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Original Article

Clinical significance of NQO1 polymorphism and expression of p53, SOD2, PARP1 in limited-stage small cell lung cancer

Ho-Cheol Kim[1*], Joon Seon Song[2*], Jae Cheol Lee[3], Dae Ho Lee[3], Sang-We Kim[3], Jung-Shin Lee[3], Woo Sung Kim[1], Jin Kyung Rho[1,4], Sun Ye Kim[4], Chang-Min Choi[1,3]

[1]Department of Pulmonary and Critical Care Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea; [2]Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea; [3]Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea; [4]Asan Institute for Life Sciences, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea. [*]Equal contributors.

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Abstract: Background: Small cell lung cancer (SCLC) is one of highly aggressive cancers with poor prognosis. Unfortunately, there are as yet no molecular targets that can be exploited to prolong survival in patients with SCLC. This study aimed to investigate possible molecular markers associated with prognosis in limited-stage small cell lung cancer (LS-SCLC). Methods: The demographic and clinical data for LS-SCLC patients treated in a tertiary care hospital between January 2008 and December 2012 were retrospectively reviewed. NQO1 polymorphism and the expression of p53, SOD2, PARP1 were examined in biopsy specimens, and the factors affecting prognosis were identified. Results: 79 patients with LS-SCLC having available pathologic tissues were analyzed. 84.8% of them received both chemotherapy and radiotherapy. NQO1 polymorphism was detected in 60.0% (45/79; heterozygous in 26 patients, homozygous in 19 patients). Over-expression of p53, SOD2, PARP1 was seen in 45.6% (36/79), 38.0% (30/79) and 41.8% (33/79) of the patients, respectively. The univariate Cox proportional hazards model revealed that serum lactate dehydrogenase (LDH) levels and PARP1 expression were associated with disease progression. In the multivariate analysis, only PARP1 expression was a significant independent prognostic factor for progression-free survival (hazard ratio: 0.494; 95% CI, 0.267-0.913, $P = 0.025$). Conclusions: PARP1 expression is correlated with longer progression-free survival in LS-SCLC requiring further studies to clarify the precise role of PARP1 and the relevance of PARP1-targeted therapy.

Keywords: Small cell lung cancer, NQO1 polymorphism, PARP1, prognosis

Introduction

Small cell lung cancers (SCLCs) represent approximately 15% of all lung cancers [1]. Although initial treatment response is favorable, overall prognosis is very poor, with median survival of less than 2 years [2, 3]. A simple staging system that classifies patients into a limited or an extensive stage of SCLC is generally accepted in clinical practice and affects treatment decisions [4]. In limited-stage small cell lung cancer (LS-SCLC), combined radiotherapy and chemotherapy improve clinical outcomes significantly more than chemotherapy alone [5, 6]. Although there have been many studies of the optimal timing of radiation and the total radiation dose [7-9], 5-year survival of LS-SCLC patients remains below 25% at best [10]. Therefore, many workers have sought to identify molecular targets for treating SCLC [11, 12]. However, currently there are no targeted therapies with beneficial effects on SCLC patients.

NAD(P)H: quinoneoxidoreductase 1 (NQO1) is a cytosolic flavoenzyme that reduces quinones to less toxic hydroquinones in a single two-electron step [13]. It protects cells against endogenous and exogenous quinines, and there is some evidence that NQO1 activity can induce apoptotic cell death in cancer tissues by regu-

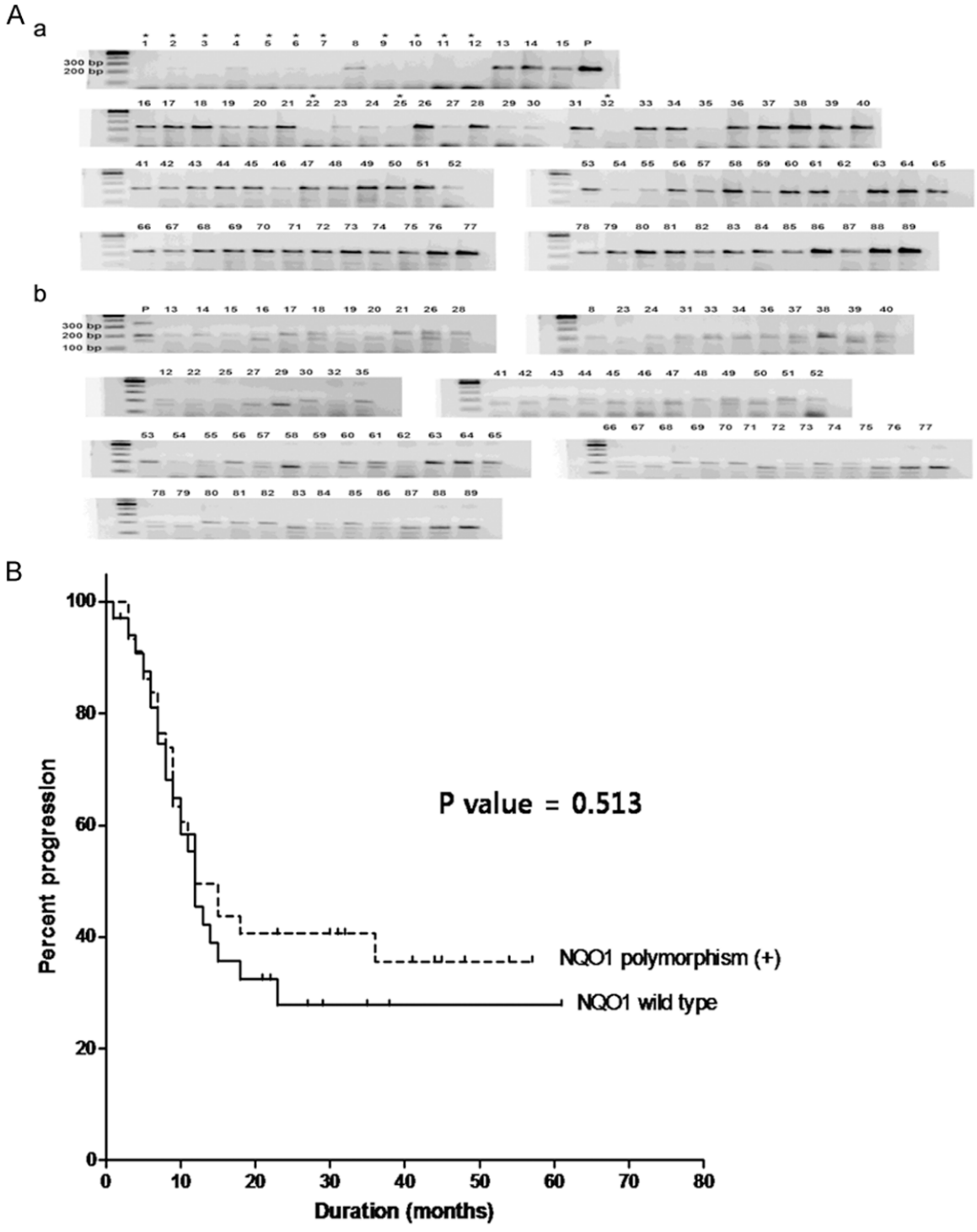

Figure 1. NQO1 polymorphism (A) and progression-free survival in 79 patients with LS-SCLC according to NQO1 polymorphism (B). NQO1 gene fragments were amplified (A) and digested with HInf1 endonuclease (B). *insufficient DNA available for analysis; P: positive control (heterozygous NQO1).

lating the tumor suppressor gene p53 [14, 15]. Recent studies have revealed an NQO1 polymorphism that affects NQO1 activity: the NQO1*2 allele is a C to T alteration in codon 609 of NQO1 DNA that leads to weak NQO1 activity [16, 17], and there is evidence that the C609T polymorphism can increase cancer risk and reduce treatment responses [18-20].

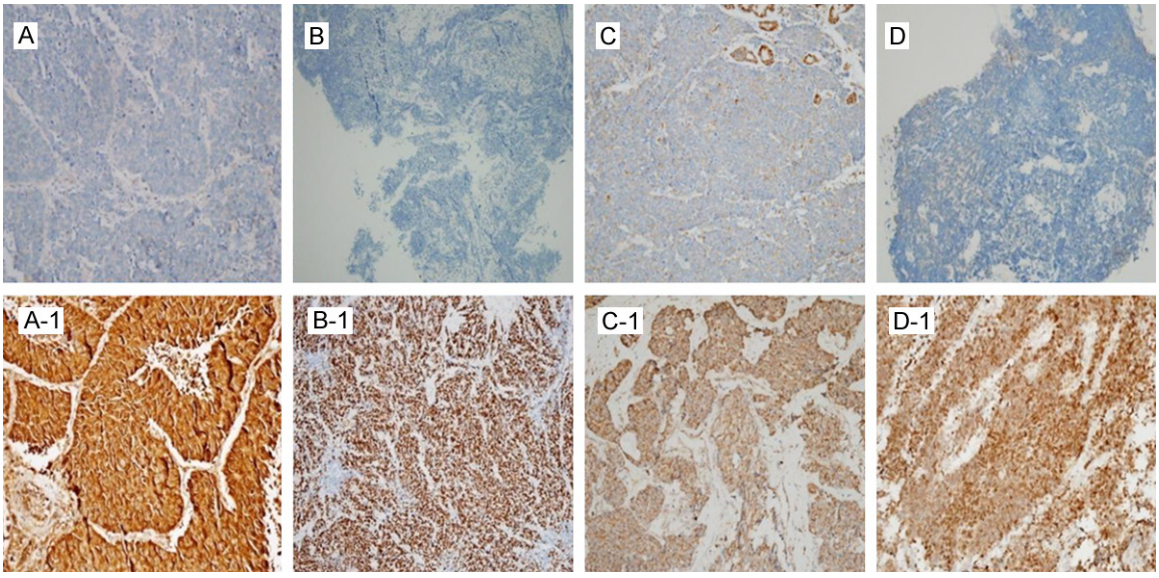

Figure 2. Immunohistochemical staining of SCLC specimens. TOP, (A-D) Negative results for NQO1, p53, SOD2 and PARP1. Bottom, (A1-1-D1-1) Positive results for NQO1, p53, SOD2 and PARP1.

Superoxide dismutase 2 (SOD2) is an antioxidant enzyme that transforms toxic superoxide into hydrogen peroxide and diatomic oxygen [21], and studies suggest that it is associated with the development and prognosis of cancers [22, 23]. There have also been many studies on the effect of p53 expression on cancer susceptibility, with conflicting results [24, 25].

Poly (ADP-Ribose) polymerase 1 (PARP1) is a DNA binding enzyme involved in DNA repair via the base excision repair (BER) pathway [26]. It is overexpressed in many cancers and its level has been associated with prognosis [27, 28]. There are indications that PARP1 inhibitors may be novel therapeutic tools in many cancers [29], and one study showed that the antitumor agent β-lapachone induced PARP1-mediated cell death in an NQO1-dependent manner [30].

In this study we aimed to investigate the frequency of the NQO1 C609T polymorphism and expression of p53, SOD2, PARP1 in LS-SCLC, and to examine their effects on prognosis.

Methods

Study population

The study population consisted of 79 patients with limited stage small cell lung cancer with available pathologic tissue. These patients were treated at the Asan Medical Center, a 2,700-bed tertiary referral hospital in Seoul, Republic of Korea, between January 2008 and December 2012. The data were retrospectively collected from medical records and a special data system in the Medical Center designated ABLE (Asan Biomedical Research Environment). Patients were followed-up until May 2014. As this was a retrospective study written informed consent was waived. The study protocol was approved by the Institutional Review Board of Asan Medical Center.

Evaluation of NQO1 polymorphism

DNA purification and detection of NQO1 gene polymorphism were performed as previously reported [31]. The following forward and reverse primers were used to amplify a 230 bp NQO1 gene fragment: 5'-TCCTCAGAGTGGCATTCTGC-3' and 5'-TCTCCTCATCCTGTACCTCT-3'. Amplification was carried with an AccuPower TagPCR PreMix (Bioneer Corp., Daejeon, Korea). PCR mixtures contained primers (0.5 pmoL, each) and 50 ng of genomic DNA in a final volume of 20 μL. PCR conditions consisted of initial denaturing at 94°C for 5 min, 35 amplification cycles (95°C for 30 s, 58°C for 30 s, and 72°C for 30 s), and a final extension at 72°C for 5 min. To examine restriction fragment length polymorphism (RFLP), the amplified fragments were digested with Hinf1 (Thermo Scientific, Rockford, IL) and analyzed by agarose gel electrophoresis. Wild type (Pro187Ser) NQO1

Table 1. Baseline characteristics of small cell lung cancer limited stage (79 patients)

Characteristics	
Age (mean), years ± SD	62.6 ± 8.0
Gender, male	71 (89.9)
Body mass index, kg/m^2 (mean ± SD)	23.8 ± 4.0
Smoking (Ever smoker)	73 (92.4)
Pack years	42.1 ± 22.1
ECOG performance	
1	62 (78.5)
2	17 (21.5)
3	0
4	0
Underlying disease	
Diabetes mellitus	14 (17.7)
Heart failure	1 (1.3)
Chronic kidney disease	0
Liver cirrhosis	1 (1.3)
Cerebrovascular accident	4 (5.1)
Previous tuberculosis history	6 (7.6)
Paraneoplastic syndrome	4 (5.1)
LDH levels* (median, interquartile range)	222 (191-253)
TNM stage	
1	5 (6.3)
2	9 (11.4)
3	65 (82.3)
4	0

Values are reported as mean ± standard deviation or as frequency (%), ECOG = Eastern cooperative oncology group; LDH = Lactate dehydrogenase. *LDH levels were checked in 44 patients.

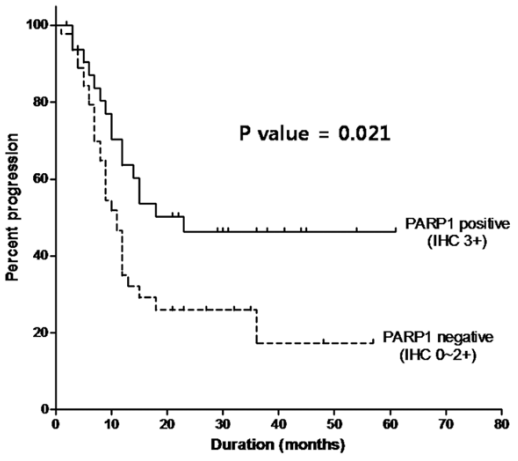

Figure 3. Progression-free survival in 79 patients with LD-SCLC according to PARP1.

formed a 191 bp band while the homozygous (Ser/Ser) and heterozygous (Pro/Ser) NQO1*2 variants produced a single 151 bp band and a191 bp and 151 bp band, respectively (**Figure 1A**).

Immunohistochemistry of NQO1, p53, SOD2, and PARP1

Immunohistochemistry was performed with anautomated IHC staining device (Benchmark XT; Ventana Medical Systems, Tucson, AZ). Briefly, 4-μm thick sections of formalin-fixed paraffin-embedded (FFPE) tissue were made and subjected to antigen retrieval by microwave boiling in 0.01 M sodium citrate buffer (pH 6.0) for 30 min. Endogenous peroxidase activity was eliminated by exposure to 3% hydrogen peroxide for 30 min. The slides were then incubated sequentially with antibodies toNQO1 (sc-32793, 1:400, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), p53 (M7001; 1:1,500; Dako, Glostrup, Denmark), SOD2 (sc-133134, 1:5,000, Santa Cruz Biotechnology Inc.) and PARP1 (IHC-00279, 1:200, Bethly Laboratories Inc., Montgomery, TX, USA) followed byperoxidase-labeled secondary antibody. Peroxidase activity was visualized using an aminoethylcarbazole substrate kit (Zymed Laboratories, Inc., San Francisco, California), and the slides were counterstained with hematoxylin.

Antibody staining was analyzed semiquantitatively by classifying the area of tissue stained into four levels (0, < 10% of tumor area stained; 1, 10%-30% of the area stained; 2, 30-60% of the area stained; and 3, > 60% of the area stained). P53 and PARP1 expression was evaluated at the nuclear level and NQO1 and SOD2 expressions at the cytoplasmic level (**Figure 2**).

Statistical analysis

For statistical analysis antibody staining was divided into two levels (negative including the above scores 0, 1 and 2 vs. positive including score 3). Comparisons of baseline characteristics and treatments were made using Student's *t*-test or the Mann-Whitney U test for continuous variables, and the chi-square or Fisher's exact test for categorical data. All *P*-values were two-tailed, with statistical significance set at $P < 0.05$. Kaplan-Meier analysis was used to evaluate differences in progression-free survival, and risk factors for progression were analyzed with Cox proportional hazards models. Statistical analyses were performed with SPSS

Table 2. Treatment and clinical course of small cell lung cancer limited stage (79 patients)

Characteristics	
Initial chemotherapy regimen	
Etoposide + Cisplatin	14 (17.7)
Etoposide + Carboplatin	65 (82.3)
Thoracic radiotherapy	67 (84.8)
Operation	6 (7.6)
Sequential chemotherapy only	4
Sequential chemotherapy and radiation therapy	2
Concurrent chemoradiation therapy	46 (58.2)
Chemotherapy + sequential radiation therapy	21 (26.6)
Prophylactic cranial irradiation	25 (31.6)
Median follow up days (range, interquartile range)	535 (301-874)
Overall death	40 (50.6)

Values are reported as frequency (%).

Table 3. Prediction factors for progression in patients with small cell lung cancer limited stage assessed by Cox proportional hazard model

Parameters	Hazard ratio	95% CI	*P* value
Univariate analysis			
Age	1.004	0.966-1.044	0.825
Gender, male	1.101	0.466-2.603	0.826
Smoking (Ever smoker)	0.710	0.279-1.806	0.472
ECOG performance	1.327	0.658-1.327	0.430
Paraneoplastic syndrome	0.044	0.000-6.186	0.215
LDH value	1.003	1.000-1.005	0.020
TNM stage	1.615	0.388-6.725	0.804
Thoracic radiotherapy	1.491	0.534-4.164	0.446
Prophylactic cranial irradiation	0.603	0.321-1.133	0.116
Concurrent chemoradiation therapy	0.897	0.466-1.727	0.745
NQO1 polymorphism	0.670	0.299-1.502	0.331
Positive p53	0.675	0.370-1.231	0.200
Positive SOD2	0.866	0.475-1.578	0.638
Positive PARP1	0.500	0.271-0.925	0.027
Multivariate analysis			
Age	1.001	0.964-1.039	0.964
ECOG performance	1.163	0.566-2.391	0.681
Prophylactic cranial irradiation	0.611	0.317-1.178	0.141
Positive PARP1	0.494	0.267-0.913	0.025

version 18.0 for Windows (SPSS, Inc., Chicago, IL).

Results

Patient characteristics

The mean age of the 79 LS-SCLC patients was 62.6 ± 8.0 years, and 89.9% (71/79) were male. Eastern cooperative oncology group (ECOG) performance was 1 (78.5%) or 2 (21.5%), and diabetes mellitus was the most common comorbidity (17.7%). Other characteristics are presented in **Table 1**.

Treatments and clinical course of the patients

Table 2 gives the treatment and clinical courses of the LS-SCLC patients. All 79 LS-SCLC patients received chemotherapy. The first chemotherapy regimen was chosen by the attending physician (Etoposide + Cisplatin in 17.7% of the patients, Etoposide + Carboplatin in 82.3%). 84.8% of the patients (67/79) received thoracic radiotherapy, and 31.6% (25/79) underwent prophylactic cranial irradiation treatment. 15.2% of patients (12/79) did not receive thoracic radiotherapy because of refusal (5 patients), old age (3 patients), or poor general condition (4 patients). The median follow-up duration was 535 days (interquartile range 301-874 days). All-cause mortality was 50.6% (40/79).

NQO1 polymorphism, expression of p53, SOD2, PARP1

NQO1 polymorphism was detected in 57.0% of the patients (45/79, heterozygous in 26 patients, homozygous in 19 patients). NQO1 IHC staining was positive in 29.4% of the wild type tissues (10/34), 11.5% of the heterozygous tissues (3/26) and 5.3% of the homozygous tissues (1/19). P53, SOD2, and PARP1 IHC staining was positive in 45.6% (35/79), 38.0% (30/79) and 41.8% (33/79) of the total study population, respectively.

Factors associated with progression-free survival

The univariate Cox proportional hazards model revealed that only serum lactate dehydroge-

Table 4. Association with NQO1 polymorphism and other gene expression, TNM stage

	Homozygote (N = 19)	Heterozygote (N = 26)	Wild type (N = 34)	*P* value
P53 expression	9 (47.4)	10 (38.5)	17 (50.0)	0.663
SOD2 expression	8 (42.1)	10 (38.5)	12 (35.3)	0.886
PARP1 expression	5 (26.3)	10 (38.5)	18 (52.9)	0.155
TNM stage				0.700
1	2 (10.5)	2 (7.7)	1 (2.9)	
2	1 (5.3)	3 (11.5)	5 (14.7)	
3	16 (84.2)	21 (80.8)	28 (82.4)	
4	0	0	0	

Values are reported as frequency (%).

nase (LDH) levels and PARP1 expression were associated with progression. In the multivariate analysis, PARP1 expression was a significant independent prognostic factor for disease progression (hazard ratio: 0.494; 95% CI, 0.267-0.913, $P = 0.025$). Covariates in the full model were age, ECOG performance, prophylactic cranial irradiation and PARP1 expression. LDH was not included because values were frequently missing (44.3%), and TNM stage was excluded because of its strong correlation with ECOG performance (**Table 3**). Also, PARP1 expression was related to progression-free survival of the LS-SCLC patients (**Figure 3**).

Relationship between NQO1 polymorphism, expression of other genes and progression-free survival

NQO1 genotype was not associated with p53, SOD2, PARP1 expression or TNM stage (see **Table 4**). Also, NQO1 polymorphism was not related to progression-free survival of the LS-SCLC patients (**Figure 1B**).

Discussion

Our current study showed that while NQO1 polymorphism did not affect prognosis and expression of the other genes examined, PARP1 expression was related to longer progression-free survival. We focused on the limited stage of small cell lung cancer, and found that age, sex, ECOG performance, TNM stage, and prophylactic cranial irradiation were unrelated to cancer progression. This underscores the importance of identifying new genetic markers in small cell lung cancer.

Although chemotherapy and radiation therapy have developed remarkably, the treatment of SCLC remains a challenge. It is difficult to develop novel therapeutic strategies because SCLC can arise by numerous distinct routes [11]. Also, there is still a lack of data concerning prognosis and survival in SCLC patients; our study was aimed at establishing the clinical significance of several genetic markers.

We found that NQO1 polymorphism was not associated with p53, SOD2, or PARP1 expression or with TNM stage. Also, we did not detect any association between NQO1 polymorphism and prognosis. There is still controversy concerning the relation between NQO1 polymorphism and non-small cell lung cancer (NSCLC). Studies have suggested that the presence of the NQO1*2 allele is associated with NSCLC susceptibility [19], locoregional recurrence [20] or even poor overall survival [32] whereas others have reported no relationship with NSCLC susceptibility [33] or that it has a protective effect on NSCLC [34]. In the case of SCLC, there is a lack of evidence in relation to NQO1 polymorphism, but one study has claimed that it is associated with the development of SCLC [35]. Thus, further study of NQO1 polymorphism in SCLC patients is needed.

There have been many studies of PARP1 expression in human cancers [36-38]. In breast cancer, there is growing evidence that PARP1 expression is related to a worse prognosis [27] and PARP1 inhibitors are potential treatments especially in patients harboring BRCA mutations [39]. There are very little data on PARP1 expression in lung cancer. One study has suggested that PARP1 expression is associated with a poor prognosis in NSCLC [28], but another found PARP1 and other marker expression related with prolonged survival within untreated patients [40]. In the case of SCLC, there is limited evidence that PARP1 expression is higher in SCLC than NSCLC, and PARP1 inhibitors could provide potential treatment strategies via several pathways [41, 42]. PARP1 inhibition causes down-regulation of essential DNA repair genes including RAD51, and PARP1 inhibitor

alone or in combination with chemotherapy seem to be effective in cell lines. However, the study referred to only compared protein expression in cell lines and there were no clinical data. To the best of our knowledge, our study is the first to evaluate the association between PARP1 expression and prognosis in SCLC.

There are limitations to this study. First, since it was retrospective in design and non-interventional, there could be selection bias. Second, as it was performed at a single center in Korea and involved a relatively small number of patients, it would be unwise to generalize our results. Further studies with larger numbers are needed.

In summary, NQO1 polymorphism and p53 and SOD2 expression were not associated with prognosis in LS-SCLC. However, PARP1 expression was independently related to longer progression-free survival.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chang-Min Choi, Department of Pulmonary and Critical Care Medicine, Asan Medical Center, College of Medicine, University of Ulsan, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 138-736, South Korea. Fax: 02-3010-5902; E-mail: ccm9607@gmail.com

References

[1] Stupp R, Monnerat C, Turrisi AT 3rd, Perry MC, Leyvraz S. Small cell lung cancer: state of the art and future perspectives. Lung Cancer 2004; 45: 105-117.

[2] Govindan R, Page N, Morgensztern D, Read W, Tierney R, Vlahiotis A, Spitznagel EL, Piccirillo J. Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. J Clin Oncol 2006; 24: 4539-4544.

[3] Chua YJ, Steer C, Yip D. Recent advances in management of small-cell lung cancer. Cancer Treat Rev 2004; 30: 521-543.

[4] Micke P, Faldum A, Metz T, Beeh KM, Bittinger F, Hengstler JG, Buhl R. Staging small cell lung cancer: Veterans Administration Lung Study Group versus International Association for the Study of Lung Cancer-what limits limited disease? Lung Cancer 2002; 37: 271-276.

[5] Turrisi AT 3rd, Kim K, Blum R, Sause WT, Livingston RB, Komaki R, Wagner H, Aisner S, Johnson DH. Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. N Engl J Med 1999; 340: 265-271.

[6] Takada M, Fukuoka M, Kawahara M, Sugiura T, Yokoyama A, Yokota S, Nishiwaki Y, Watanabe K, Noda K, Tamura T, Fukuda H, Saijo N. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with cisplatin and etoposide for limited-stage small-cell lung cancer: results of the Japan Clinical Oncology Group Study 9104. J Clin Oncol 2002; 20: 3054-3060.

[7] Fried DB, Morris DE, Poole C, Rosenman JG, Halle JS, Detterbeck FC, Hensing TA, Socinski MA. Systematic review evaluating the timing of thoracic radiation therapy in combined modality therapy for limited-stage small-cell lung cancer. J Clin Oncol 2004; 22: 4837-4845.

[8] Huncharek M, McGarry R. A meta-analysis of the timing of chest irradiation in the combined modality treatment of limited-stage small cell lung cancer. Oncologist 2004; 9: 665-672.

[9] Watkins JM, Fortney JA, Wahlquist AE, Shirai K, Garrett-Mayer E, Aguero EG, Sherman CA, Turrisi AT 3rd, Sharma AK. Once-daily radiotherapy to > or =59.4 Gy versus twice-daily radiotherapy to > or =45.0 Gy with concurrent chemotherapy for limited-stage small-cell lung cancer: a comparative analysis of toxicities and outcomes. Jpn J Radiol 2010; 28: 340-348.

[10] Sculier JP, Lafitte JJ, Efremidis A, Florin MC, Lecomte J, Berchier MC, Richez M, Berghmans T, Scherpereel A, Meert AP, Koumakis G, Leclercq N, Paesmans M, Van Houtte P. A phase III randomised study of concomitant induction radiochemotherapy testing two modalities of radiosensitisation by cisplatin (standard versus daily) for limited small-cell lung cancer. Ann Oncol 2008; 19: 1691-1697.

[11] D'Angelo SP, Pietanza MC. The molecular pathogenesis of small cell lung cancer. Cancer Biol Ther 2010; 10: 1-10.

[12] Sattler M, Salgia R. Molecular and cellular biology of small cell lung cancer. Semin Oncol 2003; 30: 57-71.

[13] Ross D, Kepa JK, Winski SL, Beall HD, Anwar A, Siegel D. NAD(P)H:quinone oxidoreductase 1 (NQO1): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. Chem Biol Interact 2000; 129: 77-97.

[14] Asher G, Lotem J, Kama R, Sachs L, Shaul Y. NQO1 stabilizes p53 through a distinct pathway. Proc Natl Acad Sci U S A 2002; 99: 3099-3104.
[15] 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 U S A 2001; 98: 1188-1193.
[16] Anwar A, Siegel D, Kepa JK, Ross D. Interaction of the molecular chaperone Hsp70 with human NAD(P)H:quinone oxidoreductase 1. J Biol Chem 2002; 277: 14060-14067.
[17] 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: 995-996.
[18] Liu Y, Zhang D. The NQO1 C609T polymorphism and risk of lung cancer: a meta-analysis. Asian Pac J Cancer Prev 2011; 12: 3091-3095.
[19] Tian G, Wang M, Xu X. The role of NQO1 polymorphisms in the susceptibility and chemotherapy response of Chinese NSCLC patients. Cell Biochem Biophys 2014; 69: 475-479.
[20] Song SY, Jeong SY, Park HJ, Park SI, Kim DK, Kim YH, Shin SS, Lee SW, Ahn SD, Kim JH, Lee JS, Choi EK. Clinical significance of NQO1 C609T polymorphisms after postoperative radiation therapy in completely resected non-small cell lung cancer. Lung Cancer 2010; 68: 278-282.
[21] Mates JM, Segura JA, Alonso FJ, Marquez J. Oxidative stress in apoptosis and cancer: an update. Arch Toxicol 2012; 86: 1649-1665.
[22] Li N, Huang HQ, Zhang GS. Association between SOD2 C47T polymorphism and lung cancer susceptibility: a meta-analysis. Tumour Biol 2014; 35: 955-959.
[23] Chen PM, Wu TC, Wang YC, Cheng YW, Sheu GT, Chen CY, Lee H. Activation of NF-kappaB by SOD2 promotes the aggressiveness of lung adenocarcinoma by modulating NKX2-1-mediated IKKbeta expression. Carcinogenesis 2013; 34: 2655-2663.
[24] Paik KH, Park YH, Ryoo BY, Yang SH, Lee JC, Kim CH, Ki SS, Kim JM, Park MJ, Ahn HJ, Choi W, Chung JH. Prognostic value of immunohistochemical staining of p53, bcl-2, and Ki-67 in small cell lung cancer. J Korean Med Sci 2006; 21: 35-39.
[25] Lee JS, Yoon A, Kalapurakal SK, Ro JY, Lee JJ, Tu N, Hittelman WN, Hong WK. Expression of p53 oncoprotein in non-small-cell lung cancer: a favorable prognostic factor. J Clin Oncol 1995; 13: 1893-1903.
[26] Rouleau M, Patel A, Hendzel MJ, Kaufmann SH, Poirier GG. PARP inhibition: PARP1 and beyond. Nat Rev Cancer 2010; 10: 293-301.
[27] Rojo F, Garcia-Parra J, Zazo S, Tusquets I, Ferrer-Lozano J, Menendez S, Eroles P, Chamizo C, Servitja S, Ramirez-Merino N, Lobo F, Bellosillo B, Corominas JM, Yelamos J, Serrano S, Lluch A, Rovira A, Albanell J. Nuclear PARP-1 protein overexpression is associated with poor overall survival in early breast cancer. Ann Oncol 2012; 23: 1156-1164.
[28] Xie KJ, He HE, Sun AJ, Liu XB, Sun LP, Dong XJ. Expression of ERCC1, MSH2 and PARP1 in non-small cell lung cancer and prognostic value in patients treated with platinum-based chemotherapy. Asian Pac J Cancer Prev 2014; 15: 2591-2596.
[29] Kummar S, Chen A, Parchment RE, Kinders RJ, Ji J, Tomaszewski JE, Doroshow JH. Advances in using PARP inhibitors to treat cancer. BMC Med 2012; 10: 25.
[30] Bey EA, Bentle MS, Reinicke KE, Dong Y, Yang CR, Girard L, Minna JD, Bornmann WG, Gao J, Boothman DA. An NQO1- and PARP-1-mediated cell death pathway induced in non-small-cell lung cancer cells by beta-lapachone. Proc Natl Acad Sci U S A 2007; 104: 11832-11837.
[31] Sameer AS, Shah ZA, Syeed N, Rasool R, Afroze D, Siddiqi MA. NAD(P)H:quinone oxidoreductase 1 (NQO1) Pro187Ser polymorphism and colorectal cancer predisposition in the ethnic Kashmiri population. Asian Pac J Cancer Prev 2010; 11: 209-213.
[32] Kolesar JM, Dahlberg SE, Marsh S, McLeod HL, Johnson DH, Keller SM, Schiller JH. The NQO1*2/*2 polymorphism is associated with poor overall survival in patients following resection of stages II and IIIa non-small cell lung cancer. Oncol Rep 2011; 25: 1765-1772.
[33] Guo S, Gao M, Li X, Li Y, Chu S, Zhu D, Niu W. Lack of association between NADPH quinone oxidoreductase 1 (NQO1) gene C609T polymorphism and lung cancer: a case-control study and a meta-analysis. PLoS One 2012; 7: e47939.
[34] Bock CH, Wenzlaff AS, Cote ML, Land SJ, Schwartz AG. NQO1 T allele associated with decreased risk of later age at diagnosis lung cancer among never smokers: results from a population-based study. Carcinogenesis 2005; 26: 381-386.
[35] Lewis SJ, Cherry NM, Niven RM, Barber PV, Povey AC. Polymorphisms in the NAD(P)H: quinone oxidoreductase gene and small cell lung cancer risk in a UK population. Lung Cancer 2001; 34: 177-183.
[36] Csete B, Lengyel Z, Kadar Z, Battyani Z. Poly (adenosine diphosphate-ribose) polymerase-1 expression in cutaneous malignant melanomas as a new molecular marker of aggressive tumor. Pathol Oncol Res 2009; 15: 47-53.
[37] Nosho K, Yamamoto H, Mikami M, Taniguchi H, Takahashi T, Adachi Y, Imamura A, Imai K, Shinomura Y. Overexpression of poly (ADP-ribose)

polymerase-1 (PARP-1) in the early stage of colorectal carcinogenesis. Eur J Cancer 2006; 42: 2374-2381.

[38] Brustmann H. Poly (adenosine diphosphate-ribose) polymerase expression in serous ovarian carcinoma: correlation with p53, MIB-1, and outcome. Int J Gynecol Pathol 2007; 26: 147-153.

[39] Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JH, de Bono JS. Inhibition of poly (ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 2009; 361: 123-134.

[40] Olaussen KA, Adam J, Vanhecke E, Vielh P, Pirker R, Friboulet L, Popper H, Robin A, Commo F, Thomale J, Kayitalire L, Filipits M, Le Chevalier T, Andre F, Brambilla E, Soria JC. PARP1 impact on DNA repair of platinum adducts: preclinical and clinical read-outs. Lung Cancer 2013; 80: 216-222.

[41] Byers LA, Wang J, Nilsson MB, Fujimoto J, Saintigny P, Yordy J, Giri U, Peyton M, Fan YH, Diao L, Masrorpour F, Shen L, Liu W, Duchemann B, Tumula P, Bhardwaj V, Welsh J, Weber S, Glisson BS, Kalhor N, Wistuba II, Girard L, Lippman SM, Mills GB, Coombes KR, Weinstein JN, Minna JD, Heymach JV. Proteomic profiling identifies dysregulated pathways in small cell lung cancer and novel therapeutic targets including PARP1. Cancer Discov 2012; 2: 798-811.

[42] Rosell R, Wannesson L. A genetic snapshot of small cell lung cancer. Cancer Discov 2012; 2: 769-771.