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# p53 Protein Expression and its Prognostic Importance in Patients with Nodal non-Hodgkin's Lymphoma

To determine whether the p53 expression might be a predictor for treatment response and overall survival in nodal non-Hodgkin's lymphoma (NHL), we analyzed the expression of p53 in 69 NHL patients. p53 protein expression was analyzed by immunohistochemistry with long-term follow up (1-148 months: median 12.2). p53 expression was noted in 23/69 (33.3%) patients. Complete response (CR) rate to systemic chemotherapy was correlated with stage (I/II) ($p$=0.038), but not with p53 expression ($p$=0.2856). Poor overall survival was associated with stage ($p$=0.0010) or IPI score ($p$=0.0076), but not with p53 expression ($p$=0.8601). From stratification analysis by stage, in stage III/IV patients, the p53 positive group had a trend to be associated with poor overall survival than the p53 negative group. Multivariate analysis revealed that p53 positive group was associated with less CR rate compared to the p53 negative group ($p$=0.046), whereas overall survival was correlated with stage ($p$=0.0320), not with p53 status. p53 expression was associated with less CR rate in patients with DLBL. Further studies with large numbers of samples and homogenous group of NHL are needed to determine the prognostic value of cell cycle regulator, p53 in NHL.

Key Words: *Lymphoma, Non-Hodgkin's; Genes, p53; Gene Expression*

**Myung-Ju Ahn, Hawk Kim, In-Soon Kim, Jin Kyung Park*, Mo-Ran Ki*, Chan-Kum Park†**

Departments of Internal Medicine, Preventive Medicine*, and Pathology†, Hanyang University College of Medicine, Seoul, Korea

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**Address for correspondence**
Chan-Kum Park, M.D.
Department of Pathology, Hanyang University College of Medicine, 17 Hangdang-dong, Sungdong-gu, Seoul 133-791, Korea
Tel: +82.2-2290-8250, Fax: +82.2-
E-mail: parkcg@email.hanyang.ac.kr

## INTRODUCTION

It has been well established that *p53* plays a central role in controlling functions such as G1/S transition, DNA damage repair and apoptosis of a cell (1-4). *p53* has been found to be altered in a wide variety of tumors and it has been associated with a poor prognosis in some tumors (5-7). In various hematologic malignancies, *p53* mutations have been reported in 5% to 50% and a mutated *p53* has been associated with advanced disease or with the development of resistance to therapy (8-11). Non-Hodgkin's lymphoma (NHL) form a heterogenous group in terms of clinical presentation, histology, immunophenotype, genetic alterations, response to treatment and prognosis. A few studies have examined gross structural changes or mutations in *p53* in selected histologic groups of lymphoma. Although *p53* mutations are reported in many studies, the significance of p53 in NHL is still controversial. Recent investigations of lung cancer, breast cancer, and bladder cancer have indicated that *p53* mutations or accumulation of p53 protein correlated with poor prognosis (12-15). Normal p53 protein is almost undetectable by immunohistochemistry. Overexpression of aberrant nonfunctional p53 protein might, therefore, be used to mark genetic alterations of p53 (16-19). To investigate the prognostic significance of cell cycle regulators, p53 in nodal NHL we analyzed 69 nodal NHL using an immunohistochemical method.

## MATERIALS AND METHODS

### Tissue samples and patient population

Between January 1987 and December 1998, 69 biopsy samples of lymph node diagnosed histologically as nodal NHL as described in the Working Formulation and in the revised European-American lymphoma (REAL) classification were studied by cell surface markers (20, 21). The group of patients consisted of 23 female and 46 male patients. Age, clinical stage, performance status, serum lactate dehydrogenase (LDH) level and the numbers of extranodal sites of the disease were used as criteria which was determined by the IPI (Table 1) (22). International prognostic index (IPI) was grouped as Low/Low Intermediate and High Intermediate/High. Fifty-six of the 69

patients received systemic chemotherapy with cytoxan adriamycin vincristine prednisone combination chemotherapy (CHOP) like regimen. Complete response (CR) was defined as the resolution of clinical and radiologic evidence of disease for a minimum of four weeks. Information concerning diagnosis date, other clinical characteristics and deaths was obtained from a retrospective study. Subjects were followed until the earliest of the following: their date of death, the date they were last known to be alive, or the end of the follow up period. Observations were censored at either the date of last known follow-up or the end date of the follow-up period if death had not occurred. The median follow-up period was 12.2 months.

### Immunophenotypic studies

Immunophenotypic studies were performed on formalin-fixed paraffin-embedded sections, using monoclonal antibodies which are reactive in routinely processed paraffin-embedded tissues by a avidin-biotin complex (ABC) method: a panel of monoclonal antibodies against T cells [CD3 (DAKO, Santa Babara, CA, U.S.A.), CD43 (MT-1, BioGenex, San Ramon, U.S.A.), CD45RO (UCHL-1, DAKO)], B cells [CD20 (L-26, DAKO), MB-2 (BioGenex)]; macrophage [CD68 (DAKO)], NK cells [CD56 (DAKO)] and activated T, B, and Reed-Sternberg cells [CD30 (Ki-1, DAKO)].

### Immunohistochemical staining for p53

Overexpression of p53 was assessed using the DO7 monoclonal antibody (MoAb) (Novocastra, Newcastle, U.K.), which specifically detects human wild type and mutant p53. Because the half-life of normal p53 is short and the amount of normal p53 expressed is low, the detection of stainable levels of p53 in a tumor cell suggests a *p53* mutation. The 4-$\mu$m thick sections from formalin-fixed paraffin-embedded tissue blocks were deparaffinized in xylene and rehydrated. After blocking endogenous peroxidase activity with 3% hydrogen peroxidase in methanol, the sections were incubated in 0.01 M citrate buffer pH 6.0 for two 5-min cycles in a microwave oven at 800 W for antigen retrieval. All the slides were preincubated with 10% normal goat serum for 20 min. The DO7 antibody was used at a concentration of 1:50 and applied for 60 min at room temperature. Each of the biotinylated secondary antibodies was added for 30 min followed by the streptavidin-biotin peroxidase reagent (DAKO) for an additional 30 min. After washing with phosphate buffered saline, the antibody was detected by means of an ABC method and developed with 3,3'-diaminobenzidine (DAKO). The slides were counterstained with hematoxylin. All slides were evaluated without knowledge of patient identity or clinical status by an experienced pathologist. Each experiment was independently performed twice. A case was regarded as immunostaining positive if greater than 10% of the malignant cells stained with the antibody. Cases of p53 positive were arbitrarily divided into three groups according to the number of p53 positive cells and p53 immunoreactivity: Grade 0: 0-10% nuclear or cytoplasmic stain; Grade 1: 11-30% nuclear stain; Grade 2: 31-60% nuclear stain; Grade 3: 61-100% nuclear stain. Reactive hyperplastic lymph node and known p53 positive invasive ductal carcinoma of the breast were used as a negative and positive control, respectively. A threshold of 10% of positive cells was used for statistical analysis, because in previous studies this threshold separated groups that had different clinical behavior (16, 23).

Table 1. Characteristics of 69 nodal non-Hodgkin's lymphoma patients

| | No. of patients | (%) |
|---|---|---|
| Sex | | |
| Male | 46 | (66.7) |
| Female | 23 | (33.3) |
| Age (yrs) | | |
| Mean | 44.5 | |
| Median | 50.0 | |
| ≤60 | 48 | (69.6) |
| >60 | 21 | (30.4) |
| Stage (Ann Arbor) | | |
| I + II | 21 | (30.4) |
| III + IV | 48 | (69.6) |
| IPI score | | |
| L/LI | 28 | (40.6) |
| HI/H | 41 | (59.4) |
| Response to therapy | | |
| CR | 31/56 | (55.4) |
| Overall survival (months) | | |
| Range | 1-148.1 months | |
| Median | 12.17 months | |

IPI score, international prognostic index score; L, low; LI, low intermediate; HI, high intermediate; H, high risk, respectively (18)

### Statistical analysis

Frequencies of p53 overexpression were compared with various clinical characteristics and pathological variables using Pearson's chi-square test. Survival curves were calculated using the Kaplan-Meier method and compared with the frequencies of p53 expression using log-rank test. Univariate analysis and multivariate stepwise Cox's regression analysis were performed to identify prognostic factors for survival. All statistical analyses were two-sided at a significance level of $p$=0.05, and performed using SPSS 7.5® statistical software.

## RESULTS

Using the anti-p53 antibody, the reactive lymph node showed up as either absent or present only in rare, activated cells in the follicular and interfollicular zones (Fig. 1). p53 positive invasive ductal carcinoma of the breast served as a positive control. We arbitrarily defined p53 positive in more than 10% of nuclear staining. p53 protein expression was noted in 23/69 (33.3%) of patients. There were 13% (9/69) of Grade 3, 5.7% (4/69) of Grade 2, and 14.5% (10/69) of Grade 1 p53 expression, respectively (Table 2). Representative results for p53 expression are shown in Fig. 2. p53 expression was found in 5 of 22 cases (22.7%) of T cell NHL, 18 of 47 (38.2%) B cell NHL. According to the REAL classification, p53 expression was noted in 13 of 33 diffuse large B cell lymphoma (DLBL) (39.4%), 1 of 9 lymphoblastic lymphoma (LB; 11.1%), 2 of 6 Burkitt's lymphoma (33.3%), 2 of 12 peripheral T cell lymphoma (PTCL; 18.2%), 3 of 6 follicle center lymphoma (FCL 50%; 1/3 FCL grade II, 2/3 FCL grade III), 1 of 1 angiocentric lymphoma, 0 of 2 anaplastic large cell lymphoma, respectively. There was no correlation between p53 expression status and age, sex, stage, T/B cell type or IPI score (Table 3).

Thirty-one out of 56 patients (55.4%) treated with systemic chemotherapy achieved CR. No correlation was noted between CR and p53 expression. By multiple logistic regression analysis, CR rate was correlated with stage (I/II) ($p$=0.038), but not with p53 expression ($p$=0.2856).

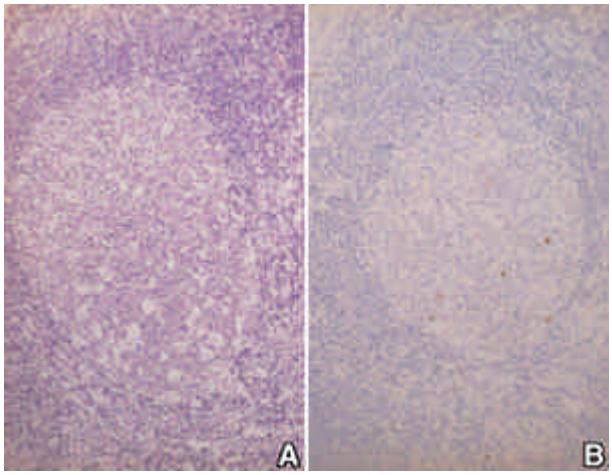


**Fig. 1.** **A:** Light microscopic findings of reactive hyperplasia of the lymph node (H&E, ×100). **B:** Immunoperoxidase staining with anti-p53 monoclonal antibody shows absent or rare p53 positive cells (×100).

Multivariate analysis of factors that influence overall survival by Cox's proportional hazard model also showed that poor overall survival was associated with stage ($p$=0.0010) or IPI score ($p$=0.0076), but not with p53 expression ($p$=0.8601) (Table 4). But from the stratification analysis by stage, among the patients group with high stage (III/IV), p53 positive group showed a trend to be associated with poor overall survival than the p53 negative group ($p$=0.3552) (Fig. 3).

We also analyzed 33 cases of DLBL subgroup to determine the clinical prognostic significance of p53 expres-

**Table 2.** Characteristics in nodal non-Hodgkin's lymphoma patients according to p53 expression and complete response to chemotherapy

| | p53 expression | | | | Complete response | | | |
|---|---|---|---|---|---|---|---|---|
| | Positive (n=23) | | Negative (n=46) | | CR (n=31) | | No CR (n=25) | |
| | No | (%) | No | (%) | No | (%) | No | (%) |
| Age (yr) | | | | | | | | |
| <60 | 18/48 | (37.5) | 30/48 | (62.5) | 22/38 | (57.9) | 16/38 | (42.1) |
| >60 | 5/21 | (23.8) | 16/21 | (76.2) | 9/18 | (50.0) | 9/18 | (50.0) |
| Sex | | | | | | | | |
| Male | 15/46 | (32.6) | 31/46 | (67.4) | 22/34 | (64.7) | 12/34 | (35.3) |
| Female | 8/23 | (34.8) | 15/23 | (65.2) | 9/22 | (40.9) | 13/22 | (59.1) |
| Stage | | | | | | | | |
| I + II | 7/21 | (33.3) | 14/21 | (66.7) | 13/19 | (68.4) | 6/19 | (31.6) |
| III + IV | 16/48 | (33.3) | 32/48 | (66.7) | 18/37 | (48.6) | 19/37 | (51.4) |
| IPI | | | | | | | | |
| L/LI | 8/28 | (28.6) | 20/28 | (71.4) | 17/26 | (65.4) | 9/26 | (34.6) |
| HI/H | 15/41 | (36.6) | 26/41 | (63.4) | 14/30 | (46.7) | 16/30 | (53.3) |
| T/B cell | | | | | | | | |
| T | 5/22 | (22.7) | 17/22 | (77.3) | 19/40 | (47.5) | 21/40 | (52.5) |
| B | 18/47 | (38.3) | 29/47 | (61.7) | 12/16 | (75.0) | 4/16 | (25.0) |
| p53 | | | | | | | | |
| <5% | | | | | 23/37 | (62.2) | 14/37 | (37.8) |
| >5% | | | | | 8/19 | (42.1) | 11/19 | (57.9) |

IPI, international prognostic index; L, low; LI, low intermediate; HI, high intermediate; H, high

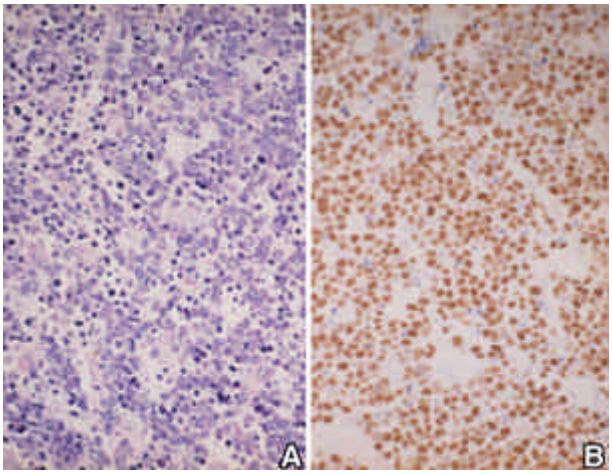


**Fig. 2. A:** Representative result for p53 positive non-Hodgkin's lymphoma. Light microscopic findings of Burkitt's lymphoma (H&E, ×200). **B:** Immunoperoxidase staining with anti-p53 monoclonal antibody shows diffuse strong nuclear immunoreactivity (Grade 3) for the p53 protein (×200).

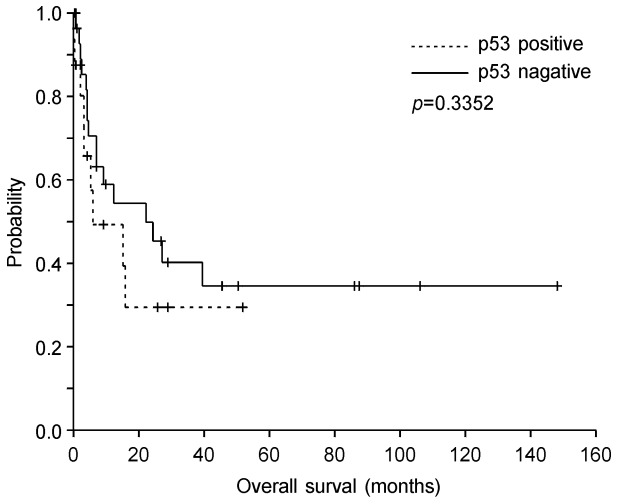


**Fig. 3.** Overall survival for 48 stage III/IV non-Hodgkin's lymphoma patients with (n=16) or without (n=32) p53 expression.

sion. There was also no correlation between p53 expression and other clinical variables. But, p53 positive group was associated with less CR rate compared to the p53 negative group ($p$=0.046) by multiple logistic regression analysis.

## DISCUSSION

The p53 transcription regulatory protein monitors the integrity of the genome by arresting cells at G1 or programming them to cell death when DNA replication is defective or when DNA is damaged (1-4). The inactivation of this gene would provide a selective advantage for clonal expansion of neoplastic cells. Mutant p53 represents a loss of function by a recessive or dominant negative process. Previous studies have shown that a majority of mutations occurs as missense changes in the highly conserved sequences of the coding region (exons 5 through 8) (24). The frequency of mutations in lymphoid malignancies varies in different histologic groups, with the highest values in adult T-cell leukemia (44%) (25), Burkitt's lymphomas (37%) (26), and DLBL (30.5%) (27). We studied a heterogenous group of nodal NHL,

**Table 3.** Multivariate analysis of factors that influence the achievement of complete response by logistic regression model and overall survival by Cox's proportional hazards model

| | Factors | b | SE(b) | *p* value | OD ratio |
|---|---|---|---|---|---|
| Complete response | | | | | |
| | Age group | 0.0712 | 0.7038 | 0.9194 | 1.0738 |
| | Sex | 1.2785 | 0.6892 | 0.6361 | 3.5914 |
| | Stage group | 1.5066 | 0.7263 | 0.038 | 4.5113 |
| | IPI group* | 0.6300 | 0.5742 | 0.2726 | 1.8777 |
| | p53 | 0.7253 | 0.6793 | 0.2856 | 2.0654 |
| | T/B | 1.3315 | 0.7600 | 0.0798 | 3.7867 |
| Overall survival | | | | | |
| | Age group | 0.0320 | 0.4440 | 0.9425 | 1.0325 |
| | Sex | 0.7813 | 0.4408 | 0.07631 | 2.1843 |
| | Stage group | 1.9075 | 0.5776 | 0.0010 | 6.7362 |
| | IPI group* | 1.0836 | 0.4061 | 0.0076 | 2.9554 |
| | p53 | 0.0724 | 0.4104 | 0.8601 | 1.0750 |
| | T/B | 0.6061 | 0.4547 | 0.1825 | 1.8333 |

Cox's proportional hazards regression model is as follows: $h_i(t)=h_0(t)\ \exp(\beta_1$ age group$+\beta_2$ sex$+\beta_3$ stage group$+\beta_4$ p53$+\beta_5$ T/B).
Regression analysis was performed using forward stepwise method.
Age: 0=≤60, 1=>60; Sex: 0=male, 1=female; Stage group: 0=stage I/II, 1=stage III/IV; p53: 0=negative, 1=positive; T/B: 0=B, 1=T
*The odds ratio was obtained from separately analyzed data using three factors: IPI, p53 and T/B

and found 33.3% of nodal NHL patients with p53 expression. These results are comparable to previously reported findings (25-27). Whereas p53 expression was noted in 39.4% of DLBL, the frequency of p53 expression was low in LB (1/9), Burkitt's lymphoma (2/6), and PTCL (2/11). Our results also showed no correlation between p53 expression and any clinicopathologic features, overall survival or complete response to chemotherapy. This result may be attributed to the heterogenous group of patients with NHL studied. We found that p53 positive group had a tendency to be associated with poor overall survival than the p53 negative group among the patients group with high stage (III/IV) (Fig. 3). Although the difference was not significant, large number of samples would elucidate the prognostic values in this group of patients.

Recently, many reports showed that aggressive B cell lymphoma is associated with high incidence of p53 expression and also with poor overall survival and poor response to chemotherapy (28, 29). An analysis of 47 cases of aggressive B cell lymphoma in our study did not reveal any significant difference between p53 expression and overall survival ($p$=0.4196) or response to treatment ($p$=0.0656). Our studies have reached contradictory conclusions on this point, and some of these different results may be attributed to the different subtypes of NHL studied or different methodologies used. Ichikawa et al. (28) reported that when the mutations of the p53 gene were identified by polymerase chain reaction-mediated analysis of single-strand conformation polymorphism (SSCP) and by direct sequencing, mutations of the p53 gene were associated with a poor prognosis in patients with aggressive B cell lymphoma. Since we performed the p53 mutation studies by immunostaining, the results might be different from other studies. However, Koduru et al. (29) reported that positive correlations were found between p53 mutation detected by SSCP or by direct sequencing and p53 protein expression by immunostaining, suggesting that immunohistochemical stain for p53 might be an effective way to screen for p53 changes in NHL.

The prognostic importance of p53 expression in DLBL still remains controversial. In our study, when analyzing 33 cases of DLBL, significant correlation of p53 expression was found with the achievement of complete response. Only the clinical parameters included within the IPI were significant in overall survival, but p53 expression did not correlate with shorter overall survival. Kramer et al. (30) reported that p53 expression is related to a high tumor burden, but is not an independent risk factor for complete response and overall survival in DLBL. Sanchez et al. (27) also indicated that p53 expression studied by immunohistochemical stain lacked any significant relationship with any of the clinical variables analyzed. However, Ichikawa et al. (28) showed a clear adverse effect of *p53* gene mutations on the survival probability, independent of clinical parameters. These differing results from the mutational p53 studies in aggressive NHL could be dependent on the lack of a clear correlation between *p53* mutation and p53 protein expression in NHL, as other studies have already demonstrated (19, 31-33). Marinez-Delgado et al. (33) reported that analyzing exons 5-9, seven out of 94 lymphoma cases had mutations in the *p53* gene, whereas overexpression of p53 protein was observed in 16 of 87 cases. This discrepancies between overexpression and presence of mutations suggest the existence of another mechanism to stabilize the p53 protein. Although p53 protein expression is not always related to *p53* gene mutations, immunohistochemical assessment of p53 might be a simple and effective way to screen for p53 changes in non-Hodgkin's lymphoma (29, 34).

In conclusion, our data indicate that p53 protein expression was frequently noted in nodal NHL. Especially in DLBL, p53 expression was associated with less CR rate, whereas no significant relation of p53 expression was found with overall survival. Further studies with large numbers of samples and homogeneous group of NHL are needed to determine the prognostic value of p53 in NHL. And also studies with other biological markers involved in cell cycle regulation, such as bcl2, MDM2, p21 or p27 might elucidate their true roles in NHL.

## REFERENCES


1. Levine AJ, Momand J, Finlay CA. *The p53 tumor suppressor gene. Nature 1991; 351: 453-6.*
2. Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A. *Wild type p53 induces apoptosis of myeloid leukemic cells that is inhibited by interleukin-6. Nature 1991; 352: 345-7.*
3. Shaw P, Bavey R, Tardy S, Sahli R, Sordat B, Costa J. *Induction of apoptosis by wild type p53 in human colon tumors derived cell line. Proc Natl Acad Sci USA 1992; 89: 4495-99.*
4. Fritsche M, Haessler C, Brandner G. *Induction of nuclear accumulation of the tumor suppressor protein p53 by DNA damaging agents. Oncogene 1993; 8: 307-11.*
5. Nigro JM, Baker SJ, Preisinger AC, Jessup JM, Hostetter R, Cleary K, Bigner SH, Vogelstein B. *Mutations in the p53 gene occur in diverse human tumor types. Nature 1989; 342: 705-6.*
6. Hollstein M, Sidransky D, Vogelstein B, Harris CC. *P53 mutations in human cancers. Science 1991; 253: 49-53.*
7. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. *Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994; 54: 4855-78.*

8. Imamura J, Miyoshi I, Koeffler HP. *p53 in hematologic malignancies. Blood 1994; 84: 2412-21.*
9. Diccianni MB, Yu J, Hsiao M, Mukherjee S, Shao LE, Yu AL. *Clinical significance of p53 mutations in relapsed T-cell acute lymphoblastic leukemia. Blood 1994; 84: 3105-12.*
10. Wattel E, Preudhomme C, Hecquet B, Vanrumbeke M, Quesnel B, Dervite I, Morel P, Fenaux P. *p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. Blood 1994; 84: 3148-57.*
11. Kaneko H, Misawa S, Horiike S, Nakai H, Kashima K. *p53 mutations emerge at early phase of myelodysplastic syndrome and are associated with complex chromosome abnormalities. Blood 1995; 85: 2189-93.*
12. Horio Y, Takahashi T, Kuroishi T, Hibi K, Suyama M, Niimi T, Shimokata K, Yamakawa K, Nakamura Y, Ueda R. *Prognostic significance of p53 mutations and 3p deletions in primary resected non-small cell lung cancer. Cancer Res 1993; 53: 1-4.*
13. Tholacius S, Borresen AL, Eyfjord JE. *Somatic p53 mutations in human breast cancer in an Icelandic population: a prognostic factor. Cancer Res 1993; 53: 1637-41.*
14. Thor AD, Moore DH, Edgerton SM, Kawasaki ES, Reihsaus E, Lynch HT, Marcus JN, Schwartz L, Chen LC, Mayall BH. *Accumulation of p53 tumor suppressor gene protein: an independent marker of prognosis in breast cancer. J Natl Cancer Inst 1992; 84: 845-55.*
15. Sarkis AS, Dalbagni G, Cordon-Cardo C, Zhang ZF, Sheinfeld J, Fair WR, Herr HW, Reuter VE. *Nuclear overexpression of p53 protein in transitional cell bladder carcinoma: a marker for disease progression. J Natl Cancer Inst 1993; 85: 53-9.*
16. Villuendas R, Piris MA, Orradre JL, Mollejo M, Algara P, Sanchez L, Martinez JC, Martinez P. *p53 protein expression in lymphomas and reactive lymphoid tissues. J Pathol 1992; 166: 235-41.*
17. Pezzella F, Morrison H, Jones M, Gatter KC, Lane D, Harris AL, Mason DY. *Immunohistochemical detection of p53 and bcl-2 protein in non-Hodgkin's lymphoma. Histopathology 1993; 22: 39-44.*
18. Said JW, Barrera R, Shintaku IP, Nakamura H, Koeffler HP. *Immunohistochemical analysis of p53 expression in malignant lymphomas. Am J Pathol 1992; 141: 1343-8.*
19. Villuendas R, Piris MA, Algara P, Sanchez-Beato M, Sanchez-Verde L, Martinez JC, Orradre JL, Garcia P, Lopez C, Martinez P. *The expression of p53 protein is not always dependent on p53 gene mutations. Blood 1993; 82: 3151-6.*
20. The non-Hodgkin's Lymphoma Pathologic Project. *National Cancer Institute sponsored study of classification of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. Cancer 1982; 49: 2112-7.*
21. Harris NL, Jeffe ES, Stein H, Banks PM, Chan JKC, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B, Gatter KC. *A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994; 84: 1361-7.*
22. The International non-Hodgkin's Lymphoma Prognostic Project. *A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med 1993; 329: 987-94.*
23. Nakamura S, Akazawa K, Kinukawa N, Yao T, Tsuneyoshi M. *Inverse correlation between the expression of bcl-2 and p53 proteins in primary gastric lymphoma. Hum Pathol 1996; 27: 225-33.*
24. Caron FC, Soussi T. *TP53 tumor suppressor gene: a model for investigation human mutagenesis. Genes Chrom Cancer 1992; 4: 1-15.*
25. Sakashita A, Hattori T, Miller CW, Suzushima H, Asou N, Takatsuki K, Koeffler HP. *Mutations of the p53 gene in adult T-cell leukemia. Blood 1992; 79: 477-80.*
26. Ichikawa A, Hotta T, Takagi N, Tsushita K, Kinoshita T, Nagai H, Murakami Y, Hayashi K, Saito H. *Mutation of p53 gene and their relation to disease progression in B-cell lymphomas. Blood 1992; 79: 2701-7.*
27. Sanchez E, Chacon I, Plaza MM, Murioz E, Cruz MA, Martinez B, Lopez L, Piris MA. *Clinical outcome in diffuse large B-cell lymphoma is dependent on the relationship between different cell cycle regulator proteins. J Clin Oncol 1998; 16: 1931-9.*
28. Ichikawa A, Kinoshita T, Watanabe T, Kato H, Nagai H, Tsushita K, Saito H, Hotta T. *Mutations of the p53 gene as a prognostic factor in aggressive B cell lymphoma. N Engl J Med 1997; 337: 529-34.*
29. Koduru P, Raju K, Vadmal V, Menezes G, Shah S, Susin M, Kolitz J, Broome JD. *Correlation between mutation in p53, p53 expression, cytogenetics, histologic type, and survival in patients with B cell non-Hodgkin's lymphoma. Blood 1997; 90: 4078-91.*
30. Kramer MHH, Hermans J, Krol PA, Kluin-Nelsmans JC, Haak HL, Groningen K, Kluin PM. *Clinical significance of bcl2 and p53 protein expression in diffuse large B cell lymphoma: a population based study. J Clin Oncol 1996; 14: 2131-8.*
31. Inghirami G, Macri L, Cesarman E, Chadburn A, Zhong J, Knowles DM. *Molecular characterization of CD30+ anaplastic large cell lymphoma: high frequency of c-myc proto-oncogene activation. Blood 1994; 83: 3581-90.*
32. Oka T, Sarker AB, Teramoto N, Yoshino T, Akagi T. *p53 protein expression in non-Hodgkin's lymphomas is infrequently related to p53 gene mutations. Pathol Int 1998; 48: 15-21.*
33. Martinez-Delgado B, Robledo M, Arranz E, Infantes F, Echezarreta G, Marcos B, Sanz C, Benitez J. *Correlation between mutations in p53 gene and protein expression in human lymphomas. Am J Hematol 1997; 55: 1-8.*
34. Navaratnam S, Williams GJ, Rubinger M, Pettigrew NM, Mowat MR, Begleiter A, Johnston JB. *Expression of p53 predicts treatment failure in aggressive non-Hodgkin's lymphomas. Leuk Lymphoma 1998; 29: 139-44.*