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Lymphocyte DNA adducts and polymorphism in the DNA repair enzyme XPD

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The effect of genetic polymorphism of DNA repair enzyme on the DNA adduct levels was evaluated in this study. We explored the relationship between polymorphism in the nucleotide excision repair enzyme XPD and DNA adduct levels in lymphocytes. Lymphocyte DNA adducts were measured by a ^{32}P-postlabelling method from 42 subjects. XPD ex23 genotypes were identified by the PCR-RFLP method. Subjects with the XPD ex23 heterozygous genotype (AC) showed significantly lower DNA adduct levels (0.63 – 0.19 per 10^8 nucleotides, $n = 6$) than those (0.92 – 0.38, $n = 36$) with major homozygous genotypes (AA). This result suggests that the genotype of the repair enzyme may be one of the important determinants for DNA adduct levels.

Keywords: DNA adduct, repair.

Introduction

Environmental carcinogens are enzymatically activated to form intermediates that can react with cellular DNA and form DNA adducts. The measurement of DNA adducts is a useful indicator for environmental carcinogen exposure monitoring. There are several studies in which smoking-related DNA adducts were measured by the ^{32}P-postlabelling method in peripheral blood samples (Beach and Gupta 1992). However, the inter-individual variation of adduct levels was very large (Hemminki 1995). The genetic polymorphisms of metabolic enzymes have been thought by several workers to clarify the inter-individual variation of DNA adduct levels (Ichiba *et al.* 1994, 1997, Hou *et al.* 1995, Rothman *et al.* 1995, Nielsen *et al.* 1996, Hemminki *et al.* 1997) but their results were not consistent. On the other hand, the DNA repair system is also thought to be an important determinant for DNA adduct levels. Bulky aromatic DNA adducts were repaired by the nucleotide excision repair system. Enzymes for the nucleotide excision repair are composed of nine groups: XPA, XPB (ERCC3), XPC, XPD (ERCC2), XPE, XPF, XPG (ERCC5), CSB (ERCC6) and ERCC1. With these groups, the damaged part of DNA is recognized and cut. Then the DNA chain with adducts is

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removed and complementary DNA chain is synthesized. The repair rate shows a very large inter-individual variation (Harris 1989). Polymorphisms in several DNA repair genes have been identified. Shen *et al*. (1998) reported the extent of DNA sequence variation in coding proteins of the nucleotide excision repair enzyme among 12 healthy individuals. They found nine different amino acid substitution variants in resequencing of exons of three nucleotide excision repair genes (ERCC1, XPD and XPF). However, their impact for DNA repair is unknown. We explored the relationship between polymorphism in the DNA excision repair enzyme XPD and DNA adduct levels in lymphocytes.

Subjects and methods

Subjects were 42 primary lung cancer patients, 27 men and 15 women. They gave written informed consent and completed a short questionnaire on recent diet, illness and smoking habit. Their mean age was 64.1 years old; 67% of subjects were smokers. DNA was extracted from their peripheral blood lymphocytes. Aromatic DNA adducts was measured by the ^{32}P-postlabelling nuclease P1 method (Reddy and Randerath 1986). The results were given as a total number of adducts per 10^8 normal nucleotides. The DNA adducts data from these subjects have been previously reported (Kiyohara *et al*. 1999).

There is an A to C polymorphism at codon 751 (Lys to Gln) on XPD ex23 (Shen *et al*. 1998). XPD ex23 polymorphism was measured by the PCR-restriction fragment length polymorphism method (Dybdahl *et al*. 1999). Subjects were divided into three groups; AA (Lys/Lys), AC (Lys/Gln), and CC (Gln/Gln).

Results

Characteristics of subjects and the distribution of genotype for XPD ex23 polymorphism are given in table 1. Genotype distributions were 86% (AA) and 14% (AC), respectively. We did not find a CC genotype. A significant difference in age, sex ratio and prevalence of smokers was not found between AA and AC genotypes.

To clarify whether the XPD polymorphism is associated with differences in DNA repair, we compared DNA adduct levels in the different genotypes. DNA adducts reflect levels of genotoxic damage. Figure 1 shows the result concerning lymphocyte DNA adduct levels and genotypes. The adduct levels of subjects with the AC genotype were significantly lower than those of subjects with AA genotype ($p = 0.048$, Mann–Whitney test).

Figure 2 shows correlations between number of cigarettes smoked per day and DNA adduct levels. Subjects with the AC type tended to show low adduct levels when compared with the same smoking dose, however the correlations between smoking dose and DNA adduct levels were not significant.

Table 1. Characteristics of subjects and distribution of genotypes.

	All	AA	AC
Number of subjects (%)	42 (100%)	36 (86%)	6 (14%)
Male/Female	27/15	23/13	4/2
Mean age (years)[a]	64 – 10	63 – 10	71 – 8
Smokers (%)	67%	64%	83%
Lymphocyte DNA adduct (/10^8)[a]	0.88 – 0.37	0.92 – 0.38	0.63 – 0.19

[a]Mean – SD.

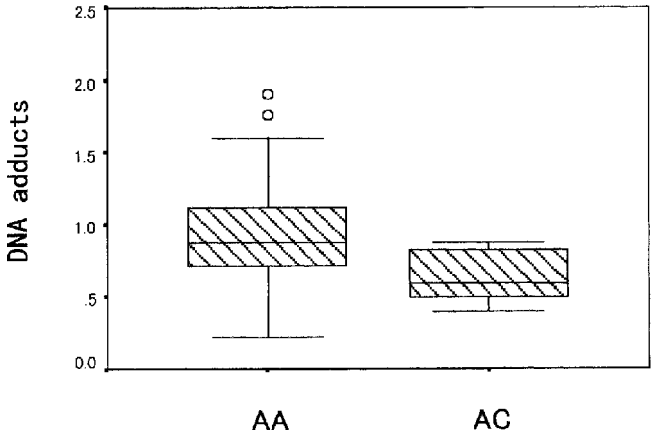

Figure 1. DNA adducts distribution in AA genotype (AA) and AC genotype (AC). Adduct levels were expressed as number of adducts per 10^8 nucleotides. Box extends from the 25th to 75th percentiles with median levels indicated with horizontal line. Outer horizontal lines indicate the 10th and 90th percentiles. Small circles indicate data over the 10th and 90th percentiles.

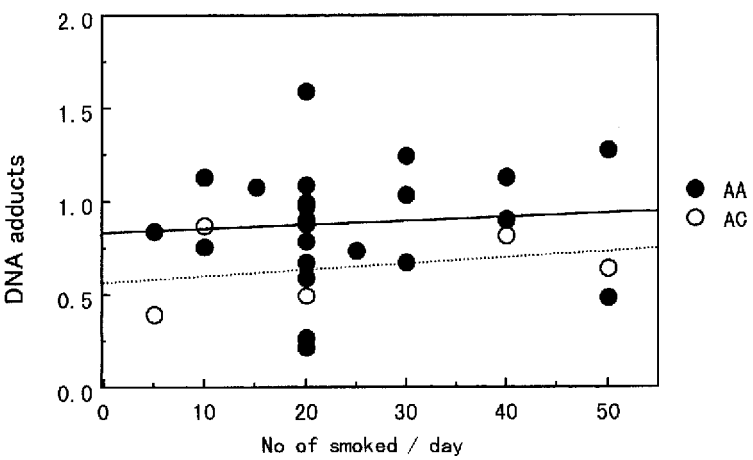

Figure 2. Linear regression analysis between number of cigarettes smoked per day and lymphocyte DNA adducts in smokers. Adduct levels were expressed as number of adducts per 10^8 nucleotides. AA: closed circle and solid line ($n = 23$); AC: open circle and dotted line ($n = 5$).

Discussion

The DNA repair system plays an important role in protecting genes from cancer incidence by environmental carcinogens. DNA repair polymorphism may alter the structure of the DNA repair enzymes and modulate cancer susceptibility.

Shen *et al*. (1998) identified four coding polymorphisms in the nucleotide excision repair gene XPD; ex8 codon 199 (Ile to Met), codon 201 (His to Tyr), ex10 codon 312 (Asp to Asn), and ex23 codon 751 (Lys to Gln). They also identified three coding XRCC1 repair enzyme polymorphisms. XRCC1 was thought to have role of the base excision repair. Ruth *et al*. (1999) measured afiatoxin B_1–DNA

adducts in placental DNA samples and compared them between genotypes of XRCC1. They found that the XRCC1 399Gln allele was significantly associated with higher levels of AFB_1–DNA adducts.

However, there are no reports about the effect of XPD polymorphism on DNA adduct levels. We analysed XPD polymorphism among 42 Japanese persons. The XPD genotype distributions were 86% AA ($n = 36$) and 14% AC ($n = 6$). This distribution showed a large difference in comparison with a previous report by Dybdahl *et al.* (1999) who found distributions of 48% AA, 41% AC and 11% CC in Caucasians. This difference may be due to racial differences. We found that subjects with the XPD ex23 AC genotype showed significantly lower adduct levels than those with the AA genotype. This result suggested that the AA genotype is associated with increased levels of DNA adducts which may be due to reduced DNA repair function. Individuals with the XPD AC genotype were more likely to have low levels of DNA adduct. Why did minor genotypes show a resistance to the exposure? We cannot explain the reason. As a similar example, the GSTM1 null genotype has been associated with increased susceptibility to cancer. The GSTM1 null frequency is about 50% in Caucasians and ranges between about 30% and 90% in different ethnic groups (Taningher *et al.* 1999).

On the other hand, Dybdahl *et al* (1999) found that subjects with the AA genotype in XPD exon 23 had a 3.7 or 4.3-fold higher risk of basal cell carcinoma (BCC) than those with the AC or CC genotype, respectively. In addition, the mean age at first skin tumour for BCC cases with the AA genotype was significantly lower than that for BCC cases with the AC or CC genotype. Lunn *et al.* (2000) also reported that individuals with the Lys/Lys (AA) codon 751 XPD genotype had a higher number of chromatid aberrations than those with the 751 Gln (AC or CC) allele. Thus, the variant C allele of exon 23 may be protective. These results may agree theoretically with our finding that subjects with the AC genotype showed lower DNA adduct levels.

Another viewpoint of evaluating the effect of repair enzyme on DNA adduct levels is the measurement of repair gene mRNA expression. However, there are few reports on the relationship between DNA adducts and repair enzyme mRNA expression. Previously, we measured mRNA levels of repair enzymes ERCC1 and XPCC and compared with their DNA adduct levels (Ichiba *et al.* 2000). The DNA adduct levels showed a positive correlation with ERCC1 and a negative correlation with XPCC mRNA.

In conclusion, we demonstrated that subjects with the XPD ex23 AC genotype showed significantly lower DNA adduct levels than those with AA genotypes. However, the numbers of heterozygous individuals were very small, limiting the interpretation of our finding. The study dealing with large numbers may help to distinguish whether this positive difference is due to chance or not. Further studies are needed to characterize the role of the XPD in the functioning of the DNA repair system, and to estimate whether it affects the levels of other biomarkers on DNA damage.

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