

Original Article

Prognostic significance of NQO1 expression in intrahepatic cholangiocarcinoma

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Abstract: This study aimed to evaluate the association between the immunohistochemical expression of NAD(P)H:quinone oxidoreductase-1 (NQO1) and nuclear factor erythroid 2-related factor 2 (Nrf2) in resected specimens of intrahepatic cholangiocarcinoma (ICC) and to elucidate the prognostic value of NQO1 and Nrf2 expression. A retrospective analysis was conducted of 34 consecutive patients who underwent surgical resection for ICC. Immunohistochemistry of the resected specimens was conducted using each of the following primary monoclonal antibodies against NQO1 and Nrf2. Of the 34 patients, 23 were classified as having tumors with NQO1-positive expression and 11 had tumors with loss of NQO1 expression, whereas 22 patients had tumors with Nrf2-positive expression and 12 had tumors with loss of Nrf2 expression. NQO1 expression showed a positive association with Nrf2 expression ($p=0.005$). Loss of NQO1 expression was more frequent in tumor specimens that were moderately or poorly differentiated (11/26; 42%) than in well-differentiated tumors (0/8; 0%; $p=0.034$). Post-resection survival was significantly worse in patients with tumors with loss of NQO1 expression than in patients with NQO1-positive tumors (cumulative 5-year survival rate of 0% and 51%, respectively; $p=0.005$). Nrf2 expression was not associated with survival after resection ($p=0.287$). The Cox proportional hazards regression analysis revealed that lymph node involvement ($p<0.001$) and loss of NQO1 expression ($p<0.001$) had an independent adverse effect on survival. Loss of NQO1 expression reflects dedifferentiation and thus indicates a poor prognosis for patients undergoing resection for ICC.

Keywords: NAD (P)H:quinone oxidoreductase-1 (NQO1), intrahepatic cholangiocarcinoma, nuclear factor erythroid 2-related factor 2 (Nrf2), surgical resection, prognosis

Introduction

NAD(P)H:quinone oxidoreductase-1 (NQO1), also known as DT-diaphorase, menadiol reductase, or quinone reductase 1, is a flavoprotein that catalyses the two-electron reduction of quinones and related compounds [1]. Oxidative stress promotes nuclear accumulation of nuclear factor erythroid 2-related factor 2 (Nrf2) and activates transcription of NQO1 [2]. In normal cells, NQO1 protects cells against redox cycling and oxidative stress [1, 2], as well as against carcinogenesis by stabilization of the p53 tumor suppressor [3-5]. Radjendirane *et al.* [1] have provided evidence of NQO1 protection against carcinogenesis in a mouse model with targeted disruption of the *nqo1* gene. Furthermore, altered NQO1 expression has been reported in tumor cells from malignancies arising

from the breast [6, 7], colon [6, 7], lung [6-8], liver [6, 9], and pancreas [10]. Although some authors have reported NQO1 expression in human cholangiocarcinoma cell lines [11, 12], there is a paucity of information in the literature regarding NQO1 expression in patients with intrahepatic cholangiocarcinoma (ICC).

In human, a polymorphic form of the oxidoreductase exists, encoded by *NQO1*2*, which is characterized by a C609T substitution [13]. *NQO1*2* is a missense variant that is homozygous in 4.4%-20.3% of the human population [14] and is associated with an increased risk of breast cancer [14]. In 2008, Fagerholm *et al.* [16] proposed that the homozygous *NQO1*2* genotype (P187S), which disables NQO1, is a strong adverse prognostic factor in patients with breast cancer. The presence of a homozygous C

-T mutation at position 609 results in a loss of NQO1 protein and activity due to accelerated protein degradation (the half-life of mutant NQO1 is 1.2 hours) via the ubiquitin proteasomal system [13, 17]. However, the prognostic value of NQO1 expression in ICC tumor cells has yet to be determined.

In the present study, we hypothesized that loss of NQO1 expression in ICC tumor cells may function as an adverse prognostic factor. We evaluated the immunohistochemical expression of NQO1 and Nrf2 in surgically resected specimens of ICC. The aim of this study was to evaluate the association between the expression of NQO1 and Nrf2 and to elucidate the prognostic value of NQO1 and Nrf2 expression in patients with ICC.

Materials and methods

Patients

The present study was performed on 34 consecutive Japanese patients who underwent surgical resection for ICC at Niigata University Medical and Dental Hospital, Niigata, Japan, from January 1992 through to December 2010. The patient group included 25 men and nine women with a median age of 67 years (range: 31-78 years). All patients provided written informed consent and the study itself was approved by the Institutional Review Board of Niigata University Medical and Dental Hospital.

At Niigata University Medical and Dental Hospital, hepatic resection is the standard treatment for ICC when the tumor is considered resectable and the patient is deemed to have an acceptable surgical risk. The hepatectomy procedures performed included left hemihepatectomy extended to an inferior part of the right anterior section in 14 patients, left hemihepatectomy in six patients, left trisectionectomy in four patients, right hemihepatectomy extended to an inferior part of Couinaud segment IV in five patients, right hemihepatectomy in 2 patients, central hepatectomy (removal of Couinaud segments IV, V, and VIII) in one patient, right posterior sectionectomy in one patient and right trisectionectomy in one patient. Twenty-seven patients also underwent combined resection and reconstruction of contiguous tissues comprising the extrahepatic bile duct ($n = 27$), portal vein ($n = 6$), hepatic artery ($n = 1$), and infe-

rior vena cava ($n = 1$). None of the patients underwent portal vein embolization prior to resection. All 34 patients underwent en bloc dissection of the regional lymph nodes. The regional lymph nodes of the liver were classified according to the Union for International Cancer Control (UICC) TNM Classification of Malignant Tumours (7th edition, 2009) [18].

Adjuvant treatment after resection was administered at the discretion of the individual surgeon. Three patients were given 5-fluorouracil orally, with another 12 patients receiving intravenous gemcitabine. None of the patients was given adjuvant radiotherapy. The median follow-up time after resection was 63 months (range: 1-230 months). At the time of assessment of disease status, 19 patients had died from tumor recurrence, five patients had died from some other cause with no evidence of disease and the remaining 10 patients were alive with no evidence of disease.

Pathologic evaluation

Resected specimens were submitted to the Department of Surgical Pathology, Niigata University Medical and Dental Hospital, and the pathologic findings followed the UICC TNM classification [18]. All patients had adenocarcinoma and the median tumor size was 4.0 cm (range: 1.2-7.2 cm). Hepatic tumors were well differentiated in eight patients, moderately differentiated in 12 patients and poorly differentiated in 14 patients. Histologic grade was assigned according to the area(s) with the highest grade. Regional lymph node metastases were identified in 12 patients (35%). Periaortic lymph node involvement was identified in three patients and another three patients had localized peritoneal metastases, classified as pathologic distant metastasis (pM1). Based on the UICC TNM classification [18], eight patients had Stage I tumors, 11 had Stage II tumors, one had a Stage III tumor, eight had Stage IVA tumors and six had Stage IVB tumors.

Immunohistochemistry

Immunohistochemistry was performed on one to three paraffin-embedded blocks (median: two blocks) from each specimen resected. Four serial 3- μ m sections were re-cut and prepared from each block: one for hematoxylin-eosin staining; one for immunohistochemical staining

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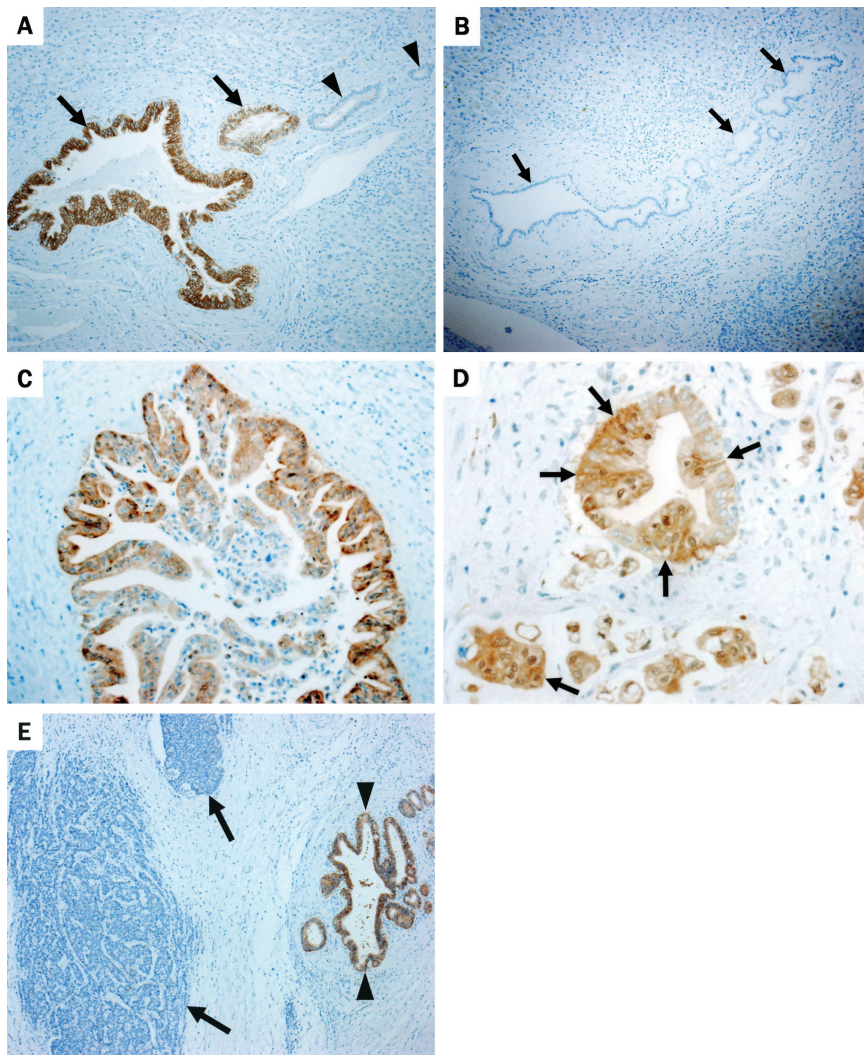


Figure 1. NAD(P)H:quinone oxidoreductase-1 (NQO1) expression. (A) NQO1-positive expression in non-neoplastic interlobular biliary epithelial cells (arrows) and no NQO1 immunoreactivity in small intrahepatic bile ducts (arrowheads). (original magnification x125). (B) Loss of NQO1 expression in non-neoplastic interlobular biliary epithelial cells (arrows). (original magnification x100). (C) Intrahepatic cholangiocarcinoma (ICC) with cytosolic NQO1-positive expression (original magnification x200). (D) Cytosolic and nuclear (arrows) staining of NQO1 in a tumor specimen of ICC (original magnification x400). (E) Loss of NQO1 expression (arrows) in a moderately to poorly differentiated ICC despite NQO1-positive expression in non-neoplastic intrahepatic bile ducts (arrowheads). (original magnification x80).

with a rabbit monoclonal antibody against NQO1 (Epitomics, Burlingame, CA, USA); one for immunohistochemical staining with a rabbit monoclonal antibody against Nrf2 (Epitomics, Burlingame, CA, USA); and one used as a negative control. Two independent surgical pathologists blinded to the clinical details assessed each section.

For immunohistochemistry, sections were deparaffinized and rehydrated before being microwaved at 500 W for 21 minutes in 10 mM sodium citrate buffer (pH 6.0) to retrieve antigenic activity. Endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide in methanol for 20 minutes. After blocking any non-specific reactions with 10% normal goat serum, sections were incubated overnight at 4°C with each of the following primary anti-

bodies: NQO1 rabbit monoclonal antibody (Epitomics; 1:200 dilution) and Nrf2 rabbit monoclonal antibody (Epitomics; 1:200 dilution). Sections were then incubated with goat anti-rabbit IgG polymerized horseradish peroxidase-labelled secondary antibody (Epitomics) at room temperature for 30 minutes. Diaminobenzidine was used as the chromogen and sections were counterstained with hematoxylin. As a negative control, normal rabbit immunoglobulin was substituted for the primary antibody.

NQO1 expression was defined as the presence of cytosolic and/or nuclear staining according to the description of Winski *et al.* [19]. Non-neoplastic interlobular biliary epithelial cells showed cytosolic immunopositive staining for NQO1 (Figure 1A), whereas no NQO1 immunoreactivity was observed occasionally in non-

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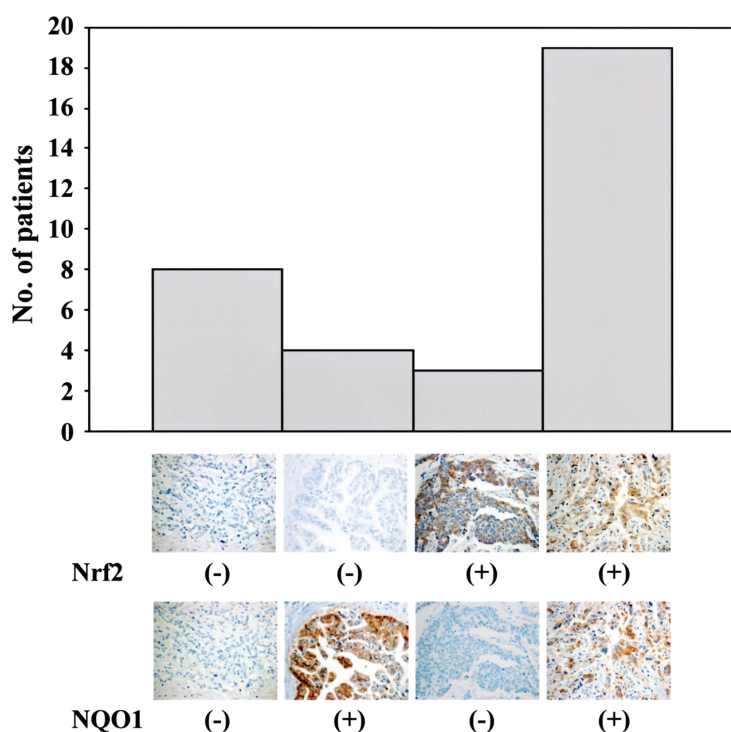


Figure 2. Immunohistochemical expression of NAD(P)H:quinone oxidoreductase-1 (NQO1) and nuclear factor erythroid 2-related factor 2 (Nrf2). NQO1 expression showed a positive association with Nrf2 expression ($p=0.005$). (-) = loss of expression; (+) = positive expression.

neoplastic interlobular biliary epithelial cells (Figure 1B), probably because homozygosity for the variant NQO1 allele is associated with a loss

of NQO1 protein [13, 17]. NQO1 expression in tumor specimens was classified as either positive expression (Figure 1C, D) or 'loss of expression' (Figure 1E). Nrf2 expression was defined as the presence of cytosolic and/or nuclear staining according to the previous reports [20, 21]. Nrf2 expression in tumor specimens was classified as either positive expression or "loss of expression" (Figure 2).

Prognostic factors

To elucidate factors influencing long-term survival after surgical resection, 15 conventional variables (Table 1) in addition to the expression of NQO1 and Nrf2 were entered into univariate and multivariate analyses. The cut-off level for patient age (65 years) was determined based on respective median values, whereas the size of the primary tumor (cut-off: 5 cm) was determined according to the UICC TNM classification (22). The cut-off values for preoperative serum carcinoembryonic antigen (CEA; 5 ng/ml) and carbohydrate antigen 19-9 (CA19-9; 37 U/ml) were determined on the basis of reference ranges of serum CEA and CA19-9 levels (≤ 5 ng/ml and ≤ 37 U/ml, respectively).

Table 1. Patient characteristics according to NQO1 expression in tumor cells

Variable	No. of patients with		p value
	Loss of NQO1 expression	NQO1-positive expression	
Age (≤ 65 / >65 years)	6/5	8/15	0.458
Gender (M/F)	10/1	15/8	0.214
Serum CEA level (≤ 5 / >5 ng/ml)	2/9	16/7	0.009
Serum CA19-9 level (≤ 37 / >37 U/ml)	3/8	5/18	>0.999
Tumor size (≤ 5 / >5 cm)	6/5	17/6	0.434
Histologic grade (G1/G2-G3)*	0/11	8/15	0.034
Lymphatic vessel invasion (absent/present)*	3/8	9/14	0.705
Vascular invasion (absent/present)*	1/10	11/12	0.053
Perineural invasion (absent/present)*	4/7	11/12	0.715
pT classification (pT1-pT2/pT3-pT4)*	10/1	19/4	>0.999
pN classification (pN0/pN1)*	7/4	15/8	>0.999
pM classification (pM0/pM1)*	9/2	19/4	>0.999
TNM stage (I-II/III-IV)*	5/6	14/9	0.475
Residual tumor status (R0/R1)*	6/5	18/5	0.232
Adjuvant chemotherapy (absent/present)	6/5	12/11	>0.999

*According to the Union for International Cancer Control (UICC) TNM classification [18]; Abbreviations: NQO1 (NAD(P)H:quinone oxidoreductase-1); CEA (carcinoembryonic antigen); CA19-9 (carbohydrate antigen 19-9)

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Table 2. Factors significantly influencing long-term survival after surgical resection

Variable	Modality	No. of patients	5-year survival rate (%)	Univariate analysis	Multivariate analysis	
				p value	Relative risk (95% CI)	p value
pN classification*	pN0	22	50	0.001	1.000	<0.001
	pN1	12	0		18.576 (4.173-82.696)	
pM classification*	pM0	28	43	0.003		
	pM1	6	0			
TNM stage*	I-II	19	63	<0.001		
	III-IV	15	0			
Residual tumor status*	R0	24	48	0.019		
	R1	10	0			
NQO1 expression	Loss	11	0	0.005	12.132 (3.119-47.197)	<0.001
	Positive	23	51		1.000	

*According to the Union for International Cancer Control (UICC) TNM classification [18]; Abbreviations: NQO1 (NAD (P)H:quinone oxidoreductase-1); CI (confidence interval)

Statistical analysis

Medical records were obtained for all 34 patients. Categorical variables were compared by Fisher's exact test. The cause of death was determined from the medical records and the follow-up period was defined as the interval between the resection and the last follow-up. Deaths from other causes were treated as censored cases. The Kaplan-Meier method was used to estimate the cumulative incidence of events and differences in the incidence of events were evaluated using the log-rank test. The Cox proportional hazards regression model was used to identify factors that were independently associated with survival. In this model, a stepwise selection is used for variable selection, with entry and removal limits of $p < 0.05$ and $p > 0.1$, respectively. The stability of this model was confirmed using a step-backward and step-forward fitting procedure. The variables identified as having an independent influence on survival were identical using both procedures. All statistical evaluations were performed using the PASW Statistics 17 software package (SPSS Japan, Tokyo, Japan). All tests were two tailed and $p < 0.05$ was considered significant.

Results

Factors associated with NQO1 expression in tumor cells

Twenty-three patients had tumors that were NQO1 positive, whereas 11 had tumors that exhibited loss of NQO1 expression. Of the 11 surgically resected specimens showing loss of

NQO1 expression in the ICC tumor cells, seven showed no immunoreactivity to NQO1 in the non-neoplastic interlobular biliary epithelial cells (**Figure 1B**), whereas the remaining four showed NQO1-positive expression in the intrahepatic bile ducts (**Figure 1E**). Twenty-two patients had tumors with Nrf2-positive expression and 12 had tumors with loss of Nrf2 expression. NQO1 expression showed a positive association with Nrf2 expression ($p = 0.005$, **Figure 2**). There was a significant correlation between NQO1 expression in tumor cells and both preoperative serum CEA levels and histologic grade (**Table 1**). High preoperative serum CEA concentrations were more frequent in patients with tumors showing loss of NQO1 expression (9/11; 82%) than in patients with tumors that were NQO1 positive (7/23; 30%, $p = 0.009$). Loss of NQO1 expression was more frequent in tumors that were moderately or poorly differentiated (11/26; 42%) than in tumors that were well differentiated (0/8; 0%, $p = 0.034$).

Factors influencing long-term survival after surgical resection

The overall cumulative survival rate after resection was 33% at 5 years, with a median survival of 19 months. Univariate analysis revealed that TNM stage ($p < 0.001$), pN classification ($p = 0.001$), pM classification ($p = 0.003$), NQO1 expression ($p = 0.005$), and residual tumor status ($p = 0.019$) were significantly associated with long-term survival after resection (**Table 2**). Survival after surgical resection was significantly worse in patients with tumors that exhibited loss of NQO1 expression than in patients

with tumors that were NQO1 positive ($p=0.005$, **Figure 3**), whereas Nrf2 expression was not associated with survival after resection ($p=0.287$). These significant variables were entered into multivariate analyses, which revealed that pN classification ($p<0.001$) and NQO1 expression ($p<0.001$) remained as significant independent predictors of survival (**Table 2**).

Discussion

NQO1 functions primarily to protect normal cells against oxidative stress [1, 2] and carcinogenesis [3-5]. In the present study it was hypothe-

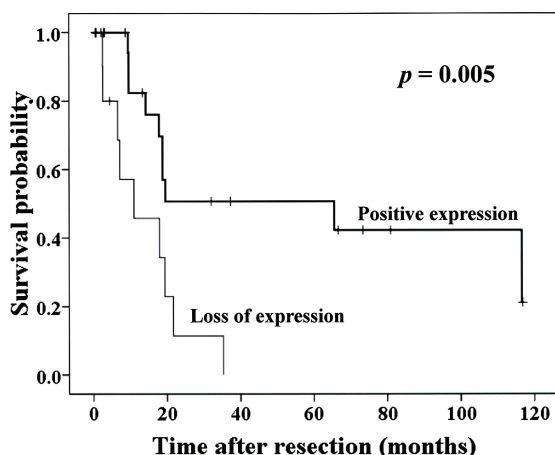


Figure 3. Kaplan-Meier survival estimates according to NAD(P)H:quinone oxidoreductase-1 (NQO1) expression. Survival after surgical resection was significantly worse in patients who had tumors exhibiting loss of NQO1 expression (median survival time, 11 months; cumulative 5-year survival rate, 0%) than in patients who had tumors that were NQO1 positive (median survival time, 66 months; cumulative 5-year survival rate, 51%; $p=0.005$).

sized that loss of NQO1 expression in ICC tumor cells may be an adverse prognostic factor. This prompted us to investigate the immunohistochemical expression of NQO1 in surgically resected specimens of ICC to clarify the prognostic value of NQO1 expression using multivariate analysis. To our knowledge, the present study is the first such study to demonstrate that NQO1 expression in tumor specimens is an independent prognostic factor in patients with ICC. Loss of NQO1 expression may predict poor survival after resection because it reflects aggressive tumor biology characterized by high serum CEA

levels and high histologic grade.

There is limited, and sometimes contradictory, clinical information in the literature regarding the association between NQO1 and histologic grade. For example, although Siegelmann-Danieli *et al* [23] reported that a significant association between NQO1 genotype and histologic grade of breast cancer, Fragerholm *et al* [16] failed to find an association. In addition, Basu *et al.* [24] reported no significant association between NQO1 expression and the histologic grade of superficial bladder tumors, although Gan *et al* [25] reported higher expression of DT-diaphorase (also termed NQO1) in lower-grade and superficial bladder tumors compared with high-grade and invasive tumors. In the present study, loss of NQO1 expression was significantly associated with moderately or poorly differentiated ICC, whereas NQO1-positive expression was observed in all eight tumors classified as well-differentiated ICC. Given that in the present study four tumor specimens showed loss of NQO1 expression in moderately or poorly differentiated ICC tumor cells despite NQO1-positive expression in the non-neoplastic interlobular biliary epithelial cells from the same specimen being NQO1 positive (**Figure 1E**), the loss of NQO1 expression appears to be associated with dedifferentiation of ICC tumor cells.

Although Nrf2 is retained in the cytoplasm by a repressor protein Keap1 [2], low Keap1 activity due to mutations or low-level expression leads to nuclear localization and constitutive activation of Nrf2 [20]. Nrf2, a redox-sensitive transcription factor, regulates the induction of NQO1 gene in response to antioxidants and xenobiotics [26]. In the present study dealing with tumor specimens of ICC, we confirmed that NQO1 expression shows a positive association with Nrf2 expression (**Figure 2**) and loss of NQO1 expression independently predicts poor survival after resection (**Table 2**). Fagerholm *et al* [16] also reported that a homozygous common missense variant (NQO1*2) that disables NQO1 strongly predicts poor survival among two independent series of women with breast cancer. Taken together, these findings suggest prognostic significance of NQO1.

NQO1 plays a role in the bioactivation of anti-cancer quinines, such as mitomycin C (MMC). It is now widely acknowledged that MMC is a sub-

strate for NQO1, but only under mildly acidic conditions [27]. Recently, Buranrat *et al* [12] reported that dicoumarol, a potent inhibitor of NQO1, enhances gemcitabine cytotoxicity in human cholangiocarcinoma cell lines with high NQO1 activity and proposed that NQO1 may contribute to the sensitivity of cholangiocarcinoma cells to gemcitabine. Given that in the present study the loss of NQO1 expression in ICC tumor cells adversely affected survival after resection, patients with tumors that exhibit loss of NQO1 expression appear to be candidates for adjuvant chemotherapy. Powis *et al* [28] reported that the inhibition of cell growth by MMC was not significantly increased in murine NIH 3T3 cell lines stably transfected with human NQO1. Thus, the role of NQO1 and related inhibitors in chemosensitivity appears questionable and future studies should investigate the relationship between NQO1 enzyme activity and chemosensitivity in greater detail.

The two main limitations of the present study are the retrospective analysis of a small number of patients and the short follow-up time for some patients. However, the authors believe that these limitations do not greatly influence the outcome of the study because the differences between groups were too marked to have resulted from these biases.

In conclusion, loss of NQO1 expression may reflect the dedifferentiation of ICC tumor cells and thus indicates a poor prognosis for patients undergoing surgical resection for ICC.

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References

- [1] Radjendirane V, Joseph P, Lee YH, Kimura S, Klein-Szanto AJ, Gonzalez FJ, Jaiswal AK. Disruption of the DT diaphorase (NQO1) gene in mice leads to increased menadione toxicity. *J Biol Chem* 1998; 273: 7382-9.
- [2] Jaiswal AK. Regulation of genes encoding NAD (P)H:quinone oxidoreductases. *Free Radic Biol Med* 2000; 29: 254-62.
- [3] Asher G, Lotem J, Cohen B, Sachs L, Shaul Y. Regulation of p53 stability and p53-dependent apoptosis by NADH quinone oxidoreductase 1. *Proc Natl Acad Sci USA* 2001; 98: 1188-93.
- [4] Asher G, Lotem J, Kama R, Sachs L, Shaul Y. NQO1 stabilizes p53 through a distinct pathway. *Proc Natl Acad Sci USA* 2002; 99: 3099-104.
- [5] Anwar A, Dehn D, Siegel D, Kepa JK, Tang LJ, Pietenpol JA, Ross D. Interaction of human NAD(P)H:quinone oxidoreductase 1 (NQO1) with the tumor suppressor protein p53 in cells and cell-free systems. *J Biol Chem* 2003; 278: 10368-73.
- [6] Schlager JJ, Powis G. Cytosolic NAD(P)H: (quinone-acceptor)oxidoreductase in human normal and tumor tissue: effects of cigarette smoking and alcohol. *Int J Cancer* 1990; 45: 403-9.
- [7] Siegel D, Ross D. Immunodetection of NAD(P)H:quinone oxidoreductase 1 (NQO1) in human tissues. *Free Radic Biol Med* 2000; 29: 246-53.
- [8] Siegel D, Franklin WA, Ross D. Immunohistochemical detection of NAD(P)H:quinone oxidoreductase in human lung and lung tumors. *Clin Cancer Res* 1998; 4: 2065-70.
- [9] Cresteil T, Jaiswal AK. High levels of expression of the NAD(P)H:quinone oxidoreductase (NQO1) gene in tumor cells compared to normal cells of the same origin. *Biochem Pharmacol* 1991; 42: 1021-7.
- [10] Ough M, Lewis A, Bey EA, Gao J, Ritchie JM, Bornmann W, Boothman DA, Oberley LW, Cullen JJ. Efficacy of beta-lapachone in pancreatic cancer treatment: exploiting the novel, therapeutic target NQO1. *Cancer Biol Ther* 2005; 4: 95-102.
- [11] Prawan A, Buranrat B, Kukongviriyapan U, Sripa B, Kukongviriyapan V. Inflammatory cytokines suppress NAD(P)H:quinone oxidoreductase-1 and induce oxidative stress in cholangiocarcinoma cells. *J Cancer Res Clin Oncol* 2009; 135: 515-22.
- [12] Buranrat B, Prawan A, Kukongviriyapan U, Kongpetch S, Kukongviriyapan V. Dicoumarol enhances gemcitabine-induced cytotoxicity in high NQO1-expressing cholangiocarcinoma cells. *World J Gastroenterol* 2010; 16: 2362-70.
- [13] Ross D, Traver RD, Siegel D, Kuehl BL, Misra V, Rauth AM. A polymorphism in NAD(P)H:quinone oxidoreductase (NQO1): relationship of a homozygous mutation at position 609 of the NQO1 cDNA to NQO1 activity. *Br J Cancer* 1996; 74: 995-6.
- [14] Kelsey KT, Ross D, Traver RD, Christiani DC, Zuo ZF, Spitz MR, Wang M, Xu X, Lee BK, Schwartz BS, Wiencke JK. Ethnic variation in the prevalence of a common NAD(P)H quinone oxidoreductase polymorphism and its implications for anti-cancer chemotherapy. *Br J Cancer* 1997; 76: 852-4.
- [15] Sarmanová J, Sůsová S, Gut I, Mrhalová M, Kodet R, Adámek J, Roth Z, Soucek P. Breast

- cancer: role of polymorphisms in biotransformation enzymes. *Eur J Hum Genet* 2004; 12: 848-54.
- [16] Fagerholm R, Hofstetter B, Tommiska J, Aaltonen K, Vrtel R, Syrjäkoski K, Kallioniemi A, Kilpivaara O, Mannermaa A, Kosma VM, Uusitupa M, Eskelinen M, Kataja V, Aittomäki K, von Smitten K, Heikkilä P, Lukas J, Holli K, Bartkova J, Blomqvist C, Bartek J, Nevanlinna. NAD(P)H:quinone oxidoreductase 1 NQO1*2 genotype (P187S) is a strong prognostic and predictive factor in breast cancer. *Nat Genet* 2008; 40: 844-53.
- [17] Siegel D, Anwar A, Winski SL, Kepa JK, Zolman KL, Ross D. Rapid polyubiquitination and proteasomal degradation of a mutant form of NAD(P)H:quinone oxidoreductase 1. *Mol Pharmacol* 2001; 59: 263-8.
- [18] Sobin LH, Gospodarowicz MK, Wittekind Ch (eds): UICC. TNM Classification of Malignant Tumours. 7th edition. New York, John Wiley and Sons, Inc., 2009.
- [19] Winski SL, Koutalos Y, Bentley DL, Ross D. Subcellular localization of NAD(P)H:quinone oxidoreductase 1 in human cancer cells. *Cancer Res* 2002; 62: 1420-4.
- [20] Ohta T, Iijima K, Miyamoto M, Nakahara I, Tanaka H, Ohtsuji M, Suzuki T, Kobayashi A, Yokota J, Sakiyama T, Shibata T, Yamamoto M, Hirohashi S. Loss of Keap1 function activates Nrf2 and provides advantages for lung cancer cell growth. *Cancer Res* 2008; 68: 1303-9.
- [21] Wang J, Zhang M, Zhang L, Cai H, Zhou S, Zhang J, Wang Y. Correlation of Nrf2, HO-1, and MRP3 in gallbladder cancer and their relationships to clinicopathologic features and survival. *J Surg Res* 2010; 164: e99-105.
- [22] Sobin LH, Wittekind Ch (eds): UICC. TNM Classification of Malignant Tumours. 6th edition. New York, John Wiley and Sons, Inc., 2002.
- [23] Siegelmann-Danieli N, Buetow KH. Significance of genetic variation at the glutathione S-transferase M1 and NAD(P)H:quinone oxidoreductase 1 detoxification genes in breast cancer development. *Oncology* 2002; 62: 39-45.
- [24] Basu S, Brown JE, Flannigan GM, Gill JH, Loadman PM, Martin SW, Naylor B, Scally AJ, Seargent JM, Shah T, Puri R, Phillips RM. Immunohistochemical analysis of NAD(P)H:quinone oxidoreductase and NADPH cytochrome P450 reductase in human superficial bladder tumours: relationship between tumour enzymology and clinical outcome following intravesical mitomycin C therapy. *Int J Cancer* 2004; 109: 703-9.
- [25] Gan Y, Mo Y, Kalns JE, Lu J, Danenberg K, Danenberg P, Wientjes MG, Au JL. Expression of DT-diaphorase and cytochrome P450 reductase correlates with mitomycin C activity in human bladder tumors. *Clin Cancer Res* 2001; 7: 1313-9.
- [26] Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. *Proc Natl Acad Sci USA* 1996; 93: 14960-5.
- [27] Siegel D, Beall H, Kasai M, Arai H, Gibson NW, Ross D. pH-dependent inactivation of DT-diaphorase by mitomycin C and porfiromycin. *Mol Pharmacol* 1993; 44: 1128-34.
- [28] Powis G, Gasdaska PY, Gallegos A, Sherrill K, Goodman D. Over-expression of DT-diaphorase in transfected NIH 3T3 cells does not lead to increased anticancer quinone drug sensitivity: a questionable role for the enzyme as a target for bioreductively activated anticancer drugs. *Anticancer Res* 1995; 15: 1141-5.