A polymorphism in NAD(P)H:quinone oxidoreductase (NQO1): relationship of a homozygous mutation at position 609 of the NQO1 cDNA to NQO1 activity

Sir – NQO1 has attracted considerable attention owing to its ability to deactivate a broad range of xenobiotics while activating certain anti-tumour quinones (Ross *et al.*, 1994). A number of recent reports have highlighted the occurrence and potential significance of a point mutation in the NQO1 gene which is associated with a loss of NQO1 activity in both normal and tumour tissue (Traver *et al.*, 1992; Eickelmann *et al.*, 1994*a,b*; Rosvold *et al.*, 1995; Kolesar *et al.*, 1995). The mutation is a C to T point mutation at position 609 of the NQO1 cDNA which codes for a proline to serine substitution in the amino acid sequence of the protein (Traver *et al.*, 1992). The mutation was originally characterised in the BE human colon adenocarcinoma cell line by SSCP analysis and sequencing (Traver *et al.*, 1992) and subsequently in the H596 human non-small-cell lung cancer cell line by a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique (Traver *et al.*, 1995). Both the BE and H596 cell lines had moderate NQO1 mRNA expression but extremely low or non-detectable NQO1 activity. Purified, recombinant BE and H596 mutant NQO1 proteins expressed in *E. coli* have residual activity (<10% of wild-type). While both BE and H596 recombinant mutant proteins reacted with a mouse monoclonal antibody raised against human NQO1, immunoblot analysis of BE and H596 cell lines did not detect NQO1 protein expression (Siegel *et al.*, 1995). This suggests that in cells the mutant protein may not be made or that the mutant protein may be made but rapidly degraded. Kuehl *et al.* (1995) have recently examined the relationship between the heterozygous mutation at position 609 and NQO1 activity. These data demonstrate a wide range of NQO1 activity in ^{609}C to T heterozygotes and suggests a significant role for post-transcriptional modification in determination of NQO1 activity.

In the paper by Kuehl *et al.* (1995), the BE cell line is described as heterozygous for the ^{609}C–T point mutation with very low NQO1 activity. An additional fibroblastoid cell line, the 3701T line, was reported as homozygous for the mutation with very low NQO1 activity. Since these results differed, with respect to the BE cell line, from the original report of Traver *et al.* (1992), the two laboratories involved have exchanged stocks of BE cells and repeated the *Hinf*1 PCR–RFLP assay for the mutation at position 609 (Eickelmann *et al.*, 1994*b*). Both stocks of BE cells have consistently been found to be homozygous for the 609 point mutation. The reasons for the appearance of the BE cells as heterozygous for the mutation in the report of Kuehl *et al.* (1995) are unclear. In addition, both the BE cells and the 3701T cells were found to have no detectable NQO1 protein by immunoblot analysis (employing a human monoclonal antibody to NQO1) and extremely low or non-detectable NQO1 activity using standard activity assays [dicoumarol inhibition of dichlorophenolindophenol (DCPIP) reduction]. The NQO1 activity of ^{609}C–T homozygous mutants, such as BE and 3401T cells, has been reported as either non-detectable or as extremely low (Siegel *et al.*, 1992; Traver *et al.*, 1992; Kuehl *et al.*, 1995). Because of the somewhat non-specific nature of both DCPIP as a substrate and dicoumarol as an inhibitor (Ross *et al.*, 1993), it is possible that the extremely low rates of dicoumarol-inhibitable DCPIP reduction obtained in BE, H596 or 3701T cells may reflect the presence of reductases other than NQO1. Our joint findings confirm previous results that the BE cells are homozygous for the ^{609}C–T mutation and that the presence of the homozygous mutation is associated with a loss of NQO1 protein activity (Traver *et al.*, 1992, 1995). The same conclusion was reached by Eickelmann *et al.* (1994) who reported that a cell line and three human kidney carcinoma samples without detectable NQO1 activity were all homozygous for the ^{609}C to T mutation.

An alternatively spliced form of NQO1 lacking exon 4 and the quinone binding site has recently been reported (Gasdaska *et al.*, 1995). This form of NQO1 has minimal enzyme activity with traditional model substrates for NQO1 but retains immunoreactivity and can be detected using a polyclonal antibody to NQO1 (Gasdaska *et al.*, 1995). We have also examined BE and H596 cells for NQO1 protein using the same polyclonal antibody used by Gasdaska and colleagues and were unable to detect NQO1 protein. We have recently suggested a different form of alternate splicing as a possible explanation for the appearance of marked NQO1 activity in a fibroblastoid cell line, G38-8X, in the absence of detectable protein by immunoblot analysis (Kuehl *et al.*, 1996). We reasoned that an alternatively spliced form of NQO1 might retain catalytic activity but have lost the epitope required for immunoreactivity with the antibody against NQO1 (Kuehl *et al.*, 1996). This would not be a plausible explanation for the lack of NQO1 protein in homozygous ^{609}C to T mutants since these cells are essentially devoid of both NQO1 immunoreactivity and NQO1 activity.

In conclusion, although a heterozygous C to T mutation at position 609 of the NQO1 cDNA may be associated with widely differing NQO1 activities, the presence of a homozygous C–T mutation at position 609 results in a loss of NQO1 protein and activity. The homozygous mutation represents a polymorphism in NQO1 which may be of significance since its prevalence in various populations has been reported to be between 6% and 17% (Rosvold *et al.*, 1995; Kuehl *et al.*, 1995; Eickelmann *et al.*, 1994*a*; Traver *et al.*, 1996). The role of this polymorphism with respect to impaired protection from xenobiotic toxicity is under investigation (Rothman *et al.*, 1996).

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