPG locus significantly deviate from the 1:2 proportion expected under random loss: interestingly, this loss almost exclusively concerned the XPG frequent G allele. In sarcomas with specific translocation, the frequency of LOH for the XPD polymorphism was the same for primary (11/47), metastases (3/8) and recurrences (3/9) (χ^2=0.94, 2 df). For the XPG polymorphism, the LOH frequency was also not significantly different between tumor types: 19/47 for primary tumors, 5/8 for metastases, and 7/9 for recurrences (χ^2 = 4.95, 2 df).

As shown in Table I, a substantial number of patients received a chemotherapy prior to sample collection. Since the majority of treated patients belong to synovial sarcoma subgroup (20 out of 34), we tested whether our results could be influenced by adjuvant or neoadjuvant therapy. We found that treatments did not affect the deviation from Hardy-Weinberg proportions for both XPD and XPG polymorphisms and had no effect on the differences in LOH that we observed (data not shown). Thus, it is unlikely that chemotherapy would contribute to the exclusive loss of the frequent allele that was observed in the case of XPG for synovial sarcomas and myxoid liposarcomas.

DISCUSSION

A growing number of studies are suggesting that genetic variability such as single nucleotide polymorphisms (SNPs) may play an important role in cancer predisposition. Recently, a particular emphasis was made on SNPs in DNA repair genes because of their implication in the maintenance of genome integrity.[1] In the present study we have analyzed the genetic polymorphisms in genes coding for two DNA repair enzymes involved in Nucleotide Excision Repair, XPG Asp1104His (G>C) and XPD Lys751Gln (A>C) in normal and tumoral samples of patients with sarcomas. XPG is a structure-specific nuclease that excises the DNA 24 to 32 nucleotides 3' to the lesion.[1] XPD is an ATPase with a 5'-3' helicase activity that is part of the TFIIH complex and participates to the opening of the double-stranded DNA at the site of the lesion.[1]

We specifically focused on these two frequent polymorphisms because they both lead to an amino-acid change that could potentially impair NER efficacy. Though it is difficult to determine the relationship between the presence of a single NER polymorphism in coding regions and DNA repair activity at the constitutional level, it is possible that amino-acid changes could affect protein structure and enzyme activity and participate to DNA recombination and tumorigenesis.[4] In the case of XPD Lys751Gln polymorphism, most studies reported a lower DNA repair efficiency for the Gln variant allele.[4] In the case of the XPG Asp1104His polymorphism however, only marginal differences were found in term of DNA repair between the two alleles, with an apparent higher repair capacity associated with the variant allele.[7, 8] This lack of significance may be attributable to the multifactorial nature of NER and measurements of DNA repair rates in isogenic systems will be needed to confirm this functional difference. Thus, it is possible that the presence of the XPG and/or the XPD variant alleles at the constitutional level may be associated with an alteration and/or a reduction of the NER-mediated DNA repair capacity.

The frequency of the variant allele of XPD (751Gln) was found associated with increased risk of lung cancer among non smokers,[12] of esophageal squamous cell carcinoma,[13] of melanoma in older subjects,[14] and of breast cancer risk for smokers.[15] An association between the XPG variant genotype and a significantly decreased risk of squamous cell carcinoma and small cell lung cancer was also reported.[16] A marginally significant increased frequency of the variant allele

was also seen in breast cancers.[8] Interestingly, these associations concerned two coding polymorphisms of crucial NER genes.

To our knowledge, we present here the first report of a significant link between NER genes polymorphisms and predisposition to specific types of sarcomas. Comparison of XPG genotype distributions between sarcomas associated with specific translocations and sarcomas with complex genetics, concluded to a high increase of carriers of the variant allele in the first group of sarcomas. This comparison was not significant for the XPD polymorphism. These results are in accordance with other associations between XPD and XPG and a higher risk to develop various types of cancers.

Despite the low number of patients in each subgroup, we found a highly significant departure from Hardy-Weinberg proportions for the XPG polymorphism with a strong excess of heterozygotes in sarcomas with specific translocations, but not for sarcomas with complex genetics. An excess of heterozygotes was also found for the XPD polymorphism for several sarcoma types. With respect to synovial sarcoma, in which the excess of heterozygotes for both XPG and XPD was the strongest, the great majority of patients (29/34) carry both rare alleles. There are only two individuals who are not carriers for the XPG variant allele and both are carriers of the XPD variant allele. Similarly, the three individuals who are not carriers for XPD are all carriers of the XPG variant allele. In other words, all synovial sarcoma patients are carriers of a variant allele for at least one of the two genes.

It is unlikely that this pronounced difference, in particular for XPG, could be due to the relative increase in median age of patients with complex genetics sarcomas, since no effect of age or sex on genotype distribution was observed within the reference population, neither for XPG ($\chi^2 = 5.10$, 5 df) nor for XPD ($\chi^2 = 4.28$, 5 df).

This finding suggests the possibility that the heterozygous status of XPG Asp1104His might facilitate the occurrence of a translocation by a mechanism that remains to be investigated. Interestingly, previous *in vitro* data showing that XPG and XPF/ERCC1 nucleases could cleave the R-loop structures formed during the immunoglobulin heavy chain class switch recombination further suggest that NER could play a role in genomic instability.[17] It is also possible that XPG functions other than its 3'-endonuclease activity in NER, could play a role in the formation of these chromosomal rearrangements. Whether NER deficiencies and concomitant accumulation of

unrepaired single-strand breaks could lead to DNA breakage and translocation, leading in turn to tumor development, remains to be further investigated. Moreover, further studies are warranted to strengthen the association between XPG status and the occurrence of chromosomal translocations in sarcomas. We also cannot exclude that other polymorphisms of NER genes or genes involved in other repair pathways could be functionally associated with these chromosomal rearrangements. Indeed, models involving recombinational repair have been proposed to explain the mechanism of their formation.[2] For instance, the loss of a single allele of ligase IV was shown to result in sufficiently reduced NHEJ activity to engender chromosomal alterations associated with sarcomas in a murine model.[18]

XPG locus is located on 13q22-33 and it has been reported that among the various genetic anomalies in synovial sarcomas, loss of the 13q21-31 region is one of the most frequent.[19] Genotyping of the tumor samples revealed indeed a high frequency of LOH for XPG in synovial sarcomas and in myxoid liposarcomas. Interestingly, LOH concerned almost exclusively the wild-type allele of XPG. Even though it is difficult to explain this "targeted" loss at the molecular level, its consequences at the clinical level may be of high importance since it could affect the DNA repair capacity of the tumor cells following treatment with NER-targeted chemotherapies. In this line, a recent retrospective study in sarcoma patients showed that the highest response rate to Et-743, a new alkylating agent specifically targeting transcription-coupled NER, was seen for tumors homozygotes for the XPD most common alleles and that no response could be observed for variant homozygotes, heterozygotes showing an intermediate response rate.[20] Therefore, depending on the histological type of sarcoma, the determination of XPD and XPG genotypes at the tumor level may be predictive of drug response to alkylating agents targeting nucleotide excision repair.

Akgnowledgements: This work was supported by the Ligue Nationale contre le Cancer (Comité de la Dordogne).

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Table 1: Patients characteristics

	N	Men	Women	Age range (average)	Chemotherapy*
Reference population	53	30	23	14-80 (47)	0
Synovial sarcomas	34	16	18	8-74 (39)	20
Myxoid liposarcomas	15	11	4	29-76 (48)	2
DFSP	15	9	6	9-53 (35)	0
MFH	15	7	8	41-86 (66)	3
Leiomyosarcomas	14	8	6	29-83 (60)	0

* Number of patients who have received adjuvant chemotherapy prior to sample collection.

Table 2: Distribution of constitutional genotypes for XPG Asp1104His exon 15 and XPD Lys751Gln exon 23 polymorphisms of (**A**) reference populations (see Material and Methods section) and (**B**) in the different sarcoma groups

A

	XPG				**XPD**			
	genotypes			Hardy-Weinberg p value	genotypes			Hardy-Weinberg p value
	G/G (%)	G/C (%)	C/C (%)		A/A (%)	A/C (%)	C/C (%)	
Kumar et al. 2003 (N=308) [8]	182 (59)	107 (35)	19 (6)	N.S.				
Zhou et al. 2002 (N=1240) [12]					499 (40)	575 (46)	166 (13)	N.S.
Reference population (N=53)	31 (58)	21 (40)	1 (2)	N.S	20 (38)	27 (51)	6 (11)	N.S

B

		XPG				**XPD**			
		genotypes			Hardy-Weinberg p value*	genotypes			Hardy-Weinberg p value*
	N	G/G	G/C	C/C		A/A	A/C	C/C	
Synovial sarcomas	34	2	30	2	**1.5×10^{-5}**	3	30	1	**3×10^{-6}**
Myxoid liposarcomas	15	0	15	0	**1.5×10^{-4}**	7	7	1	N.S.
DFSP	15	3	12	0	0.028	1	14	0	**0.0014**
MFH	15	11	4	0	N.S.	6	9	0	N.S.
Leiomyo-sarcomas	14	10	4	0	N.S.	2	12	0	0.0096

Tests of departure from Hardy-Weinberg proportions were performed (p-value obtained from a χ^2 distribution or by using simulations when expected values were too small). * Due to a high number of comparisons only p-values < 0.005 are considered significant (in bold). N.S.: not significant.

Table 3: Distribution (%) of constitutional genotypes (carriers or non carriers of the variant allele) for XPG Asp1104His and XPD Lys751Gln polymorphisms in normal tissues from sarcoma patients, and p-values for comparison between sarcoma types

	XPG genotypes			XPD genotypes		
	G/G	G/C or C/C	p value	A/A	A/C or C/C	p value
Sarcomas with specific translocation (n=64)	7.8%	92.2%	**$< 10^{-9}$**	17.7%	82.3%	N.S.
Sarcomas with complex genetics (n=29)	72.4%	27.6%		27.6%	72.4%	

Table 4: LOH of XPG Asp1104His and XPD Lys751Gln polymorphisms in tumor samples and test for random loss

XPG Asp1104His	Synovial sarcomas	Myxoid liposarcomas	DFSP	MFH	Leiomyo-sarcomas
Number of tumors	34	15	15	15	14
Number of LOH	20	8	3	0	2
Lost allele	20G	7G + 1C	3C	-	1G + 1C
p value	$p<10^{-6}$	p=0.035	N.S.	-	N.S.

XPD Lys751Gln	Synovial sarcomas	Myxoid liposarcomas	DFSP	MFH	Leiomyo-sarcomas
Number of LOH	13	3	1	7	4
Lost allele	8C + 5A	1C + 2A	1A	5C + 2A	3C + 1A
p value	N.S.	N.S.	-	N.S.	N.S.

N.S.: not significant