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Lack of involvement of nucleotide excision repair gene polymorphisms in colorectal cancer

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DNA repair has an essential role in protecting the genome from damage by endogenous and environmental agents. Polymorphisms in DNA repair genes and differences in repair capacity between individuals have been widely documented. For colorectal cancer, the loss of mismatch repair gene activity is a key genetic determinant. Nucleotide excision repair (NER), recombination repair (RR) and base excision repair (BER) pathways have critical roles in protection against other cancers, and we wished to investigate their role in colorectal cancer. We have compared the frequency of polymorphisms in the NER genes, XPD, XPF, XPG, ERCC1; in the BER gene, XRCC1; and in the RR gene, XRCC3; in colorectal cancer patients and in a control group. No significant associations were found for any of the NER gene polymorphisms or for the XRCC1 polymorphism. The C allele (position 18067) of the XRCC3 gene was weakly but significantly associated with colorectal cancer (odds ratio 1.52, 95% confidence interval 1.04–2.22, P = 0.03). For all patients who were heterozygous for any of the repair genes studied, tumour tissue was investigated for loss of heterozygosity (LOH). Only one example of LOH was found for all the genes examined. From the association and LOH data, we conclude that these genes do not have an important role in protection against colorectal carcinogenesis.

Keywords: DNA damage; DNA repair; loss of heterozygosity; xeroderma pigmentosum

Several complementary DNA repair mechanisms have evolved to protect the genome from DNA damage caused by endogenous or environmental agents, which could lead to mutations and carcinogenesis (Friedberg, 2001). DNA repair capacity varies between individuals in the general population (for a review, see Berwick and Vineis, 2000). An increasing number of DNA repair gene polymorphisms are being described and their involvement in carcinogenesis is being investigated. For colorectal cancer, the importance of mutations in mismatch repair (MMR) genes has been extensively documented. MMR gene defects account for 15% of sporadic colorectal cancer, and germline mutations in MMR genes are the cause of hereditary nonpolyposis colon cancer (for a review, see Jiricny and Nystrom-Lahti, 2000). The role of additional low-penetrance genes in colorectal cancer susceptibility has been recently reviewed (de Jong et al, 2002). We wished to investigate the hypothesis that alterations in other DNA repair pathways were also important genetic determinants of colorectal carcinogenesis.

The nucleotide excision repair (NER) pathway deals with UV light-induced DNA damage (for a review, see Wood, 1997). In the inherited disorder, xeroderma pigmentosum, NER deficiency results in a 1000-fold increased incidence of skin cancer, but also a 20-fold increase in internal tumours (Friedberg et al, 1995), indicating that NER is also important in the repair of endogenous DNA damage. Indeed, the digestive tract contains materials such as lipid peroxidation by-products that can react with DNA to generate bulky adducts that are recognised by NER (Friedberg et al, 1995). Amino-acid variants in NER genes are common in the general population (Shen et al, 1998) and some, such as the XPD exon 23 polymorphism, have been associated with reduced DNA repair capacity (Lunn et al, 2000; Qiao et al, 2002). We have reported a significant association between polymorphisms in exons 6, 22 and 23 of the XPD gene and melanoma in patients under 50 (Tomescu et al, 2001). Significant association between NER gene polymorphisms and other cancers, but not colorectal, have also been reported: glioma (Chen et al, 2000); lung (Chen et al, 2002; Park et al, 2002); squamous cell carcinoma of the head and neck (SCCHN; Sturgis et al, 2002).

The XRCC1 gene is involved in the repair of single-strand DNA breaks and in base excision repair (BER) of damaged bases caused by endogenous and exogenous oxidants, including tobacco smoke. XRCC1 polymorphisms have been associated with SCCHN (Sturgis et al, 1999), pancreatic adenocarcinoma (Duell et al, 2002), lung cancer (Chen et al, 2002) and bladder cancer (Stern et al, 2002). There is also a single report of a significant association with colorectal cancer (reviewed by de Jong et al, 2002). The XRCC3 gene, a parologue of RAD51, is involved in recombination repair (RR) and is required for genome stability (Griffin et al, 2000). XRCC3 polymorphisms have been associated with melanoma (Winsey et al, 2000), bladder cancer (Matullo et al, 2001a; Stern et al, 2002) and SCCHN (Shen et al, 2002).

Loss of heterozygosity (LOH) of tumour suppressor genes, such as p53, APC and BRCA1, is an important step in carcinogenesis. LOH for NER genes has been reported as a common occurrence in a range of carcinomas (Takebayashi et al, 2001), and we also...
wished to study LOH of NER genes in our colorectal cancer samples.

In this study, we have compared the frequency of polymorphisms in the NER genes (XPD, XPF, XPG, ERCC1), and in XRCC1 and XRCC3 in colorectal cancer patients and a control group. Furthermore, for patients heterozygous at any of these loci, we have looked for LOH in a biopsy of tumour tissue.

MATERIALS AND METHODS

Subjects

Subjects were colorectal cancer patients (mean age 69 years) attending the Western General Hospital, Edinburgh, UK. Biopsy material, from cancerous and adjacent noncancerous tissue, was collected between 1994 and 1997 by Professor Andrew Wyllie for histology and DNA extraction. At the same time, control blood samples were selected entirely at random from donors to the Scottish National Blood Transfusion Service (mean age of donors is 42 years), and DNA was extracted as described (Tomescu et al, 2001).

PCR and RFLP assays

The polymorphisms studied are shown in Table 1. All, apart from the XPG exon 15 polymorphism (Emmert et al, 2001), were originally described by Shen et al (1998). Polymorphisms were chosen for study because, in each case, the variant allele was common and the single-nucleotide change resulted in the gain, or loss, of a restriction site so that the polymorphism could be easily typed by PCR and RFLP analysis. In addition, significant associations between some of these polymorphisms and cancer have previously been reported. Details of the RFLPs are shown in Table 1. Details of the PCR products, primers and cycle conditions used are shown in Table 2. Genomic DNA (~100 ng) was amplified in a 50 μl reaction volume containing 300 ng of each primer and 2.5 U Taq DNA polymerase (Promega, UK) in 50 mM KCl, 1.5 mM MgCl2, 10 mM Tris-HCl pH 8.3, 0.45% Triton X-100, 0.45% Tween 20, 0.4 mM Na2EDTA, 0.1 mM dNTPs.

Statistical analysis

We first investigated whether the observed genotype distributions at each locus were consistent with a Hardy–Weinberg equilibrium. Having confirmed that this was the case, we assumed that alleles were independent at each locus, and compared allele counts among cases and controls. A χ2-test with Yates’ correction was used to give a conservative test for the significance of any association between a polymorphism and colorectal cancer.

RESULTS

Initially, we set out to genotype a minimum of 40 patient and control samples for the eight DNA repair gene RFLPs shown in Table 1. Where less genotypes than this are reported for each sample group, it is because some genotyping reactions failed. In the one instance (XRCC3 exon 7), where a significant association was found during this first round of genotyping, additional patient and control samples were then genotyped to increase the power of the statistical analysis. All the genotyping data obtained from the patient and control samples analysed are presented below. The patterns obtained for XPF exon 11, XPG exon 15, XRCC1 exon 17 and XRCC3 exon 7 are illustrated in Figure 1. The patterns for the XPD (exons 6, 22 and 23) and ERCC1 exon 4 polymorphisms have been described previously (Tomescu et al, 2001). For each polymorphism investigated in the control and patient groups, the observed genotype distributions were compared with the expected frequencies under the Hardy–Weinberg equilibrium. In each case, there were no significant deviations from the expected values. We therefore assumed independence of alleles at these loci and compared allele counts, rather than genotypes, in patient and control groups because of the resulting increased power of the statistical analysis.

Table 1 Details of RFLPs studied and fragment sizes

| Gene/exon | Enzyme | Polymorphism | Position* | Genotype | Fragment sizes (bp) |
|-----------|--------|--------------|-----------|----------|--------------------|
| XPD exon 6 | HinfI | C to A | 22541 | CC | 288 (no cut) |
| XPD exon 22 | FokI | C to T | 3532 | CC | 229 (no cut) |
| XPD exon 23 | PstI | A to C | 35931 | AA | 234+110 |
| ERCC1 exon 4 | BsmDI | G to A | 19007 | AC | 234+172+110+62 |
| XPG exon 15 | NolI | G to C | 3507 | GA | 252+179+73 |
| XPF exon 11 | AlwNI | T to C | 30028 | TC | 709+442+267 |
| XRCC1 exon 17 | Stul | G to A | 36189 | GA | 501+343+158 |
| XRCC3 exon 7 | NolI | C to T | 18067 | CT | 346+242+104 |

*Nucleotide positions are from the GenBank entries: XPD, L47234; ERCC1, M63796; XPG, NM_000123; XPF, L76568; XRCC1, L34079; XRCC3, AF037222.
**Table 2** Details of PCR products for polymorphism analysis

| Product          | Primer sequence          | Size (bp) | Conditions |
|------------------|--------------------------|-----------|------------|
| XPD exon 6       | (F) GTGCCAAAGCCGCGCCGCTGTG (R) CGACGGTCAAGGGAGCTGGGTGTC | 288       | 30 cycles: 94°C 1 min, 69°C 1 min, 72°C 30 s |
| XPD exon 22      | (F) AATGACCTTCTGGCCCGCGG (R) AGAACTCACTGCGGTGCCTCAG | 229       | 35 cycles: 94°C 1 min, 72°C 30 s |
| XPD exon 23      | (F) TCACATGCGCTGGGCTGAC (R) AGGATCAGCTGCGGCTGTG | 344       | 35 cycles: 94°C 1 min, 72°C 30 s |
| ERCC1 exon 4     | (F) CATTCCATTTGATGGTCTCAGTCC (R) CTGTCACCTGGAAGAGCACAGTCC | 252       | 34 cycles: 94°C 1 min, 69°C 1 min, 72°C 30 s |
| XPG exon 15      | (F) GACCTGCCTCTCCAGATCATC (R) CTCGGACGTTTATGTTT | 271       | 35 cycles: 94°C 1 min, 62°C 1 min, 72°C 1 min |
| XPG exon 11      | (F) TCTGCTGTGCTGACTACTAC (R) GCAGGCTCGGCCAGGTCGAACAA | 709       | 35 cycles: 94°C 1 min, 67°C 1 min, 72°C 1 min |
| XRCC1 exon 17    | (F) CTACATCAGCTGCGGTGGTGTG (R) CAGAGTGTGGGAGGCTGAGG | 501       | 35 cycles: 94°C 1 min, 69°C 1 min, 72°C 1 min |
| XRCC3 exon 7     | (F) GCTGCGCTGTTTGTCTGATGCT (R) GCTGCTCAGCTGGAGGTCGAGTCC | 346       | 35 cycles: 94°C 1 min, 69°C 1 min, 72°C 1 min |

*Primer sequences were derived from the GenBank entries.

**Table 3** Lack of association of XPD polymorphisms and colorectal cancer

| Product       | A | C | T | A | C |
|---------------|---|---|---|---|---|
| XPD exon 6    | 41 | 49 | 56 | 34 | 50 | 40 |
| XPD exon 22   | 56 | 86 | 56 | 32 | 60 | 34 |
| XPD exon 23   | OR 1.28, P = 0.43 | OR 0.94, P = 0.97 | OR 0.71, P = 0.32 |
| (95% CI 0.73–2.27) | (95% CI 0.49–1.81) | (95% CI 0.38–1.34) |

*Patients: n = 45. **Controls: n = 71; exon 22, n = 44; exon 23, n = 47. \( \chi^2 \) analysis with Yates’ correction.

**Figure 1** RFLP analysis of DNA repair gene polymorphisms. The patterns obtained for the polymorphisms in XPF exon 11, XRCC1 exon 17, XPG exon 15 and XRCC3 exon 7 are indicated. In each case, the sizes (bp) of the fragments generated for each genotype by restriction of the PCR products are shown, along with the uncut PCR product and a molecular size standard (Std). Details of the digests used and fragment sizes are given in Table 1. Note that the 44 bp fragment for the XPG assay and the 104 bp fragment for the XRCC3 assay are not resolved on the gels used. For the XRCC3 assay, LOH in a colorectal tumour sample is also shown. Normal tissue from the patient is CT, but the tumour is T.

**Lack of association between NER gene polymorphisms and colorectal cancer**

No significant associations were found between the XPD polymorphisms (exons 6, 22 and 23) and colorectal cancer (Table 3). Similarly, no significant associations were found for XPF exon 11, XPG exon 15 and ERCC1 exon 4 (Table 4).

**Association between XRCC3 exon 7 polymorphism and colorectal cancer**

Although no significant association with colorectal cancer was found for the XRCC1 exon 17 polymorphism (Table 3), a significant association was found for the XRCC3 polymorphism in the first batch of 40 samples genotyped. This association was maintained as the sample size of patient and control groups was increased to over 120, such that the XRCC3 exon 7 C allele was significantly over-represented in the patient group (odds ratio (OR) 1.52, 95% confidence interval (CI) 1.04–2.22, \( P = 0.03 \)).

**No LOH at NER gene loci in colorectal cancer**

Where normal tissue biopsied from colorectal cancer patients showed heterozygosity at any of the DNA repair gene loci genotyped, the tumour tissue from the same patients was analysed for LOH. The numbers of heterozygous loci examined in the patient and control groups were: 16 for XRCC1 exon 11, 28 for XRCC3 exon 7.

**DISCUSSION**

We have investigated the hypothesis that NER gene polymorphisms might predispose to colorectal cancer because some forms of the encoded proteins may be less efficient at repairing DNA damage arising from exposure of the gut epithelium to genotoxic compounds in the lumen. NER is known to be active against a range of bulky DNA lesions in addition to its main role in the repair of UV-induced DNA damage. In a small study, we have previously shown a significant association between melanoma and three polymorphisms in the XPD gene (exon 6)
### Table 4  
Lack of association of NER polymorphisms and colorectal cancer

|         | XPF exon 11 |         | ERCC1 exon 4 |
|---------|-------------|---------|--------------|
|         | T C         | C G     | G A          |
| Patients | 61 19       | 13 67   | 38 52        |
| Controls| 57 9        | 22 58   | 58 86        |
| OR 0.51, P = 0.18 | OR 0.51, P = 0.13 | OR 1.08, P = 0.87 |
| (95% CI 0.19 – 1.30) | (95% CI 0.22 – 1.18) | (95% CI 0.61 – 1.92) |

* Patients: XP and XPG, n = 40; ERCC1, n = 45.  
* Controls: XP, n = 33; XPG, n = 40; ERCC1, n = 72.

### Table 5  
Association of XRCC3 exon 7 C allele and colorectal cancer

| XRCC1 exon 17 | XRCC3 exon 7 |
|---------------|--------------|
| G A           | C T          |
| Patients      | 40 40       | 161 85 |
| Controls      | 38 28       | 142 114 |
| OR 0.74, P = 0.46 | OR 1.52, P = 0.03 |
| (95% CI 0.36 – 1.50) | (95% CI 1.04 – 2.22) |

* Patients: XRCC1, n = 40; XRCC3, n = 123.  
* Controls: XRCC1, n = 33; XRCC3, n = 128.

controls. This XRCC3 exon 7 polymorphism has previously shown a significant association with melanoma (Winsey et al., 2000), bladder cancer (Matullo et al., 2001a) and SCCHN (Shen et al., 2002). However, in these studies, it was the variant T (Met) allele that was over-represented in the cancer patients and this allele has also been associated with a reduced DNA repair phenotype (Matullo et al., 2001b).

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