[8] NAD(P)H:Quinone Oxidoreductase 1 (NQO1, DT-Diaphorase), Functions and Pharmacogenetics

By DAVID ROSS and DAVID SIEGEL

Introduction

NQO1 is a flavoprotein that is known to catalyze two electron reduction of a broad range of substrates.[1–3] As the name of the enzyme suggests, a common group of substrates are quinones, which are reduced via a hydride transfer mechanism to generate the corresponding hydroquinone derivative. Elucidation of the mechanism of catalysis has been facilitated by elucidation of the crystal structure of the enzyme and has been recently summarized.[4–7] The broad substrate specificity has also been explained by structural studies demonstrating the presence of a highly plastic active site that can accommodate a range of structures.[8] Because of the many deleterious effects of quinonoid compounds, including their capacity to arylate nucleophiles and generate aggressive oxygen species via redox cycling mechanisms, removal of a quinone from a biological system by NQO1 has been considered to be a detoxification reaction.[9–12] Two electron reduction of certain antitumor quinones such as mitomycin C, E09, streptonigrin and B-lapachone by NQO1, however, results in bioactivation

[1] L. Ernster, *Methods Enzymol.* **10,** 309 (1967).
[2] C. Lind, E. Cadenas, P. Hochstein, and L. Ernster, *Methods Enzymol.* **186,** 287 (1990).
[3] L. Ernster, *in* "Pathophysiology of lipid peroxides and free radicals" (K. Yagi, ed.), p. 149. Japan Sci. Soc. Press/Karger, Tokyo/Basel, 1998.
[4] R. Li, M. Bianchet, P. Talalay, and L. M. Amzel, *FASEB J.* **9,** A1338 (1995).
[5] M. Faig, M. A. Bianchet, P. Talalay, S. Chen, S. Winski, D. Ross, and A. L. Mario, *Proc. Natl. Acad. Sci. USA* **97,** 3177 (2000).
[6] G. Cavelier and L. M. Amzel, *Proteins* **43,** 420 (2001).
[7] R. Li, M. A. Bianchet, P. Talalay, and L. M. Amzel, *Proc. Natl. Acad. Sci. USA* **92,** 8846 (1995).
[8] M. Faig, M. A. Bianchet, S. Winski, R. Hargreaves, C. J. Moody, A. R. Hudnott, D. Ross, and L. M. Amzel, *Structure. (Camb.)* **9,** 659 (2001).
[9] C. Lind, P. Hochstein, and L. Ernster, *Arch. Biochem. Biophys.* **216,** 178 (1982).
[10] H. Thor, M. T. Smith, P. Hartzell, G. Bellomo, S. A. Jewell, and S. Orrenius, *J. Biol. Chem.* **257,** 12419 (1982).
[11] D. Di Monte, G. Bellomo, H. Thor, P. Nicotera, and S. Orrenius, *Arch. Biochem. Biophys.* **235,** 343 (1984).
[12] D. Di Monte, D. Ross, G. Bellomo, L. Eklow, and S. Orrenius, *Arch. Biochem. Biophys.* **235,** 334 (1984).

0076-6879/04 $35.00

of these compounds to more toxic metabolites.[13–16] Since NQO1 is expressed at high levels throughout many human solid tumors,[17,18] compounds efficiently bioactivated by NQO1 have been designed for the therapy of tumors rich in NQO1.[8,19–21] Currently, a new NQO1-targeted aziridinylbenzoquinone, RH1,[20] is undergoing phase 1 clinical trials. The role of NQO1 in chemoprotection and its contrasting role in bioactivation of antitumor quinones[22,23] and both the gene and protein structure of NQO1[24,25] have been recently summarized. The purpose of this review is to introduce the enzyme, to review the possible functions of NQO1, to summarize current information on polymorphic forms of NQO1 and finally, to summarize the relevance of the common NQO1*2 polymorphism for chemoprotection, susceptibility to disease and cancer chemotherapy.

Possible Functions of NQO1

Early Work on Mitochondrial Electron Transport and Vitamin K Metabolism

When NQO1 was first isolated by Ernster,[26,27] there was considerable controversy regarding a potential role for NQO1 in mitochondrial electron transport. This work was summarized in a fascinating historical review of

[13] D. Siegel, N. W. Gibson, P. C. Preusch, and D. Ross, *Cancer Res.* **50,** 7483 (1990).
[14] M. I. Walton, P. J. Smith, and P. Workman, *Cancer Commun.* **3,** 199 (1991).
[15] H. D. Beall, Y. Liu, D. Siegel, E. M. Bolton, N. W. Gibson, and D. Ross, *Biochem. Pharmacol.* **51,** 645 (1996).
[16] J. J. Pink, S. M. Planchon, C. Tagliarino, M. E. Varnes, D. Siegel, and D. A. Boothman, *J. Biol. Chem.* **275,** 5416 (2000).
[17] D. Siegel, W. A. Franklin, and D. Ross, *Clin. Cancer Res.* **4,** 3083 (1998).
[18] D. Siegel and D. Ross, *Free Radic. Biol. Med.* **29,** 246 (2000).
[19] H. D. Beall, A. M. Murphy, D. Siegel, R. H. J. Hargreaves, J. Butler, and D. Ross, *Mol. Pharmacol.* **48,** 499 (1995).
[20] S. Winski, R. H. J. Hargreaves, J. Butler, and D. Ross, *Clin. Cancer Res.* **4,** 3083 (1998).
[21] S. L. Winski, E. Swann, R. H. Hargreaves, D. L. Dehn, J. Butler, C. J. Moody, and D. Ross, *Biochem. Pharmacol.* **61,** 1509 (2001).
[22] D. Ross, *in* "Comprehensive toxicology" (F. P. Guengerich, ed.), Vol. 3, p. 179. Pergamon, New York, 1997.
[23] D. Ross, J. K. Kepa, S. L. Winski, H. D. Beall, A. Anwar, and D. Siegel, *Chem. Biol. Interact.* **129,** 77 (2000).
[24] D. Ross, *in* "Encyclopedia of Molecular Medicine" p. 2208. John Wiley and Sons, New York, 2001.
[25] D. Ross, "Atlas genetics cytogenetics oncology hematology" http://www.infobiogen.fr/services/chromcancer/Genes/NQO1ID375.html, 2002.
[26] L. Ernster and F. Navazio, *Acta Chem. Scand.* **12,** 595 (1958).
[27] L. Ernster, *Fed. Proc.* **17,** 216 (1958).

the enzyme by Ernster in 1987.[28] NQO1 was found not to be a component of the mitochondrial respiratory chain, and attention turned to other possible physiological functions for the enzyme such as a potential role in vitamin K metabolism, suggested in the early 1960s by Martius.[29] The role of NQO1 in generating reduced vitamin K1 cofactor and triggering protein carboxylation critical to prothrombin synthesis was suggested in rat liver,[30] but vitamin K1 was found not to be a substrate for purified NQO1 isolated from rat liver cytosol, suggesting that other enzyme systems may be more important for vitamin K1 reduction.[31]

Detoxification of Quinones via Two Electron Reduction

A considerable body of literature exists supporting the role of NQO1 as a detoxification system. Induction of NQO1 has been demonstrated to protect against the cytotoxicity, mutagenicity and carcinogenicity of many compounds.[32–34] Induction of NQO1 occurs however via the XRE and ARE elements in the NQO1 promoter,[35] and many protective enzymes in addition to NQO1 may also be induced via this mechanism. For example, recent work using t-butylhydroquinone, a common inducer used to elevate NQO1, increased the expression of 63 different genes as indicated by microarray analysis.[36] This observation emphasizes the need to perform specific studies to define the role of a particular gene in detoxification and a good example is work performed with menadione.

Work by two independent groups a few miles apart in Stockholm in the early 1980s using the naphthoquinone menadione[9,10] defined the role of NQO1 as a detoxification enzyme in quinone metabolism. Both menadione-induced arylation of cellular nucleophiles and generation of aggressive oxygen species were diminished in the presence of functional NQO1. Detoxification of quinones by NQO1 has been confirmed by studies in NQO1 knockout mice that demonstrated increased menadione toxicity in NQO1-deficient animals.[37] NQO1 knockout animals also demonstrate

[28] L. Ernster, *Chemica Scripta*, **27A,** 1 (1987).
[29] C. Martius, *in* "The Enzymes" (P. D. Boyer, H. Lardy, and K. Myrback, eds.), Vol. 7, p. 517. Academic Press, New York, 1960.
[30] R. Wallin, S. R. Rannels, and L. F. Martin, *Chemica Scripta* **27A,** 193 (1987).
[31] P. C. Preusch and D. M. Smalley, *Free Rad. Res. Comm.* **8,** 401 (1990).
[32] C. Huggins and R. Fukunishi, *J. Exp. Med.* **119,** 923 (1964).
[33] A. M. Benson, M. J. Hunkler, and P. Talalay, *Proc. Natl. Acad. Sci. USA* **77,** 5216 (1980).
[34] P. Talalay and H. J. Prochaska, *Chemica Scripta* **27A,** 61 (1987).
[35] J. K. Kepa, R. D. Traver, D. Siegel, S. L. Winski, and D. Ross, *Rev. Toxicol.* **1,** 53 (1997).
[36] J. Li, J. M. Lee, and J. A. Johnson, *J. Biol. Chem.* **277,** 388 (2002).
[37] V. Radjendirane, P. Joseph, Y. H. Lee, S. Kimura, A. J. P. Klein-Szanto, F. J. Gonzalez, and A. K. Jaiswal, *J. Biol. Chem.* **273,** 7382 (1998).

increased benzo[a]pyrene and 7,12-dimethylbenzanthracene-induced mouse skin carcinogenesis.[38,39] A role for NQO1 in detoxification is consistent with the distribution of the enzyme in animal and human systems. In mice, rats and humans, NQO1 is mainly localized to epithelial and endothelial tissues,[17,18] which facilitates exposure of compounds entering the body to NQO1. An interesting and puzzling species difference in enzyme distribution is that both rat and mouse liver contain high levels of NQO1, but only trace levels of NQO1 could be detected by immunoblot analysis in five human liver samples.[18] This confirms earlier work demonstrating a low activity of NQO1 in human liver cytosol relative to other species.[30]

Sharpening the Double-Edged Sword: NQO1 in Bioactivation

It is often difficult to generalize whether NQO1 functions as a detoxification enzyme or a toxification system with an individual substrate. The reactions of the hydroquinone generated will determine whether NQO1 mediated reduction results in deleterious or protective effects in a cellular system. Hydroquinones are not necessarily innocuous species and may autoxidize to generate reactive oxygen species or rearrange to produce reactive arylating species.[23] Efficient detoxification of quinones via two electron reduction relies on excretion of the more water soluble hydroquinone or conjugation with glucuronide or sulfate and subsequent excretion.

NQO1 is expressed at high levels throughout many human solid tumors, and a number of compounds clinically used as antitumor agents or in development as experimental antitumor agents can be efficiently bioactivated by NQO1.[23,40–42,43] Apart from the bioactivation of antitumor quinones such as mitosenes, indolequinones, aziridinylbenzoquinones and others, NQO1 can also bioactivate simpler quinones. Even though the naphthoquinone menadione is detoxified by NQO1, closely related naphthoquinones such as 2-hydroxy-1,4-naphthoquinone[44] and β-lapachone can be activated by NQO1.[16] Interestingly, although NQO1 is predominantly a cytosolic enzyme, recent work has demonstrated a significant nuclear pool of

[38] D. J. Long, R. L. Waikel, X. J. Wang, L. Perlaky, D. R. Roop, and A. K. Jaiswal, *Cancer Res*. **60,** 5913 (2000).
[39] D. J. Long, R. L. Waikel, X. J. Wang, D. R. Roop, and A. K. Jaiswal, *J. Natl. Cancer Inst*. **93,** 1166 (2001).
[40] D. Ross, D. Siegel, H. Beall, A. S. Prakash, R. T. Mulcahy, and N. W. Gibson, *Cancer Metastasis Rev*. **12,** 83 (1993).
[41] D. Ross, H. Beall, R. D. Traver, D. Siegel, R. M. Phillips, and N. W. Gibson, *Oncol. Res*. **6,** 493 (1994).
[42] R. J. Riley and P. Workman, *Biochem. Pharmacol*. **43,** 1657 (1992).
[43] H. D. Beall and S. I. Winski, *Front Biosci*. **5,** D639 (2000).
[44] R. Munday, B. L. Smith, and C. M. Munday, *Chem. Biol. Interact*. **117,** 241 (1999).

NQO1 in human tumor cells when using both confocal microscopy and immuno-electron microscopy.[45] A nuclear pool of NQO1 may be of considerable importance for the bioactivation of NQO1-directed antitumor quinones that are designed to crosslink DNA. Whether a similar pool of nuclear NQO1 exists in normal cells is currently unclear.

NQO1 as an Antioxidant Enzyme: Role in Ubiquinone and Vitamin E Metabolism

In addition to activation and deactivation of exogenous compounds, NQO1 has been shown to play a role in the metabolism of endogenous quinones such as ubiquinone and vitamin E quinone. These quinones have very large hydrophobic tails, and in their reduced state they protect cellular membranes against lipid peroxidative injury. The reduction of ubiquinone by NQO1 has been shown to generate ubiquinol, and this compound possesses excellent antioxidant properties.[46,47] Vitamin E quinone is formed during free radical attack on vitamin E and has been shown to undergo reduction by NQO1 to generate vitamin E hydroquinone.[48] Since vitamin E quinone is devoid of antioxidant activity, in this situation NQO1 may extend the antioxidant potential of vitamin E by generation of vitamin E hydroquinone, a compound that has been suggested to have antioxidant properties superior to vitamin E.[49]

NQO1 as a Component of a Stress Response: Stabilization of p53

Studies with proteins typically considered as metabolic enzymes suggest that these proteins may have additional roles outside the range of their normal metabolic functions. For example, glutathione-S-transferase associates with c-Jun N terminal kinase leading to inhibition of kinase activity and modulation of signaling and cellular proliferation.[50–52] Recent studies

[45] S. L. Winski, Y. Koutalos, D. L. Bentley, and D. Ross, *Cancer Research* **62,** 1420 (2002).
[46] R. E. Beyer, J. Segura-Aguilar, S. Di Bernardo, M. Cavazzoni, R. Fato, D. Fiorentini, M. Galli, M. Setti, L. Landi, and G. Lenaz, *Proc. Natl. Acad. Sci. USA* **93,** 2528 (1996).
[47] L. Landi, D. Fiorentini, M. C. Galli, J. Segura-Aguilar, and R. E. Beyer, *Free Radic. Biol. Med*. **22,** 329 (1997).
[48] D. Siegel, E. M. Bolton, J. A. Burr, D. C. Liebler, and D. Ross, *Mol. Pharmacol*. **52,** 300 (1997).
[49] I. Kohar, M. Baca, C. Suarna, R. Stocker, and P. T. Southwell-Keely, *Free Rad. Biol. Med*. **19,** 197 (1995).
[50] T. Wang, P. Arifoglu, Z. Ronai, and K. D. Tew, *J. Biol. Chem*. **276,** 20999 (2001).
[51] V. Adler, Z. Yin, S. Y. Fuchs, M. Benezra, L. Rosario, K. D. Tew, M. R. Pincus, M. Sardana, C. J. Henderson, C. R. Wolf, R. J. Davis, and Z. Ronai, *EMBO J*. **18,** 1321 (1999).
[52] J. E. Ruscoe, L. A. Rosario, T. Wang, L. Gate, P. Arifoglu, C. R. Wolf, C. J. Henderson, Z. Ronai, and K. D. Tew, *J. Pharmacol. Exp. Ther*. **298,** 339 (2001).

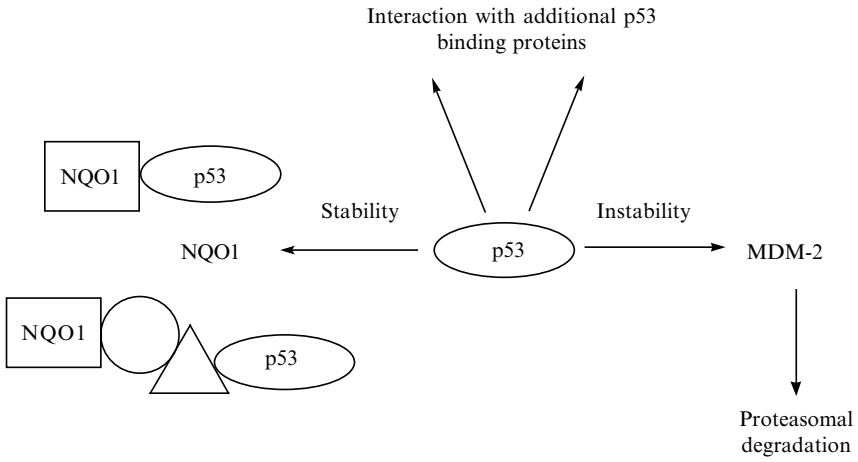

FIG. 1. Proposed mechanism of stabilization of p53 via a protein-protein interaction with NQO1. See reference 56.

have shown that NQO1 may influence the stability of the tumor suppressor protein p53 by inhibiting its degradation.[53–55] In these studies the authors hypothesized that the NQO1-mediated conversion of NADH to NAD^+ promoted stabilization of p53. Our own work has shown a direct physical interaction between p53 and NQO1.[56] In these studies, p53 was found to directly associate with NQO1 in an *in vitro* translation/transcription system and in human cancer and primary cell lines. The association between p53 and NQO1 was not affected by pretreatment with ES936, a mechanism based inhibitor of NQO1, suggesting that the catalytic activity of NQO1 was not needed for a protein-protein interaction of p53 and NQO1 proteins. Anwar *et al.* proposed that the protein-protein interaction of NQO1 and p53 may represent an alternative mechanism of p53 stabilization by NQO1,[56] as shown in Fig. 1.

[53] G. Asher, J. Lotem, B. Cohen, L. Sachs, and Y. Shaul, *Proc. Natl. Acad. Sci. USA* **98,** 1188 (2001).
[54] G. Asher, J. Lotem, R. Kama, L. Sachs, and Y. Shaul, *Proc. Natl. Acad. Sci. USA* **99,** 3099 (2002).
[55] G. Asher, J. Lotem, L. Sachs, C. Kahana, and Y. Shaul, *Proc. Natl. Acad. Sci. USA* **99,** 13125 (2002).
[56] A. Anwar, D. Dehn, D. Siegel, J. K. Kepa, L. J. Tang, J. A. Pietenpol, and D. Ross, *J. Biol. Chem.* **278,** 10368 (2003).

Future Work on Possible Physiological Roles of NQO1

A number of possible functions of NQO1 have been investigated. Many of the pharmacological studies of NQO1 have relied on the use of the inhibitor dicoumarol. The major problem with the use of dicoumarol is its nonspecific nature.[57] The recent development of a mechanism-based inhibitor of NQO1[58] offers the promise of more specific targeting of NQO1. Taken together with the application of gene targeting methods[59] and the development of NQO1 knockout animals,[37] these approaches should be able to more precisely define the role of NQO1 in cellular systems.

Pharmacogenetics of NQO1

There are two well-characterized polymorphisms in NQO1 with defined phenotypes and population frequencies—the NQO1*2[60,61] and NQO1*3[62,63] polymorphisms. A recent review by Nebert and coworkers[64] screened the SNP database at the University of Utah and identified 22 SNPs in NQO1. Three of these SNPs had been reported earlier in a study of 84 Japanese volunteers.[65] In addition to the NQO1*2 polymorphism, 5 SNPs were found in the 5′ flanking region of NQO1, 10 SNPs at 9 sites in intron 1, a synonomous mutation in exon 2, 2 SNPs in the 3′ untranslated region and 3 SNPs in the 3′ flanking region of the gene.[64] The NQO1*3 polymorphism was not detected in the SNP database study. Apart from the NQO1*2 and NQO1*3 polymorphisms, the frequency of the additional 21 variant alleles in the population is unknown and their significance, if any, for phenotype remains to be characterized. Further work needs to be performed to determine if any of the SNP's characterized in the SNP database[64] represent true polymorphisms with allele frequencies of greater than 1%.

The two NQO1 variant alleles that have been well-characterized are both coding region alleles that are of sufficient frequency to be

[57] P. C. Preusch, D. Siegel, N. W. Gibson, and D. Ross, *Free Rad. Biol. Med.* **11,** 77 (1991).
[58] S. L. Winski, M. Faig, M. A. Bianchet, D. Siegel, E. Swann, K. Fung, M. W. Duncan, C. J. Moody, L. M. Amzel, and D. Ross, *Biochemistry* **40,** 15135 (2001).
[59] T. Yoshida and H. Tsuda, *Biochem. Biophys. Res. Commun.* **214,** 701 (1995).
[60] R. D. Traver, T. Horikoshi, K. D. Danenberg, T. H. W. Stadlbauer, P. V. Danenberg, D. Ross, and N. W. Gibson, *Cancer Res.* **52,** 797 (1992).
[61] R. D. Traver, D. Siegel, H. D. Beall, R. M. Phillips, N. W. Gibson, W. A. Franklin, and D. Ross, *Br. J. Cancer* **75,** 69 (1997).
[62] S. S. Pan, G. L. Forrest, S. A. Akman, and L.-T. Hu, *Cancer Res.* **55,** 330 (1995).
[63] L. T. Hu, J. Stamberb, and S. S. Pan, *Cancer Res.* **56,** 5253 (1996).
[64] D. W. Nebert, A. L. Roe, S. E. Vandale, E. Bingham, and G. G. Oakley, *Genet. Med.* **4,** 62 (2002).
[65] A. Iida, A. Sekine, S. Saito, Y. Kitamura, T. Kitamoto, S. Osawa, C. Mishima, and Y. Nakamura, *J. Hum. Genet.* **46,** 225 (2001).

characterized as polymorphisms (allele frequency > 0.01). The most prevalent variant allele is the NQO1*2 polymorphism,[66,67] which has profound implications for phenotype[68]; individuals carrying the homozygous NQO1*2 allele have no NQO1 activity. The phenotypic implications of the NQO1*3 allele vary according to substrate,[62] and the frequency of the NQO1*3 allele in the population is very low.[66]

Research into the identification and significance of NQO1 polymorphisms is increasing, and a search of the PubMed database in March 2003 resulted in 82 hits when using the keywords NQO1 and polymorphism. Ten additional studies of NQO1 polymorphisms were obvious from additional PubMed searches of NQO1-related publications generating a total of 92 publications related to NQO1 polymorphisms. The majority of work on NQO1 polymorphisms has focused on the NQO1*2 polymorphism, and to give the reader some sense of impact of this polymorphism in different disciplines, we have classified each of these studies into a particular subgroup (Table I).

Polymorphisms in NQO1

*NQO1*2 Polymorphism*

Genotype-Phenotype Relationships

The NQO1*2 polymorphism was characterized in a collaboration between our own laboratory and that of Dr. Neil Gibson. During investigations of a series of human colon carcinoma cell lines[60] and lung tumor cell lines,[61] a good correlation was observed between NQO1 mRNA levels and NQO1 activity. In each series of tumor cell lines, however, one cell line (BE human colon carcinoma cells and H596 lung cancer cells) demonstrated high mRNA levels but no NQO1 activity. After SSCP analysis and DNA sequencing the same homozygous point mutation—a C to T mutation at position 609 of the cDNA—was identified in each cell line.[60,61] Immunoblot analysis revealed that the reason underlying the lack of NQO1 activity in each cell line was a lack of NQO1 protein. Although the recombinant mutant NQO1*2 protein demonstrated poor activity relative to wild-type protein, virtually no NQO1 protein could be detected in cells,

[66] A. Gaedigk, R. F. Tyndale, M. Jurima-Romet, E. M. Sellers, D. M. Grant, and J. S. Leeder, *Pharmacogenetics* **8,** 305 (1998).
[67] K. T. Kelsey, J. K. Wiencke, D. C. Christiani, Z. Zuo, M. R. Spitz, X. Xu, B. K. Lee, B. S. Schwartz, R. D. Traver, and D. Ross, *Br. J. Cancer* **76,** 852 (1997).
[68] D. Siegel, A. Anwar, S. L. Winski, J. K. Kepa, K. L. Zolman, and D. Ross, *Mol. Pharmacol.* **59,** 263 (2001).

TABLE I
NQO1 POLYMORPHISMS, FREQUENCY OF CITATIONS IN DIFFERENT FIELDS[a]

Field	Number of citations
Toxicological	9
Association with specific cancers (apart from leukemia)	29
Association with leukemia	10
Association with Parkinson's disease	2
Association with diabetes	1
Genotype distribution and phenotype	8
Mechanistic studies	15
Reviews	9
Relevance for chemotherapy	6
NQO2[b]	3
Total	92[a]

[a] Eighty-two studies were identified in a PubMed search using the keywords NQO1 and polymorphism and were classified into subgroups. An additional 10 studies were identified by PubMed searches using the key word NQO1. Each study was assigned to one subgroup only and since some studies could be entered in various groups, this classification solely represents the views of the authors. The table describes the wide range of studies of NQO1 polymorphisms in different disciplines. The vast majority of the citations listed are focused on the NQO1*2 polymorphism.
[b] Citations for NQO2 were also retrieved as part of this search.

and, more importantly, in human tissues genotyped as homozygous for the NQO1*2 polymorphism.[17,18,60,61,69] A number of cell lines commonly used in research are homozygous for the NQO1*2 polymorphism (Table II).

The NQO1*2 polymorphism shows a clear gene-dose effect with respect to phenotype. Individuals genotyped as NQO1*1/*1 (or wild type) had the highest levels of NQO1 protein in saliva while individuals carrying the homozygous NQO1*2/*2 polymorphism had no detectable levels of NQO1 protein.[69] Heterozygous individuals (NQO1*1/*2) had intermediate levels of NQO1 protein.[69] In other studies, cell lines and tumor samples homozygous for the NQO1*2 polymorphism had no detectable NQO1 activity.[60,61,70–73] In addition, tumor samples genotyped as NQO1*1/*2 or

[69] D. Siegel, S. M. McGuinness, S. Winski, and D. Ross, *Pharmacogenetics* **9,** 113 (1999).
[70] V. Misra, H. J. Klamut, and A. M. Rauth, *Br. J. Cancer* **77,** 1236 (1998).
[71] P. Eickelmann, T. Ebert, U. Warskulat, W. A. Schulz, and H. Sies, *Carcinogenesis* **15,** 219 (1994).
[72] P. Eickelmann, W. A. Schulz, D. Rohde, B. Schmitz-Dräger, and H. Sies, *Biol. Chem. Hoppe Seyler* **375,** 439 (1994).
[73] W. A. Schulz, A. Krummeck, I. Rosinger, P. Eickelmann, C. Neuhas, T. Ebert, B. Schmitz-Dräger, and H. Sies, *Pharmacogenetics* **7,** 235 (1997).

TABLE II
COMMONLY USED HUMAN CELL LINES WITH THE NQO1*2/*2 GENOTYPE[a]

Cell Line	Tissue
BE	Colon
Caco-2	Colon
NCI-H1570	Lung
NCI-H596	Lung
MDA-MB-231	Breast
MDA-MB-468	Breast

[a] Modified from reference 69.

TABLE III
NQO1 ALLELE FREQUENCIES[a]

	Allele		
Population	*1	*2	*3
Chinese	0.47	0.49	0.04
Inuit	0.54	0.46	<0.01
Native American	0.59	0.40	0.01
Caucasian	0.79	0.16	0.05

[a] Adapted from reference 66.

heterozygous had significantly lower NQO1 activity than samples containing the wild type or concensus sequence.[74]

Ethnic Distribution

The allele frequency of the NQO1*2 polymorphism (Table III) has been reported to be as high as 0.49 in some Asian populations.[66] The prevalence of the NQO1*2/*2 genotype in different ethnic groups is shown in Fig. 2. The NQO1*2/*2 genotype is essentially a null polymorphism and as many as 22% of individuals in some Asian populations lack NQO1 (Fig. 2).

[74] R. A. Fleming, J. Drees, B. W. Loggie, G. B. Russell, K. R. Geisinger, R. T. Morris, D. Sachs, and R. P. McQuellon, *Pharmacogenetics* **12,** 31 (2002).

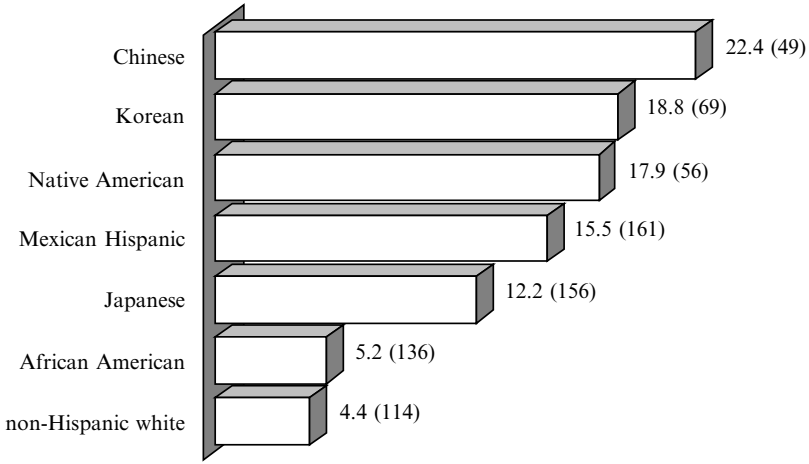

FIG. 2. Percentage of individuals in different populations with the NQO1*2/*2 genotype. Data are from healthy individuals and is primarily taken from reference 67 and unpublished data in a Japanese population (A. Sadler, M. Yano, and D. Ross, unpublished) and a Native American population (A. Sadler and D. Ross, unpublished) adapted from reference 24.

*Mechanisms Underlying the Lack of NQO1*2 Protein in Cells and Tissues*

Our studies have demonstrated that the NQO1*2 protein is rapidly degraded by the ubiquitin proteasomal system with a half-life of approximately 1.2 h.[68] The mechanisms underlying the structural instability of the NQO1*2 protein are unclear. Although we have generated a crystal structure of human wild type recombinant NQO1,[5] crystallization of the NQO1*2 mutant protein for structural elucidation studies has not been possible. However, the mutation in the NQO1*2 protein is a proline to serine at position 187, which may disrupt the structure of an external loop of the protein. A potential role for the protein chaperone Hsp70 has also been demonstrated in recent studies.[75] NQO1 has two Hsp binding sites and Hsp70 interacts with early immature forms of the wild-type NQO1 protein, but not with the mature protein, presumably to assist in correct protein folding. We were unable to detect any interaction of Hsp70 and the NQO1*2 protein (Fig. 3) and we have hypothesized that this leads to incorrect folding of the NQO1*2 mutant protein followed by ubiquitination and proteasomal degradation.[75]

[75] A. Anwar, D. Siegel, J. K. Kepa, and D. Ross, *J. Biol. Chem.* **16,** 14060 (2002).

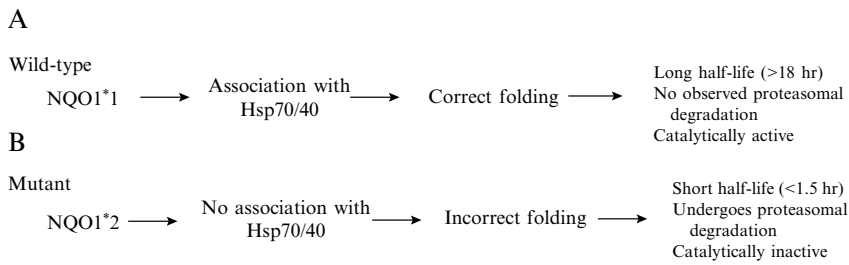

FIG. 3. The potential role of protein chaperones in the folding of the NQO1*1 protein. The protein chaperones HSP 70 and HSP 40 interact with newly-synthesized forms of the NQO1*1 protein but not the NQO1*2 protein.[75]

*NQO1*3 Polymorphism: Phenotype and Implications for NQO1 mRNA Alternative Splicing*

The NQO1*3 polymorphism was characterized by Pan and colleagues[62,63] and represents a C465T change coding for an arginine to tryptophan change in the protein. The variant protein had similar stability to wild-type protein and the implications of the NQO1*3 polymorphism for phenotype are variable according to the substrate. For example, the NQO1*3 recombinant protein demonstrated a similar ability to reduce menadione relative to wild-type protein but showed a 60% decreased ability to metabolize mitomycin C.[62]

At least three distinct transcripts of NQO1 mRNA (2.7, 1.7 and 1.2 kb) have been reported because of alternative poladenylation sites in the NQO1 gene.[76] A fourth smaller transcript of 1.1 kb was identified in human cancer cell lines as a product of alternative splicing.[77] The truncated transcript corresponds to a protein lacking exon 4 which contains the quinone binding site. Although the truncated protein could be expressed in *E. coli*, it was not detected in human tumor cells[77] or in Cos7 cells,[63] so it appears that the alternatively spliced mRNA is not translated into protein. The recombinant NQO1*3 protein purified from *E. coli* had no detectable activity when using a variety of electron acceptors,[77] as would be predicted from a protein lacking the critical quinone binding site. The truncated mRNA could be detected in patients but expressed wide interindividual variability.[78]

[76] A. K. Jaiswal, O. W. McBride, M. Adesnik, and D. W. Nebert, *J. Biol. Chem.* **263,** 13572 (1988).
[77] P. Y. Gasdaska, H. Fisher, and G. Powis, *Cancer Res.* **55,** 2542 (1995).
[78] K.-S. Yao, A. K. Godwin, C. Johnson, and P. J. O'Dwyer, *Cancer Res.* **56,** 1731 (1996).

Recently Pan *et al.*[79] have reported that in a human colon carcinoma cell line, alternative splicing of NQO1 at the 5′ splice site of intron 4 increased in cells carrying the NQO1*3 polymorphism. The NQO1*3 polymorphism disrupts the binding of small nuclear RNA (snRNA) in spliceosomes and transfection of snRNA constructs compensating for the NQO1*3 polymorphism increased NQO1 protein expression and enzymatic activity. These authors concluded that the NQO1*3 polymorphism was the major cause of increased alternate splicing and decreased expression of NQO1 protein in this particular colon carcinoma cell line.[79] The significance of the NQO1*3 polymorphism may therefore not be limited to the phenotype exhibited by the recombinant NQO1*3 protein but may also involve decreased expression of NQO1 wild type protein.

The frequency of the NQO1*3 polymorphism is low (Table III) and varied from 0 to 0.05 in different ethnic groups.[66]

*Relevance of the NQO1*2 Polymorphism*

As stated previously, a review of PubMed citations in March 2003 resulted in 92 hits related to NQO1 polymorphisms. The vast majority of these publications focus on the NQO1*2 polymorphism. We cannot discuss all of these studies in detail because of limitations of space, but we have attempted to summarize these studies in Table IV. Three studies on NQO2 were not included in Table IV, resulting in a total of 89 publications summarized. In addition, a few areas of significance of the NQO1 polymorphism are highlighted later.

*NQO1*2 Polymorphism and Benzene Toxicity*

The metabolism of benzene is complex,[80] but it is clear that metabolism is necessary for the induction of benzene toxicity. A number of reactive metabolites have been proposed as being responsible for benzene toxicity, and the evidence favoring each of these metabolites has recently been critically reviewed.[81] The conclusion of this analysis was that there was not sufficient evidence to define a particular benzene metabolite or group of metabolites as being responsible for benzene toxicity. The greatest weight of evidence, however, favors the generation of polyphenolics via cytochrome P4502E1-mediated metabolism of benzene, accumulation of metabolites such as catechol and hydroquinone in the target organ—the bone marrow—and subsequent oxidation of these metabolites to reactive quinone metabolites via a number of possible pathways.[81]

[79] S. S. Pan, Y. Han, P. Farabaugh, and H. Xia, *Pharmacogenetics* **12,** 479 (2002).
[80] D. Ross, *Eur. J. Haematol.* **57**(Suppl. 60), 111 (1996).
[81] D. Ross, *J. Toxicol. Environ. Health* **61,** 357 (2000).

TABLE IV
STUDIES ON NQO1 POLYMORPHISMS

First Author (Year)	Study	Remarks
Traver (1992)[60]	Characterization of NQO1*2	The C to T substitution at position 609 of the human NQO1 cDNA was identified in cell lines with high NQO1 mRNA expression but no NQO1 catalytic activity.
Eickelmann (1994)[71]	Kidney Tumors	No NQO1 catalytic activity was detected in three separate kidney tumor samples with normal NQO1 mRNA levels.
Eickelmann (1994)[72]	PCR-RFLP/Kidney Tumors	A PCR-RFLP assay is described for the detection of the NQO1*2 polymorphism. No NQO1 catalytic activity was detected in kidney tumor samples from patients genotype as homozygous for the NQO1*2 polymorphism.
Kolesar (1995)[82]	Colorectal Cancer	A C to T substitution at position 609 of the human NQO1 cDNA was detected in a patient with colon cancer.
Rosvold (1995)[83]	Lung Cancer and Smoking	The NQO1 codon 187 (pro→ser) mutation was identified as a polymorphism (NQO1*2). No correlation was observed between this mutant allele and the risk of lung cancer.
Rosvold (1995)[84]	Colorectal Cancer	A letter suggesting that the NQO1 mutation described by Kolesar (1995) was a polymorphism.
Kolesar (1995)[85]	Colorectal Cancer	Response by Kolesar *et al.*
Ross (1996a)[80]	Benzene	Review.
Kuehl (1995)[86]	Characterization of NQO1*2	Genotype-phenotype studies of the NQO1*2 polymorphism.
Ross (1996b)[87]	Characterization of NQO1*2	A letter indicating that the BE human colon carcinoma cell line previously genotyped by Kuehl *et al.* as NQO1*1/*2 is actually NQO1*2/*2.
Rebbeck (1996)[88]	Breast Cancer	The NQO1*2 allele was associated with an increased risk of breast cancer.
Traver (1997)[61]	Characterization of NQO1*2/Lung Cancer	The NQO1 protein was not detected in human tumor cell lines with the NQO1*2/*2 genotype. This study also suggested that the NQO1*2 polymorphism may be over represented in lung cancers.
Wiencke (1997)[89]	Lung Cancer	The NQO1*1/*1 genotype was associated with an increased risk of lung cancer.

(continued)

TABLE IV *(continued)*

First Author (Year)	Study	Remarks
Schulz (1997)[73]	Urological Malignancies	The NQO1*2/*2 genotype was associated with an increased risk of renal cell and urothelial carcinomas.
Rothman (1997)[90]	Benzene Poisoning	The NQO1*2/*2 genotype and rapid CYP4502E1 phenotypes were associated with an increased risk of benzene poisoning.
Kelsey (1997)[67]	Anticancer Chemotherapy	This study suggested that the NQO1*2 polymorphism may influence the response of tumor cells to quinone antitumor drugs. Prevalence of the NQO1*2/*2 genotype was reported in different ethnic populations.
Bartsch (1998)[91]	Pancreatic Disease	No correlation was observed between the NQO1*2 allele frequency and risk of pancreatic disease.
Gaedigk (1998)[66]	Mutant Allele Frequency	The NQO1*2 allele frequency was higher in Chinese, Canadian Native Indian and Canadian Inuit populations relative to a Caucasian population.
Kadlubar (1998)[92]	DNA Adducts/Pancreas	No correlation was observed between the NQO1*2 allele frequency and the levels of pancreatic DNA adducts in smokers and non-smokers.
Steiner (1999)[93]	Prostate Disease	The NQO1*2 allele was not associated with an increased risk of prostate disease.
Siegel (1999)[69]	Genotype/Phenotype Studies	A correlation was observed between the amount of NQO1 protein isolated from saliva and the NQO1*2 genotype. Individuals with the NQO1*2/*2 genotype had no detectable NQO1 protein whereas individuals with the NQO1*1/*2 genotype had intermediate NQO1 protein levels.
Ozawa (1999)[94]	Bronchial DNA Adducts	The NQO1*2 polymorphism was not associated with an increased level of bronchial-DNA adducts in smokers and non-smokers.
Longuemaux (1999)[95]	Renal Cell Carcinoma	The NQO1*2 polymorphism was not associated with an increased risk of renal cell carcinoma.
Clairmont (1999)[96]	Basal Cell Carcinoma	The NQO1*2/*2 genotype was associated with an increased risk of basal cell carcinoma.

(continued)

TABLE IV *(continued)*

First Author (Year)	Study	Remarks
Smith (1999)[97]	Benzene Poisoning	Commentary on Moran *et al.* 1999.
Moran (1999)[98]	Benzene Metabolism	Human bone marrow progenitor cells carrying the NQO1*2 allele have decreased NQO1 protein levels following induction by benzene metabolites.
Chen (1999)[99]	Lung Cancer	The NQO1*2 allele was protective against lung cancer in a Japanese population. Increased risk of lung cancer was associated with the NQO1*1 wild type allele.
Larson (1999)[100]	Myeloid Leukemia	The NQO1*2 allele was associated with an increased risk of therapy-related acute myeloid leukemia.
Kristiansen (1999)[101]	Type I Diabetes	No correlation was observed between the NQO1*2 polymorphism and the risk of type I diabetes in a Danish population.
Wiemels (1999)[102]	Pediatric Leukemias	The mutant NQO1*2 allele was associated with an increased risk of infant leukemias with chromosomal rearrangements.
Kelland (1999)[103]	17-Demethoxygeldanamycin	The sensitivity of tumor cells to 17-demethoxygeldanamycin was associated with expression of NQO1*1 protein.
Zheng (1999)[104]	Manganism	No correlation was observed between the NQO1*2 allele frequency and occupational chronic manganism.
Lin (1999)[105]	Lung Cancer	No correlation was observed between the NQO1*2 polymorphism and risk of lung cancer in a Taiwan population. Stratification of tumors according to histological subtype suggested that the NQO1*1/*1 genotype was more common in adenocarcinomas than controls.
Harth (2000)[106]	Colorectal Cancer	No correlation was observed between the NQO1*2 polymorphism and risk of colorectal cancer.
Gaedigk (2000)[107]	Drug Metabolism	This study describes interethnic differences in the distribution of polymorphisms including NQO1*2 in closely related populations.
Shi (2000)[108]	High-Output Genotyping	The NQO1*2 polymorphism was successfully genotyped in a high output assay utilizing Taq poymerase and fluorogenic Taqman probes.

(continued)

TABLE IV *(continued)*

First Author (Year)	Study	Remarks
Martone (2000)[109]	p53 Mutations in Bladder Cancer	No correlation was observed between the NQO1*2 allele frequency and p53 mutations in human bladder cancers.
Nakajima (2000)[110]	Susceptibility to Industrial Chemicals	Review.
Schelonka (2000)[111]	Eye Disease	The NQO1*2/*2 genotype was associated with the absence of NQO1 protein in human donor eyes.
Lafuente (2000)[112]	Colorectal Cancer	The NQO1*2 polymorphism was associated with a increased risk of colon cancers in association with K-ras codon 12 mutations.
Naoe (2000)[113]	Leukemias	The NQO1*2 polymorphism was associated with an increased risk of therapy-related leukemia and myelodysplastic syndrome.
Ross (2000)[23]	NQO1	Review.
Siegel (2001)[68]	NQO1*2 Protein Stability	The mutant NQO1*2 protein was found to have a greatly reduced protein half-life compared to the NQO1*1 protein as a result of degradation by the proteasomal pathway.
Pavanello (2001)[114]	Genotoxic Risk	No correlation was observed between the NQO1*2 polymorphism and biomarkers of genotoxic exposure.
Peters (2001)[115]	Glioma	No correlation was observed between the NQO1*2 allele frequency and risk of glioma.
Smith (2001)[116]	Leukemias	The NQO1*2 allele was associated with an increased risk of *de novo* acute myeloid leukemia in adults.
Shao (2001)[117]	Parkinson's Disease	The NQO1*2 polymorphism in combination with a polymorphism in monoamine oxidase B resulted in an additive increased risk of Parkinson's disease.
Xu (2001)[118]	Lung Cancer	No correlation was observed between the NQO1*2 allele frequency and susceptibility to lung cancer. An association, however, was observed between the NQO1*2/2 genotype and risk of lung cancer in heavy former and current smokers.

(continued)

TABLE IV *(continued)*

First Author (Year)	Study	Remarks
Iida (2001)[65]	SNPs in Quinone Reductase	A Japanese population was screened for single nucleotide polymorphisms in quinone metabolizing enzymes including NQO1 and NQO2.
Bergamaschi (2001)[119]	Effect of Ozone	The NQO1*1/*1 genotype was associated with both a decrease in pulmonary function and increased serum clara cell protein 16 in patients exposed to ozone.
Winski (2001)[21]	Chemotherapy	A human colon carcinoma cell line genotyped as NQO1*2/*2 (BE) and lacking NQO1 catalytic activity was transfected with the NQO1*1 coding region. Stable clones obtained following transfection demonstrated increased sensitivity to the DNA cross-linking agent RH-1.
Le Marchand (2001)[120]	Genotyping	The NQO1*2 polymorphism was successfully genotyped by PCR-RFLP from buccal DNA obtained in a large, community-based mail-in study.
Gallou (2001)[121]	Renal Cell Carcinoma	No correlation was observed between the NQO1*2 allele frequency and mutations in the von Hippel-Lindau tumor suppressor gene in renal cell carcinoma.
Yin (2001)[122]	Lung Cancer	The NQO1*2 allele was not associated with an increased risk of lung cancer in a Chinese population.
Lewis (2001)[123]	Lung Cancer	The NQO1*2 allele was associated with an increased risk of small cell lung cancer. No correlation was observed between the NQO1*2 allele and non-small cell lung cancer.
Harada (2001)[124]	Parkinson's Disease	The NQO1*2 polymorphism was not associated with an increased risk of Parkinson's disease.
Siegel (2001)[125]	Bone Marrow Expression	NQO1 protein was detected by immunohistochemistry in human bone marrow endothelial cells suggesting a role for NQO1 in the metabolism of xenobiotics in the bone marrow.

(continued)

TABLE IV *(continued)*

First Author (Year)	Study	Remarks
Phillips (2001)[126]	Response to Mitomycin C	No correlation was observed between the NQO1*2 allele frequency and response to mitomycin C treatment in a panel of human tumor xenografts transplanted into mice.
Verdina (2001)[127]	Urinary Biomarkers/ Benzene Exposure	No correlation was observed between the NQO1*2 allele frequency and the levels of benzene urinary metabolites in policeman exposed to low concentrations of benzene.
Fleming (2002)[74]	Response to Mitomycin C	The NQO1*2 allele was associated with significantly lower NQO1 catalytic activity and reduced survival in patients receiving intraperitoneal hyperthermic chemotherapy with mitomycin C.
Krajinovic (2002)[128]	Leukemias	The NQO1*2 and NQO1*3 alleles in combination with variant alleles in CYP2E1, MPO or GSTM1 were associated with an increased risk of acute lymphoblastic leukemia in children.
Siegelmann (2002)[129]	Breast Cancer	No correlation was observed between the NQO1*2 allele frequency and the risk of breast cancer. The NQO1*1/*1 genotype however, was associated with an increased risk of ductal carcinoma with poor histological grade.
Anwar (2002)[75]	HSP70 Binding	The NQO1*1 protein but not the NQO1*2 protein was found to associate with HSP70 and HSP40 in cellular and cell free systems. The lack of association between NQO1*2 protein and HSP proteins may explain the diminished catalytic activity of the mutant NQO1 protein.
Naoe (2002)[130]	Leukemias	The NQO1*2 polymorphism does not affect survival of patients with AML.
Nebert (2002)[64]	HUGO Review	Review.
Krajinovic (2002)[131]	Leukemias	The NQO1*2 and variant CYP1A1 alleles were associated with a poorer prognosis in children with acute lymphoblastic leukemia.

(continued)

TABLE IV *(continued)*

First Author (Year)	Study	Remarks
Brockstedt (2002)[132]	DNA Adducts Breast Cancer	No correlation was observed between the NQO1*2 and NQO1*3 allele frequencies and the level of DNA adducts detected in normal human breast tissues.
Tuominen (2002)[133]	PAH Exposure	The NQO1*2 polymorphism may influence the DNA adduct profile in control and aluminum smelter workers.
Hamajima (2002)[134]	PCR/Genotyping	This study describes the use of PCR with confronting two-pair primers as an alternative method for single nucleotide polymorphism analysis. The NQO1*2 polymorphism was utilized as a model.
Hamajima (2002)[135]	Japanese Cancers	The NQO1*2/*2 genotype was not associated with an increased risk of cancer of the stomach, colon, rectum, breast, prostate or malignant lymphoma in a Japanese population. This study suggested that the NQO1*1/*1 genotype was associated with an increased risk of lung cancer. In addition, an increased risk of lung and esophageal cancer in combination with smoking was observed for the NQO1*2/*2 genotype.
Goode (2002)[136]	Breast Cancer	No correlation was observed between the NQO1*2 allele frequency and survival in women with breast cancer.
Krajinovic (2001)[137]	Leukemias	Review.
Morgan (2002)[138]	Leukemia	Review.
Carere (2002)[139]	Urban Pollution	No correlation was observed between the NQO1*2 allele frequency and genetic biomarkers for exposure to urban air pollution in Rome traffic policemen.
Sunaga (2002)[140]	Lung Cancer	The NQO1*1/*1 genotype was associated with increased risk of lung adenocarcinoma. A greater risk was found in combination with the NQO1*1/*1 and GSTT-1 null genotypes. This enhanced risk was more evident in smokers compared to non-smokers.
Hamajima (2002)[141]	Japanese Noncancers	Polymorphisms in drug metabolizing enzymes including NQO1 were analyzed in a Japanese population.

(continued)

TABLE IV *(continued)*

First Author (Year)	Study	Remarks
Zheng (2002)[142]	Occupational Manganism	No correlation was observed between the NQO1*2 allele frequency and occupational chronic manganism.
Pan (2002)[79]	NQO1*3 Polymorphism	The NQO1*3 (465C→T) mutation was found to be associated with defective NQO1 RNA splicing and decreased NQO1 protein expression.
Asher (2002)[55]	p53 Interaction	The mutant NQO1*2 protein, unlike the wild type NQO1*1 protein, failed to stabilize and prevent degradation of wild type p53.
Kolesar (2002)[143]	Lung Cancer	NQO1*2 in normal tissue and lung tumors. The NQO1*2/*2 genotype was associated with a shorter survival time in patients treated with chemotherapy for stage II/III non-small cell lung cancer.
Seedhouse (2002)[144]	Leukemia	No correlation was observed between the NQO1*2 allele frequency and risk of *de novo* or therapy-related acute myeloblastic leukemia.
Smith (2002)[145]	Leukemias	The NQO1*2 polymorphism was found to be associated with an increased risk of *de novo* leukemias with MLL translocations in infants and children.
Soucek (2002)[146]	Lymphomas	No correlation was observed between the NQO1*2 allele frequency and patients with Hodgkin's and non-Hodgkin's lymphomas.
Sachse (2002)[147]	Colorectal Cancer	No correlation was observed between the NQO1*2 allele frequency and the risk of colorectal cancer.
Blanco (2002)[148]	Leukemia	No correlation was observed between the NQO1*2 allele frequency and the risk of therapy-related acute myeloid leukemia and myelodysplastic syndrome in children treated for ALL.
Wan (2002)[149]	Benzene Poisoning	Study found that Chinese patients with the NQO1*2/*2 genotype were at increased risk of benzene poisoning.
Wu (2002)[150]	Nasopharyngeal Carcinoma	The NQO1*2 polymorphism was found to be associated with genetic susceptibility to nasopharyngeal carcinoma.

(continued)

TABLE IV *(continued)*

First Author (Year)	Study	Remarks
Takagi (2002)[151]	Colorectal Cancer	A correlation was observed between the NQO1*2/*2 genotype and telomere shortening in colorectal cancer.
Chen (2002)[152]	Benzene Poisoning	Review.

[82] J. M. Kolesar, J. G. Kuhn, and H. A. Burris, III, *JNCI* **87,** 1022 (1995).
[83] E. A. Rosvold, K. A. McGlynn, E. D. Lustbader, and K. H. Buetow, *Pharmacogenetics* **5,** 199 (1995).
[84] E. A. Rosvold, K. A. McGlynn, E. D. Lustbader, and K. H. Buetow, *J. Natl. Cancer Inst.* **87,** 1802 (1995).
[85] J. M. Kolesar, H. A. Burris III, and J. G. Kuhn, *JNCI* **87,** 1803 (1995).
[86] B. L. Kuehl, J. W. E. Paterson, J. W. Peacock, M. C. Paterson, and A. M. Rauth, *Br. J. Cancer* **72,** 555 (1995).
[87] D. Ross, R. D. Traver, D. Siegel, B. L. Kuehl, V. Misra, and A. M. Rauth, *Br. J. Cancer* **74,** 995 (1996).
[88] T. R. Rebbeck, A. K. Godwin, and K. H. Buetow, *Mol. Carcinog.* **17,** 117 (1996).
[89] J. T. Wiencke, M. R. Spitz, A. McMillan, and K. T. Kelsey, *Cancer Epidemiol. Bio. Prev*. **6,** 87 (1997).
[90] N. Rothman, M. T. Smith, R. B. Hayes, R. D. Traver, B. A. Hoener, S. Campleman, G. L. Li, M. Dosemeci, M. Linet, L. P. Zhang, L. Q. Xi, S. Wacholder, W. Lu, K. B. Meyer, N. Titenko-Holland, J. T. Stewart, S. N. Yin, and D. Ross, *Cancer Res.* **57,** 2839 (1997).
[91] H. Bartsch, C. Malaveille, A. B. Lowenfels, P. Maisonneuve, A. Hautefeuille, and P. Boyle, *Eur. J. Cancer Prev*. **7,** 215 (1998).
[92] F. F. Kadlubar, K. E. Anderson, S. Haussermann, N. P. Lang, G. W. Barone, P. A. Thompson, S. L. MacLeod, M. W. Chou, M. Mikhailova, J. Plastaras, L. J. Marnett, J. Nair, I. Velic, and H. Bartsch, *Mutat. Res*. **405,** 125 (1998).
[93] M. Steiner, M. Hillenbrand, M. Borkowsi, H. Seiter, and P. Schuff-Werner, *Cancer Lett.* **135,** 67 (1999).
[94] S. Ozawa, B. Schoket, L. P. McDaniel, Y. M. Tang, C. B. Ambrosone, S. Kostic, I. Vincze, and F. F. Kadlubar, *Carcinogenesis* **20,** 991 (1999).
[95] S. Longuemaux, C. Delomenie, C. Gallou, A. Mejean, M. Vincent-Viry, R. Bouvier, D. Droz, R. Krishnamoorthy, M. M. Galteau, C. Junien, C. Beroud, and J. M. Dupret, *Cancer Res.* **59,** 2903 (1999).
[96] A. Clairmont, H. Sies, S. Ramachandran, J. T. Lear, A. G. Smith, B. Bowers, P. W. Jones, A. A. Fryer, and R. C. Strange, *Carcinogenesis* **20,** 1235 (1999).
[97] M. T. Smith, *Proc. Natl. Acad. Sci. USA* **96,** 7624 (1999).
[98] J. L. Moran, D. Siegel, and D. Ross, *Proc. Natl. Acad. Sci. USA* **96,** 8150 (1999).
[99] H. Chen, A. Lum, A. Seifried, L. R. Wilkens, and L. Le Marchand, *Cancer Res*. **59,** 3045 (1999).
[100] R. A. Larson, Y. Wang, M. Banerjee, J. Wiemels, C. Hartford, M. M. Beau, and M. T. Smith, *Blood* **94,** 803 (1999).
[101] O. P. Kristiansen, Z. M. Larsen, J. Johannesen, J. Nerup, T. Mandrup-Poulsen, and F. Pociot, *Hum. Mutat.* **14,** 67 (1999).

In the mid 1990s, a collaborative effort led by the National Cancer Institute examined the risk of benzene poisoning as a function of metabolic phenotype and genotype of occupationally exposed workers in China.[90] Benzene poisoning was defined by decreases in white blood cell and platelet counts after exposure and was strongly related to the subsequent development of acute nonlymphocytic leukemia and related myelodysplastic

[102] J. L. Wiemels, A. Pagnamenta, G. M. Taylor, O. B. Eden, F. E. Alexander, and M. F. Greaves, *Cancer Res.* **59,** 4095 (1999).
[103] L. R. Kelland, S. Y. Sharp, P. M. Rogers, T. G. Myers, and P. Workman, *J. Natl. Cancer Inst.* **91,** 1940 (1999).
[104] Y. Zheng, F. He, P. Chan, Z. Pan, Z. Wang, J. Pan, and X. Zhou, *Zhonghua Yu Fang Yi. Xue. Za Zhi*. **33,** 78 (1999).
[105] P. Lin, H. J. Wang, H. Lee, H. S. Lee, S. L. Wang, Y. M. Hsueh, K. J. Tsai, and C. Y. Chen, *J. Toxicol. Environ. Health* **58,** 187 (1999).
[106] V. Harth, S. Donat, Y. Ko, J. Abel, H. Vetter, and T. Bruning, *Arch. Toxicol*. **73,** 528 (2000).
[107] A. Gaedigk, *Int. J. Clin. Pharmacol. Ther*. **38,** 61 (2000).
[108] M. M. Shi, S. P. Myrand, M. R. Bleavins, and F. A. de la Iglesia, *Mol. Pathol*. **52,** 295 (1999).
[109] T. Martone, P. Vineis, C. Malaveille, and B. Terracini, *Mutat. Res*. **462,** 303 (2000).
[110] T. Nakajima and T. Aoyama, *Ind. Health* **38,** 143 (2000).
[111] L. P. Schelonka, D. Siegel, M. W. Wilson, A. Meininger, and D. Ross, *Invest. Ophthalmol. Vis. Sci*. **41,** 1617 (2000).
[112] M. J. Lafuente, X. Casterad, M. Trias, C. Ascaso, R. Molina, A. Ballesta, S. Zheng, J. K. Wiencke, and A. Lafuente, *Carcinogenesis* **21,** 1813 (2000).
[113] T. Naoe, K. Takeyama, T. Yokozawa, H. Kiyoi, M. Seto, N. Uike, T. Ino, A. Utsunomiya, A. Maruta, I. Jin-nai, N. Kamada, Y. Kubota, H. Nakamura, C. Shimazaki, S. Horiike, Y. Kodera, H. Saito, R. Ueda, J. Wiemels, and R. Ohno, *Clin. Cancer Res*. **6,** 4091 (2000).
[114] S. Pavanello and E. Clonfero, *Med. Lav*. **91,** 431 (2000).
[115] E. S. Peters, K. T. Kelsey, J. K. Wiencke, S. Park, P. Chen, R. Miike, and M. R. Wrensch, *Cancer Epidemiol. Biomarkers Prev*. **10,** 151 (2001).
[116] M. T. Smith, Y. Wang, E. Kane, S. Rollinson, J. L. Wiemels, E. Roman, P. Roddam, R. Cartwright, and G. Morgan, *Blood* **97,** 1422 (2001).
[117] M. Shao, Z. Liu, E. Tao, and B. Chen, *Zhonghua Yi. Xue. Yi. Chuan Xue. Za Zhi*. **18,** 122 (2001).
[118] L. L. Xu, J. C. Wain, D. P. Miller, S. W. Thurston, L. Su, T. J. Lynch, and D. C. Christiani, *Cancer Epidemiol. Biomarkers Prev*. **10,** 303 (2001).
[119] E. Bergamaschi, G. De Palma, P. Mozzoni, S. Vanni, M. V. Vettori, F. Broeckaert, A. Bernard, and A. Mutti, *Am. J. Respir. Crit Care Med*. **163,** 1426 (2001).
[120] L. Le Marchand, A. Lum-Jones, B. Saltzman, V. Visaya, A. M. Nomura, and L. N. Kolonel, *Cancer Epidemiol. Biomarkers Prev*. **10,** 701 (2001).
[121] C. Gallou, S. Longuemaux, C. Delomenie, A. Mejean, N. Martin, S. Martinet, G. Palais, R. Bouvier, D. Droz, R. Krishnamoorthy, C. Junien, C. Beroud, and J. M. Dupret, *Pharmacogenetics* **11,** 521 (2001).
[122] L. Yin, Y. Pu, T. Y. Liu, Y. H. Tung, K. W. Chen, and P. Lin, *Lung Cancer* **33,** 133 (2001).
[123] S. J. Lewis, N. M. Cherry, R. M. Niven, P. V. Barber, and A. C. Povey, *Lung Cancer* **34,** 177 (2001).

syndromes (relative risk = 70). A case-control study was performed employing 50 cases of benzene poisoning and 50 controls. CYP2E1 phenotyping was assessed by measuring urinary excretion of 6-hydroxy chlorzoxazone over 8 h after administration of chlorzoxazone while NQO1 genotype was assessed by PCR-RFLP analysis. Individuals with a greater capacity for CYP4502E1-mediated metabolism were at a greater risk for benzene poisoning (relative risk = 2.6) as were individuals carrying the

[124] S. Harada, C. Fujii, A. Hayashi, and N. Ohkoshi, *Biochem. Biophys. Res. Commun*. **288,** 887 (2001).
[125] D. Siegel, J. Ryder, and D. Ross, *Toxicol. Lett*. **125,** 93 (2001).
[126] R. M. Phillips, A. M. Burger, H. H. Fiebig, and J. A. Double, *Biochem. Pharmacol*. **62,** 1371 (2001).
[127] A. Verdina, R. Galati, G. Falasca, S. Ghittori, M. Imbriani, F. Tomei, L. Marcellini, A. Zijno, and V. D. Vecchio, *J. Toxicol. Environ. Health* **64,** 607 (2001).
[128] M. Krajinovic, H. Sinnett, C. Richer, D. Labuda, and D. Sinnett, *Int. J. Cancer* **97,** 230 (2002).
[129] N. Siegelmann-Danieli and K. H. Buetow, *Oncology* **62,** 39 (2002).
[130] T. Naoe, Y. Tagawa, H. Kiyoi, Y. Kodera, S. Miyawaki, N. Asou, K. Kuriyama, S. Kusumoto, C. Shimazaki, K. Saito, H. Akiyama, T. Motoji, M. Nishimura, K. Shinagawa, R. Ueda, H. Saito, and R. Ohno, *Leukemia* **16,** 203 (2002).
[131] M. Krajinovic, D. Labuda, G. Mathonnet, M. Labuda, A. Moghrabi, J. Champagne, and D. Sinnett, *Clin. Cancer Res*. **8,** 802 (2002).
[132] U. Brockstedt, M. Krajinovic, C. Richer, G. Mathonnet, D. Sinnett, W. Pfau, and D. Labuda, *Mutat. Res*. **516,** 41 (2002).
[133] R. Tuominen, P. Baranczewski, M. Warholm, L. Hagmar, L. Moller, and A. Rannug, *Arch. Toxicol*. **76,** 178 (2002).
[134] N. Hamajima, T. Saito, K. Matsuo, and K. Tajima, *J. Mol. Diagn*. **4,** 103 (2002).
[135] N. Hamajima, K. Matsuo, H. Iwata, M. Shinoda, Y. Yamamura, T. Kato, S. Hatooka, T. Mitsudomi, M. Suyama, Y. Kagami, M. Ogura, M. Ando, Y. Sugimura, and K. Tajima, *Int. J. Clin. Oncol*. **7,** 103 (2002).
[136] E. L. Goode, A. M. Dunning, B. Kuschel, C. S. Healey, N. E. Day, B. A. Ponder, D. F. Easton, and P. P. Pharoah, *Cancer Res*. **62,** 3052 (2002).
[137] M. Krajinovic, D. Labuda, and D. Sinnett, *Rev. Environ. Health* **16,** 263 (2001).
[138] G. J. Morgan and M. T. Smith, *Am. J. Pharmacogenomics*. **2,** 79 (2002).
[139] A. Carere, C. Andreoli, R. Galati, P. Leopardi, F. Marcon, M. V. Rosati, S. Rossi, F. Tomei, A. Verdina, A. Zijno, and R. Crebelli, *Mutat. Res*. **518,** 215 (2002).
[140] N. Sunaga, T. Kohno, N. Yanagitani, H. Sugimura, H. Kunitoh, T. Tamura, Y. Takei, S. Tsuchiya, R. Saito, and J. Yokota, *Cancer Epidemiol. Biomarkers Prev*. **11,** 730 (2002).
[141] N. Hamajima, T. Saito, K. Matsuo, T. Suzuki, T. Nakamura, A. Matsuura, K. Okuma, and K. Tajima, *J. Epidemiol*. **12,** 229 (2002).
[142] Y. X. Zheng, P. Chan, Z. F. Pan, N. N. Shi, Z. X. Wang, J. Pan, H. M. Liang, Y. Niu, X. R. Zhou, and F. S. He, *Biomarkers* **7,** 337 (2002).
[143] J. M. Kolesar, S. C. Pritchard, K. M. Kerr, K. Kim, M. C. Nicolson, and H. McLeod, *Int. J. Oncol*. **21,** 1119 (2002).
[144] C. Seedhouse, R. Bainton, M. Lewis, A. Harding, N. Russell, and E. Das-Gupta, *Blood* **100,** 3761 (2002).
[145] M. T. Smith, Y. Wang, C. F. Skibola, D. J. Slater, L. L. Nigro, P. C. Nowell, B. J. Lange, and C. A. Felix, *Blood* **100,** 4590 (2002).

homozygous NQO1*2 polymorphism (relative risk = 2.4). When both of these metabolic pathways were combined, rapid CYP2E1 metabolism and a lack of NQO1 caused by the homozygous NQO1*2 polymorphism, the relative risk for benzene poisoning increased to 7.6 fold.[90] This was the first study examining the significance of a sequence of metabolic pathways during the metabolism of benzene in humans, and studies in knockout animals have reinforced the potential importance of both CYP2E1 and NQO1 to benzene toxicity.[153,154] Interestingly, in NQO1 knockout animals, both male and female −/− mice were more susceptible to benzene induced hematotoxicity relative to NQO1 +/+ animals, but only female −/− animals were more susceptible to benzene-induced micronuclei relative to their NQO1 +/+ comparison group.[154] It is possible that different benzene metabolites may be responsible for hematotoxicity and genotoxicity,[154] but these data also suggest that genotoxicity as reflected by micronuclei induction may not be related to hematotoxicity.

The mechanism proposed for the role of CYP2E1 and NQO1 in benzene toxicity is shown in Fig. 4. Increased metabolism of benzene to polyphenolics or inhibited detoxification of benzene-derived reactive quinones caused by the NQO1*2 polymorphism may lead to increased toxicity. One potential problem with this mechanism was that for NQO1 to detoxify benzene-derived quinones, the enzyme would have to be present in human bone marrow, the target organ of benzene toxicity. Our studies, however, did not detect NQO1 in human bone marrow aspirates.[155,98] Two potential

[146] P. Soucek, J. Sarmanova, V. N. Kristensen, M. Apltauerova, and I. Gut, *Int. Arch. Occup. Environ. Health* **75**(Suppl. 1), 86 (2002).
[147] C. Sachse, G. Smith, M. J. Wilkie, J. H. Barrett, R. Waxman, F. Sullivan, D. Forman, D. T. Bishop, and C. R. Wolf, *Carcinogenesis* **23,** 1839 (2002).
[148] J. G. Blanco, M. J. Edick, M. L. Hancock, N. J. Winick, T. Dervieux, M. D. Amylon, R. O. Bash, F. G. Behm, B. M. Camitta, C. H. Pui, S. C. Raimondi, and M. V. Relling, *Pharmacogenetics* **12,** 605 (2002).
[149] J. Wan, J. Shi, L. Hui, D. Wu, X. Jin, N. Zhao, W. Huang, Z. Xia, and G. Hu, *Environ. Health Perspect.* **110,** 1213 (2002).
[150] D. H. Wu, *Di Yi. Jun. Yi. Da. Xue. Xue. Bao*. **22,** 1126 (2002).
[151] S. Takagi, Y. Kinouchi, N. Hiwatashi, M. Hirai, S. Suzuki, S. Takahashi, K. Negoro, N. Obana, and T. Shimosegawa, *Anticancer Res.* **22,** 2749 (2002).
[152] Y. Chen, G. Li, and S. Yin, *Wei Sheng Yan. Jiu*. **31,** 130 (2002).
[153] J. L. Valentine, S. S. T. Lee, M. J. Seaton, B. Asgharian, G. Farris, J. C. Corton, F. J. Gonzalez, and M. A. Medinsky, *Toxicol. Appl. Pharmacol*. **141,** 205 (1996).
[154] A. K. Bauer, B. Faiola, D. J. Abernethy, R. Marchan, L. J. Pluta, V. A. Wong, K. Roberts, A. K. Jaiswal, F. J. Gonzalez, B. E. Butterworth, S. Borghoff, H. Parkinson, J. Everitt, and L. Recio, *Cancer Res.* **63,** 929 (2003).
[155] D. Ross, D. Siegel, D. G. Schattenberg, X. M. M. Sun, and J. L. Moran, *Environ. Health Perspect.* **104**(Suppl. 6), 1177 (1996).

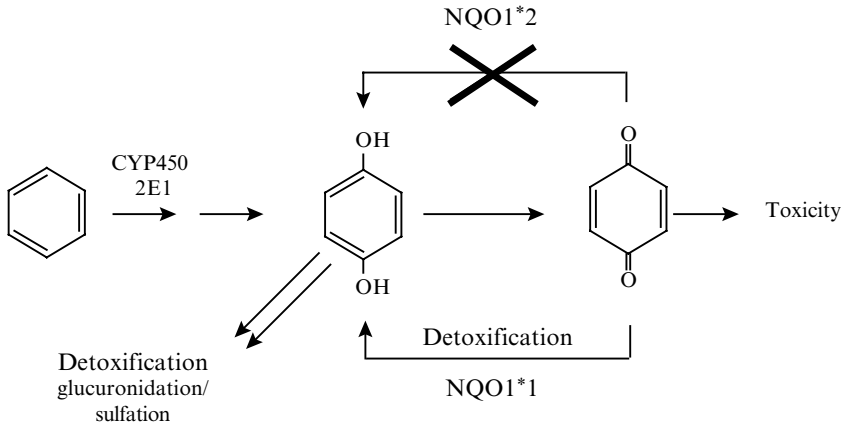

FIG. 4. The proposed role of cytochrome P4502E1 and NQO1 in benzene toxicity.

mechanisms were proposed to explain this contradiction, and both of these mechanisms have now been confirmed. The first mechanism involved induction of NQO1 by polyphenolic metabolites of benzene. Although NQO1 was not present in either human bone marrow or purified CD34+ human bone marrow cells, exposure of these cells *in vitro* to hydroquinone or catechol led to induction of NQO1 activity but *only* in cells from individuals genotyped as NQO1*1/*1 or wild-type.[98] No induction of NQO1 activity was observed in individuals genotyped as NQO1*2/*2, and intermediate levels of induction were observed in heterozygous or NQO1*1/*2 individuals.[98] Presumably, the lack of induction of NQO1 associated with the NQO1*2 polymorphism reflected the instability of the NQO1*2 protein due to proteasomal degradation[68] rather than any effect on the mechanism and extent of enzyme induction. This mechanism would therefore provide an explanation for increased benzene toxicity associated with the NQO1*2 polymorphism; benzene metabolites would induce NQO1, which could detoxify benzene-derived quinones but not in individuals with the NQO1*2/*2 genotype.

The second hypothesis that we tested was that NQO1 was indeed present in human bone marrow but was contained in cells that were not removed by conventional aspiration techniques. We performed immunohistochemistry on archived bone marrow core samples and found that NQO1 could be detected in human bone marrow but was present predominantly in bone marrow endothelial cells.[125] Endothelial cells are not readily removed from bone marrow by aspiration. The role of bone marrow endothelial cells in benzene toxicity is as yet unclear but is a subject of

investigation in our laboratory. Bone marrow endothelial cells participate in the maturation of hematopoietic progenitor cells in a number of ways, including expression of adhesion molecules and secretion of cytokines.[156,157] The potential mechanisms underlying a protective role of NQO1 against benzene toxicity in human bone marrow are summarized in Fig. 5. Since additional roles of NQO1 are being uncovered, such as its involvement in antioxidant defense and stabilization of p53 (see previous text), it is feasible that additional mechanisms may be involved in NQO1-mediated protection against benzene toxicity.

*NQO1*2 Polymorphism and Leukemia*

Since the homozygous NQO1*2 polymorphism is effectively a null polymorphism and heterozygous individuals demonstrate decreased NQO1 protein and activity relative to the wild-type or concensus sequence, the NQO1*2 polymorphism provides a convenient molecular tool to examine the role of NQO1 in disease. Of the many cancers that have been examined for an association with the NQO1*2 polymorphism (Table IV), the most frequent association has been found with leukemias.

Eight separate studies have examined the possible relationship of deficient NQO1 caused by the NQO1*2 polymorphism and leukemia. Two additional studies examined the prognosis of leukemia patients with the NQO1*2 polymorphism after chemotherapy.[130,131] Of the eight studies examining risk of leukemia, six of these found an increased risk of various types of leukemia associated with the NQO1*2 polymorphism while two found no association of therapy related leukemia. An increased risk of leukemia associated with the NQO1*2 polymorphism was found in therapy-related leukemia,[100] therapy-related leukemia/myelodysplastic syndrome,[113] pediatric leukemias (particularly with MLL gene rearrangements[102,145]), childhood acute lymphoblastic leukemia (ALL),[128] and adult *de-novo* leukemias, both acute myeloid leukemia (AML) and ALL.[116] In the study by Krajinovic *et al*.,[128] the wild-type NQO1 genotype was found to be protective against childhood ALL, whereas children carrying at least one mutant allele of NQO1 (NQO1*2 or NQO1*3) were at increased risk. Two additional studies found no association of the NQO1*2 polymorphism with therapy related leukemia.[158,159]

[156] C. Voermans, P. M. Rood, P. L. Hordijk, W. R. Gerritsen, and C. E. van der Schoot, *Stem Cells* **18,** 435 (2000).
[157] R. Mohle, M. A. Moore, R. L. Nachman, and S. Rafii, *Blood* **89,** 72 (1997).
[158] D. J. Long, A. Gaikwad, A. Multani, S. Pathak, C. A. Montgomery, F. J. Gonzalez, and A. K. Jaiswal, *Cancer Res*. **62,** 3030 (2002).
[159] K. Mikami, M. Naito, A. Tomida, M. Yamada, T. Sirakusa, and T. Tsuruo, *Cancer Res*. **56,** 2823 (1996).

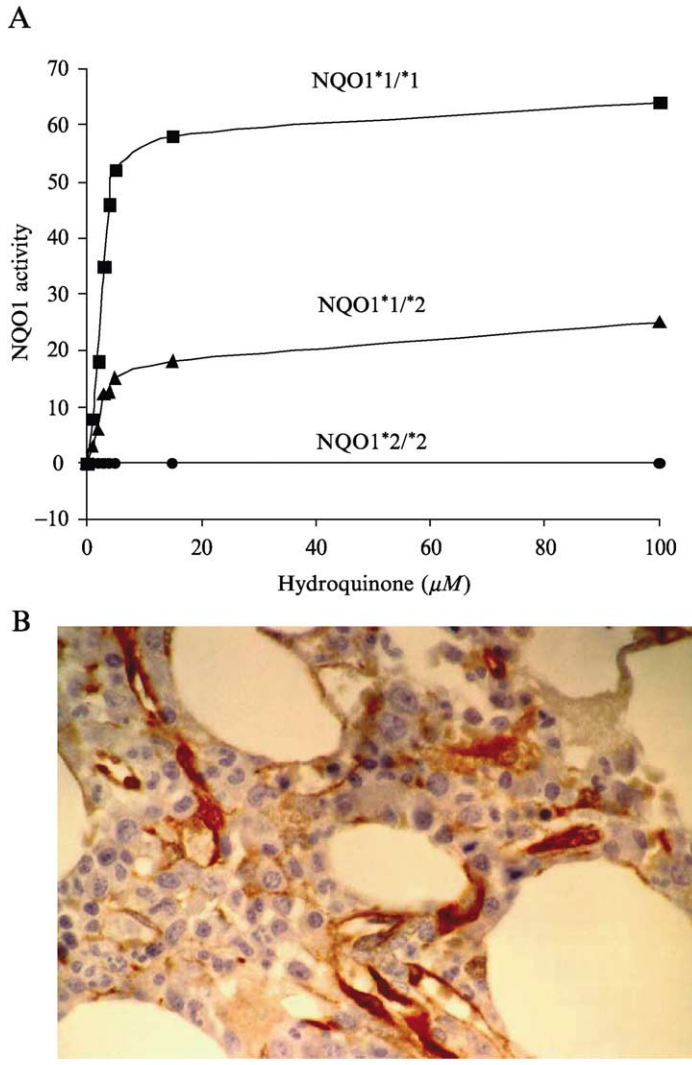

FIG. 5. Potential mechanisms underlying a protective role of NQO1 against benzene toxicity. (A) Induction of NQO1 in human bone marrow mononuclear cells after exposure to hydroquinone depends on NQO1 genotype.[98] Catechol induces a similar induction of NQO1, and induction of NQO1 by hydroquinone was also detected in bone marrow $CD34^{+}$ enriched cell populations.[98] The inability of polyphenolic metabolites of benzene to increase NQO1 activity in NQO1*2/*2 cells was presumably related to the instability of the mutant NQO1*2 protein rather than any effect on induction. (B) Presence of NQO1 in bone marrow endothelial cells. Although NQO1 could not be detected in aspirated human bone marrow cells, it could be detected by immunohistochemistry in bone marrow endothelial cells.[125]

The NQO1*2*2 genotype has also been associated with an increased risk of benzene induced myelotoxicity in occupationally exposed workers in China,[82] and biologically plausible mechanisms can be proposed to account for this increased risk (see previous text). Another important study in NQO1 knockout animals has also recently been published[158] demonstrating that NQO1 knockout animals were at a greater risk of myeloid hyperplasia and increased levels of blood neutrophils, basophils and eosinophils. NQO1 knockout mice also contained decreased levels of p53 in bone marrow relative to wild type animals,[158] reinforcing a possible role for NQO1 in stabilization of p53 (see previous text). Taken together, these data suggest that low activity NQO1 genotypes, and particularly the NQO1*2/*2 genotype, are associated with myeloid abnormalities and increase the risk of leukemias. Assessing the impact of the NQO1*2 polymorphism should be incorporated as a part of the design of any large epidemiological studies of genetic risk factors for the induction of leukemia.

*NQO1*2 Polymorphism and Chemotherapy*

Since NQO1 can bioactivate certain antitumor quinones such as mitomycin C, E09 streptonigrin, β-lapachone[13–16] and newer experimental agents such as RH1,[20,21] the NQO1*2 polymorphism would be expected to impact therapy using these agents. Pre-clinical studies using gene transfection and gene targeting approaches[20,21,59,159] have clearly demonstrated the role of NQO1 in bioactivation of antitumor quinones and provided proof of principle for this approach. The impact of the NQO1*2 polymorphism has also been demonstrated by using excised human tumors *in vitro;* tumor tissue genotyped as NQO1*2/*2 was more resistant to mitomycin C than tumors genotyped as wild-type.[160] Perhaps the most compelling study to date is a clinical study performed by Fleming *et al.*[74] In this work, patients with disseminated peritoneal cancer received surgical debulking and intraperitoneal hyperthermic perfusions of mitomycin C. In 117 patients genotyped for the NQO1*2 polymorphism, individuals with the low activity NQO1 genotypes (heterozygous or homozygous for the NQO1*2 polymorphism) had reduced survival relative to individuals with the wild type or concensus NQO1 genotype. Genotype-phenotype relationships were also confirmed in this study, and NQO1*1/*2 heterozygotes had significantly less tumor NQO1 activity than individuals with the wild-type NQO1 genotype.[74] This study demonstrates the potential importance of the NQO1*2 polymorphism to therapy using antitumor quinones such as

[160] M. Yano, Y. Akiyama, H. Shiozaki, M. Inoue, Y. Doki, Y. Fujiwara, D. Ross, A. Sadler, and M. Monden, *Proc. Amer. Assoc. Cancer Res.* **41,** 4585 (2000).

mitomycin C. The relevance of the NQO1*2 polymorphism to therapy using other compounds such as E09, RH1 and β-lapachone remains to be determined.

Acknowledgments

The authors are supported by NIH grants CA51210, ES09554 and NS44613.

[9] Structure and Mechanism of NAD[P]H:Quinone Acceptor Oxidoreductases (NQO)

By MARIO A. BIANCHET, MARGARITA FAIG, and L. MARIO AMZEL

Introduction

Cytosolic NAD(P)H:quinone acceptor oxidoreductases (NQO) are flavoenzymes that catalyze the obligatory 2-electron reduction of quinones to hydroquinones.[1] This reaction prevents the reduction of quinones by one-electron reductases that would result in the formation of reactive oxygen species (ROS), generated by redox cycling of semiquinones in the presence of molecular oxygen.[2] In addition to its possible role in the detoxification of dietary quinones, the enzyme has been shown to catalyze the reductive activation of quinolic chemotherapeutic compounds such as mitomycins, anthracyclines, and aziridinyl-benzoquinones. NQO type 1 (NQO1; EC 1.6.99.2; also called QR1 and DT-Diaphorase) is a 274-residue enzyme expressed under oxidative or electrophilic stress among a battery of phase II enzymes.[3–6] Also expressed as part of the same response is an iso-enzyme called NQO type 2 (NQO2) that is 43 residues shorter at its C-terminus. NQO2 has a 49% sequence identity with NQO1. Both enzymes can use NAD[P]H as a source of reducing equivalents, but NQO1 uses this cofactor more efficiently. NQO2 has been shown to prefer dihydronicotinamide ribosyl (NRH) as cofactor.[7,8] NQO activity is observed in many solid

[1] L. Ernster and F. Navazio, *Acta Chem. Scand.* **12,** 595–602 (1958).
[2] H. Sies and H. de Groot, *Toxicol. Lett.* **64–65,** 547–551 (1992).
[3] A. Benson, M. Hunkeler, and P. Talalay, *Proc. Natl. Acad. Sci. USA* **77,** 5216–5220 (1980).
[4] H. Wefers, T. Komai, P. Talalay, and H. Sies, *FEBS Lett.* **169,** 63–66 (1984).
[5] H. J. Prochaska, P. Talalay, and H. Sies, *J. Biol. Chem.* **262,** 1931–1934 (1987).
[6] P. Talalay, *Biofactors* **12,** 5–11 (2000).
[7] Q. Zhao, X. Yang, W. Holtzclaw, and P. Talalay, *Proc. Natl. Acad. Sci. USA* **94,** 1669–1674 (1997).

0076-6879/04 $35.00