**MDM2 gene amplification and expression in non-small-cell lung cancer: immunohistochemical expression of its protein is a favourable prognostic marker in patients without p53 protein accumulation**

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**Summary** MDM2 is an oncoprotein that inhibits p53 tumour-suppressor protein. Amplification of the MDM2 gene and overexpression of its protein have been observed in some human malignancies, and these abnormalities have a role in tumorigenesis through inactivation of p53 function. To determine the clinicopathological and prognostic value of MDM2 abnormalities in non-small-cell lung cancer (NSCLC), MDM2 gene amplification and its protein expression status were analysed in surgically resected materials. MDM2 gene amplification was detected in only 2 (7%) of the 30 tested patients. MDM2 protein was found immunohistochemically in a total of 48 (24%) of the 201 patients. MDM2 protein was slightly frequently observed in patients with adenocarcinoma, but its presence or absence was not associated with clinicopathological factors such as T-factor, N-factor, stage, tumour size, differentiation or p53 protein status. Overall, MDM2-positive patients tended to have a better prognosis (P = 0.062). In particular, among immunohistochemically p53-negative patients (n = 110), those with positive MDM2 protein expression showed significantly better prognosis (P = 0.039) and, in a multivariate analysis, MDM2 protein status was a favourable prognostic factor (P = 0.037). In contrast, among p53-positive patients (n = 91), there was no difference in prognosis depending on MDM2 protein status. Thus, in the NSCLC patients studied, MDM2 gene amplification was a minor event, but expression of its protein, which was often observed immunohistochemically, was a favourable prognostic marker, especially among patients without p53 protein accumulation. Further study is needed to determine how MDM2 protein expression results in a better prognosis.

**Keywords:** MDM2 gene; non-small-cell lung cancer; p53; prognosis; immunohistochemistry; amplification; fluorescence-based polymerase chain reaction single-strand conformation polymorphism

The MDM2 gene was originally identified and cloned by amplification in a transformed tumorigenic Balb/c 3T3 fibroblast cell line (Cahilly-Snyder et al, 1987; Fakharzadeh et al, 1991; Oliner et al, 1992). Its product, p90, is now considered to form a tight complex with both the wild-type and mutant p53 tumour-suppressor gene protein and to inactivate wild-type p53 function by masking the N-terminal acidic transactivating domain of p53 protein, indicating that abnormalities of the MDM2 gene may be closely associated with tumorigenesis and/or tumour development (Olson et al, 1993; Haines et al, 1994). Indeed, MDM2 gene amplification and overexpression of its product have been described in several types of malignancies in humans. However, the clinicopathological role of these abnormalities has yet to be determined.

In lung cancer, p53 abnormalities have been well examined but, to our knowledge, MDM2 abnormalities in this disease have been reported only by Marchetti et al (1995a). However, because of the relatively small number of samples tested in their study, its clinicopathological and prognostic significance is as yet unknown. Hence, because p53 abnormalities in this disease may be essential for biological and clinical characteristics of the tumour, we examined MDM2 abnormality status, especially in those patients without p53 abnormalities. MDM2 gene amplification and its protein expression were examined in a series of surgically resected non-small-cell lung cancer (NSCLC) cases in association with clinicopathological parameters, p53 protein accumulation and prognosis.

**MATERIALS AND METHODS**

**Clinical materials**

For immunohistochemical analysis of MDM2 protein expression and p53 accumulation, formalin-fixed paraffin-embedded tissue blocks of primary tumours removed surgically between July 1989 and June 1995 at the Department of Thoracic Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases (formerly the Center for Adult Diseases, Osaka), were obtained from 201 NSCLC patients. For amplification analysis of the MDM2 gene, 30 fresh tissue samples were immediately frozen at the time of the operation and stored at −80°C until DNA extraction.

Of the 201 patients, 146 were men and 48 were women; they were aged between 35 and 83 years (mean 63.4 years). The histological type was adenocarcinoma in 116 patients, squamous cell carcinoma in 71, large-cell carcinoma in 12 and adenosquamous cell carcinoma in two. The pathological staging was according to...
the international TNM staging system (Mountain, 1986): stage I in
115, stage II in 23, stage IIIA in 57 and stage IIIB in six. All
disease patients underwent potentially curative operations. The median
post-operative follow-up of the patients was 839 days (range
32–2469 days). Post-operative survival curves were constructed
using the Kaplan–Meier method.

**MDM2 amplification and p53 mutation analysis**

Southern blot analysis was performed to detect amplification of the
*MDM2* gene, as described previously (Takami et al, 1994). The
human MDM2 cDNA clone (c14-2; nt 1–949), which was kindly
provided by Drs B Vogelstein and KW Kinzler, was used as a
hybridization probe. pYNH132 on chromosome 6p, pLYNZ9.1 on
chromosome 2p and pMCA1-1 on chromosome 15q were used as
internal diploid standards (kindly provided by Dr Y Nakamura).
Briefly, 5 μg of high molecular weight DNA derived from tumour
tissues was digested with EcoR I and then electrophoresed on
0.8% agarose gel followed by transfer to a nylon filter. The DNAs
on the filters were sequentially hybridized with the *MDM2* gene
probe, c14-2, and three control probes, pYNH132, pLYNZ9.1 and
pMCA1-1. The intensity of the hybridization signals was measured
by densitometry. The relative signal intensities of the
*MDM2* gene were calculated by comparing the ratio of MDM2 to
three control probes and, when the samples showed a more than
two fold increase in signal intensity of *MDM2*, they were
hybridized with two probes, pYNH15 on chromosome 12q and
pHu2M9 on chromosome 12p, to determine whether or not the
increase was due to non-specific polysomies of the chromosome.

Mutations of the *p53* gene were examined using fluorescence-
based polymerase chain reaction single-strand conformation polymor-
phism (PCR-FSSCP), as described previously by Katsuragi et
al (1995). This technique was used for the detection of point mutations
in the *p53* gene in exons 5, 6, 7 and 8 by an automated DNA
sequencer and software.

**Immunohistochemical analysis**

Sections were cut at 4 μm, dewaxed and rehydrated through a
graded ethanol series. Before staining, sections were pretreated
with microwave irradiation for antigen retrieval, as described previ-
ously (Cattoretti et al, 1992; Marchetti et al, 1995b; McCann et al,
1995; Ofner et al, 1995). Incubation with the primary antibodies
(monoclonal MDM2 antibody; IF-2, Oncogene Science, USA, and
monoclonal p53 antibody, DO-7, Novocastra Laboratories, UK),
Table 1 MDM2 and p53 abnormalities in NSCLC

| No. | Histology stage | MDM2 Amplification | Protein | Mutation | p53 Protein |
|-----|-----------------|---------------------|---------|----------|-------------|
| 1   | Ad IIIB         | –                   | –       | –        | +           |
| 2   | Ad IIIB         | –                   | NT      | ++       | ++          |
| 3   | Ad IIIA         | +(5.5-fold)         | ++      | –        | –           |
| 4   | Ad IIIA         | –                   | NT      | –        | –           |
| 5   | Ad IIIA         | –                   | ++      | +(Exon6) | ++          |
| 6   | Ad IIIA         | –                   | NT      | –        | –           |
| 7   | Ad IIIA         | –                   | NT      | –        | –           |
| 8   | Ad IIIA         | –                   | NT      | –        | –           |
| 9   | Ad II           | –                   | –       | –        | +           |
| 10  | Ad II           | –                   | NT      | ++       | ++          |
| 11  | Ad II           | –                   | NT      | ++       | ++          |
| 12  | Ad I            | –                   | –       | –        | –           |
| 13  | Ad I            | –                   | –       | –        | +           |
| 14  | Ad I            | –                   | +       | –        | –           |
| 15  | Ad I            | –                   | +       | –        | –           |
| 16  | Ad I            | –                   | –       | –        | –           |
| 17  | Ad I            | –                   | –       | –        | +           |
| 18  | Ad I            | –                   | –       | –        | NT          |
| 19  | Sq IIIA         | –                   | +       | ++       | ++          |
| 20  | Sq IIIA         | –                   | +(Exon8) | ++       | ++          |
| 21  | Sq IIIA         | –                   | NT      | +        | ++          |
| 22  | Sq IIIA         | –                   | NT      | –        | –           |
| 23  | Sq II           | –                   | +       | +(Exon5) | ++          |
| 24  | Sq I            | +(2.7-fold)         | +       | –        | ++          |
| 25  | Sq I            | –                   | +       | +(Exon7) | +           |
| 26  | Sq I            | –                   | +       | NT      | +           |
| 27  | Sq I            | –                   | +       | NT      | –           |
| 28  | Sq I            | –                   | –       | NT      | +           |
| 29  | La IIIA         | –                   | +       | NT      | ++          |
| 30  | La I            | –                   | –       | NT      | –           |

Positive patients/tested patients: 2/30 (7%) 12/30 (40%) 4/15 (27%) 18/30 (60%)

Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large-cell carcinoma; NT, not tested.

the enzyme colour reaction, haematoxylin counterstaining and mounting were carried out as described elsewhere (Foulkes et al., 1995; Marchetti et al., 1995a and b; McCann et al., 1995; Ofner et al., 1995; Matsumura et al., 1996).

Immunostaining results were assessed, taking into account the cancer cells whose nuclei showed positive immunoreactivity for MDM2 (Figure 1) or p53 protein. The percentage of immunoreactive nuclei was evaluated by scanning the whole section at medium and high magnification and by counting at least 500 cells in the most densely stained tumour areas. The patients were classified into three groups: a strongly positive group (+ +), with more than 50% positive cancer cells in the tissue; a weakly positive group (+), with 10–50% positive cancer cells; and a negative group (−) with less than 10% or no positive cancer cells (McCann et al., 1995).

Statistical analysis

The chi-square test was applied for statistical analysis. For survival data, statistical significance was analysed using the log-rank test. Variables related to survival were analysed using Cox’s proportional hazards regression model with SAS software (Statistical Analysis Institute, Cary, NC, USA). P < 0.05 was considered to be significant and 0.05 ≤ P < 0.10 was considered to be marginally significant.

Table 2 Relationship between MDM2 oncoprotein and clinicopathological parameters in 201 NSCLC patients undergoing potentially curative operation

| MDM2 protein status | P-value |
|---------------------|---------|
| (n = 153)           | (n = 38) | (n = 10) |
| –                   | 76%     | 19%      | 5%       |

Gender

Male: 117 (74.4%) 5 (13.2%)
Female: 36 (25.6%) 5 (13.2%)

Age (mean year)

63.2 (65.0–61.2) NS

T-factor

T1: 42 (13.2%)
T2: 80 (23.7%)
T3.4: 31 (6.1%)

N-factor

N0: 103 (27.7%)
N1: 24 (6.1%)
N2a: 26 (7.2%)

Stage

I: 85 (27.7%)
II: 19 (5.9%)
IIIA: 44 (12.3%)
IIIB: 5 (1.5%)

Tumour size (mm)

≤ 20: 22 (6.1%)
> 20 to ≤ 40: 82 (24.2%)
> 40 to ≤ 60: 52 (17.4%)
> 60: 17 (5.9%)

Histology

Ad: 84 (27.7%)
Sq: 58 (19.9%)
La: 10 (3.3%)
As: 1 (0.3%)

p53 protein status

p53 −: 64 (21.3%)
p53 +: 31 (11.2%)
p53 ++: 38 (6.1%)

*Ad, adenocarcinoma; non-Ad, non-adenocarcinoma, including Sq (squamous cell carcinoma), La (large-cell carcinoma) and As (adenosquamous carcinoma).

RESULTS

MDM2 amplification and p53 mutation

Of the 30 patients tested, only two (7%) showed MDM2 gene amplification (Figure 2 and Table 1): one had stage IIIA adenocarcinoma and another stage I squamous cell carcinoma. Amplification grade was 5.5-fold in the former and 2.7-fold in the latter. Both showed MDM2 protein expression by immunohistochemical analysis (strongly positive in the former and weakly positive in the latter, percentage of positive cells being 90% and 48% respectively). In contrast, ten patients showed MDM2 protein expression with no evidence of its gene amplification.

In the present series, 4 (27%) of the 15 patients showed p53 mutations (exon 5, 6, 7 or 8 in each, Table 1) using the PCR-FSSCP
method. Although the two patients with MDM2 gene amplification and MDM2 protein expression also showed p53 protein accumulation, no p53 mutation was observed.

### Association between MDM2 protein expression and clinicopathological parameters

Of the 201 patients tested, a total of 48 (24%) showed positive immunostaining for MDM2 protein within the tumour tissue: ten (5%) were in the strongly positive group and 38 (19%) were in the weakly positive group. The median percentage of MDM2-positive cells was 75% (mean ± s.d. 78 ± 17%, range 55–99%) in the former and 30% (mean ± s.d. 28 ± 7%, range 11–48%) in the latter.

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**Table 3** P-value from univariate analysis using the log-rank test for 201 NSCLC patients undergoing potentially curative operation

| Variables          | P-value |
|--------------------|---------|
| Gender             | 0.260   |
| Male vs Female     |         |
| Age (year)         | 0.191   |
| <50 vs 61≤         |         |
| T-factor           | 0.048   |
| T1 vs T2 vs T3,4   |         |
| N-factor           | <0.0001 |
| N0 vs N1 vs N2,3   |         |
| Stage              | <0.0001 |
| I vs II vs III     |         |
| Tumour size (mm)   | 0.079   |
| ≤30 vs 31 ≤        |         |
| Histology          | 0.752   |
| Ad vs non-Ad*      |         |
| Differentation     | 0.002   |
| Well vs moderate vs poor |     |
| p53 protein status |         |
| − v5 + vs ++       | 0.449   |
| − v5 +, ++         | 0.746   |
| MDM2 protein status|         |
| − v5 + vs ++       | 0.163   |
| − v5 +, ++         | 0.062   |

*Ad, adenocarcinoma; non-Ad, non-adenocarcinoma, including Sq (squamous cell carcinoma), La (large-cell carcinoma) and As (adenosquamous cell carcinoma).

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**Table 4** Multivariate analysis of Cox's proportional hazards model in 110 p53-negative patients

| Variables          | Coefficient | s.e. | χ²   | P-value |
|--------------------|-------------|------|------|---------|
| T-factor           | 0.228       | 0.219| 1.085| 0.299   |
| T1.2 vs T3.4       |             |      |      |         |
| N-factor           | 0.955       | 0.204| 21.990| <0.0001 |
| No vs N1,2,3       |             |      |      |         |
| Differentation     | 1.717       | 0.264| 0.423| 0.517   |
| Well, moderate vs poor MDM2 protein | − v5 +, ++ | 0.568| 0.269| 4.477   | 0.037 |

In comparison with the non-adenocarcinoma type, including squamous cell carcinoma, large-cell carcinoma and adenosquamous cell carcinoma, adenocarcinoma type marginally frequently showed MDM2 protein expression (P = 0.09). However, MDM2 protein status was not associated with the representative clinicopathological parameters, such as T-classification (T-factor), nodal involvement (N-factor), stage, tumour size or differentiation (Table 2).

p53 protein accumulation was seen in a total of 91 patients (45%): 47 (23%) patients showed strongly positive immunoreactivity for p53 protein (median percentage of positive cells 89%, mean ± s.d. 88 ± 5%, range 55–100%) and 44 (22%) showed weak positivity (median percentage of positive cells 28%, mean ± s.d. 33 ± 8%, range 13–49%). There was no association between MDM2 protein expression and p53 protein accumulation status in the present series (Table 2). The immunohistochemical distribution of positive cells in the tumour tissue of both MDM2- and p53-positive patients was diverse: some patients showed an almost similar pattern of distribution, while others showed mosaic pattern of MDM2 protein expression and p53 protein accumulation in the tissue.

### Post-operative prognosis

Decending on MDM2 protein expression status, post-operative overall survival was analysed for 201 patients (Figure 3). Overall, there was no statistically significant difference in prognosis among the three groups (P = 0.163), but MDM2-positive patients, including those strongly and weakly positive, showed slightly better prognosis than MDM2-negative patients (P = 0.062). In addition to MDM2 protein status, T-factor, N-factor, stage, tumour size and differentiation were also significantly or marginally significantly associated with prognosis (Table 3). p53 protein status showed no influence on prognosis in the present series.

Considering a biophysiological function of MDM2 and p53 proteins (Olson et al, 1993; Haines et al, 1994), survival was separately analysed according to p53 protein status. In the p53-negative group (110 patients), MDM2-positive patients, including those strongly and weakly positive, showed a significantly more favourable prognosis than MDM2-negative patients (Figure 4A, P = 0.039). In particular, even among the p53-negative group with stage I disease (64 patients), a similar result was obtained (Figure 4B, P = 0.049). In a multivariate analysis of the p53-negative group (Table 4), the P-value of MDM2 protein expression was significant for survival (P = 0.037), in addition to N-factor. However, among the p53-positive group (91 patients), there was no difference in the post-operative survival curve with the MDM2 protein status (Figure 4C, P = 0.556).

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**DISCUSSION**

MDM2 gene amplification has been described in several types of human sarcomas (Leach et al, 1993; Florenes et al, 1994; Bueso-Ramos et al, 1995; Nakayama et al, 1995), including 15–36% of soft tissue sarcomas and 10–15% of osteosarcomas. Similarly, this amplification has been detected in 8–10% of brain tumours (Reifenberger et al, 1993). MDM2 gene amplification has also been reported in human cancers, such as breast cancer, in which its incidence is 4–13% of the primary tumours (Marchetti et al, 1995b; McCann et al, 1995), and oesophageal cancer, in which the incidence is as high as 18% (Shibagaki et al, 1995); but overall in other cancers, including urinary bladder tumours (Lianes et al, 1994), cervical cancers (Kessis et al, 1993; Ikenberg et al, 1995), head and neck tumours (Waber et al, 1993), some paediatric tumours (Waber et al, 1993) and urothelial cancers (Habuchi et al, 1994), this event is now considered to be rather infrequent. In the NSCLC cases studied here, its incidence was only 7% (2 of 30 patients tested), compatible with that (6%) reported by Marchetti et al (1995a), and, in addition, its amplification grade was relatively low (5.5-fold and 2.7-fold) in comparison with the other tumours, indicating that amplification of this oncogene in this disease may also be a relatively infrequent event through tumorigenesis and tumour development. Interestingly, Marchetti et al (1995a) emphasized that MDM2 gene amplification was observed only in patients with adenocarcinoma but, in the present study, one patient with squamous cell carcinoma showed MDM2 gene amplification.

In immunohistochemical studies using monoclonal anti-MDM2 protein antibody, the incidence of MDM2 immunohistochemical (over)expression in human cancers has been reported to be 30% in bladder cancers (Lianes et al, 1994), 20% in colorectal cancers (Ofner et al, 1995), 3% in ovarian cancers (Foulkes et al, 1995), 40% in oral carcinoma (Matsumura et al, 1996) and 22–41% in breast cancers (Marchetti et al, 1995b; McCann et al, 1995). In NSCLC, whereas Marchetti et al (1995a) reported that MDM2 oncogene product expression was detected immunohistochemically only in three (6%) patients with gene amplification, it was detected in the present study in 24% of patients, independently of MDM2 gene amplification status. This difference in incidence reported between Marchetti et al (1995a) and ourselves is probably caused by the use of frozen sections in the former study, whereas paraffin sections, possibly larger in size, were used in the present study. In addition, the criteria for the positivity of immunostaining may have been another related factor. Thus, when considering together our findings on NSCLC, we believe that its expression (in contrast to its gene amplification) is not a rare, but a more common, event in human cancer tissues.

Although Marchetti et al (1995a) reported that MDM2 protein was not detected in patients with squamous cell carcinoma, 13 patients in this study, including 12 with weak and one with strong expression, expressed its protein. Recently, Matsumura et al (1996) reported that MDM2 protein was observed immunohistochemically in 40% of oral squamous cell carcinomas. Therefore, considering these results together, MDM2 gene product expression does occur in adenocarcinoma type in NSCLC, slightly frequently.

The relationship between MDM2 gene amplification and increased expression of the product appears to be complicated and is not completely understood. In fact, in several unique cases, the gene was amplified in the absence of increased expression (McCann et al, 1995). Although we did not examine the MDM2 gene mRNA levels in NSCLC, one case exhibiting gene amplification (case no. 3) showed strong expression of its product (percentage of positive cells 90%) and another (case no. 24) showed weak expression (percentage of positive cells 48%). Marchetti et al (1995a) also reported that all of the patients in their study with MDM2 gene amplification did not show strong expression of its protein.

In the present study, there was no significant association between MDM2 expression and tumour-staged parameters (Table 2) and, even in regard to MDM2 gene amplification, one case was in stage I and another in stage IIIA. There was also no such association in the three patients with positive amplification and overexpression described by Marchetti et al (1995a). In addition, MDM2 protein status did not appear to be associated with p53 protein accumulation status (Table 2), and there was no definite distribution of positive cells in the tumour tissue of both MDM2- and p53-positive patients. Thus, the combination of MDM2 and p53
abnormalities in NSCLC may be not so simple. However, only in the two patients with MDM2 amplification, no p53 gene mutation was detected in spite of strongly positive p53 protein accumulation; this observation is compatible with that of Marchetti et al (1995a). Considering that the antibody recognizes both the wild-type and the mutant forms of p53 protein, it is possible that immunohistochemically detected protein in such patients is wild-type p53, which may be stabilized and accumulated by MDM2 protein expression (Keleti et al, 1996).

The prognostic value of MDM2 protein expression is observed only among p53-negative patients, but not among p53-positive patients. The observation that MDM2-positive patients showed marginally better prognosis than MDM2-negative patients, on the whole, reflects the findings among p53-negative patients. Thus, it is concluded that MDM2 protein status is a useful prognostic marker only in such patients. Considering a biophysiological function of MDM2 and p53 proteins (Olson et al, 1993; Haines et al, 1994), mutant-type p53 itself may have lost its p53 function, leading to the speculation that MDM2 abnormalities have little or no effect on the p53-mediated pathway. The findings observed among p53-positive patients in this study support this hypothesis.

In contrast, we had hypothesized that MDM2 abnormalities are an alternative mechanism, escaping from p53-regulated growth control in wild-type p53 tumours in the same fashion as in mutant-type p53 tumours, but the present findings obtained in NSCLCs appear to be rather paradoxical.

The reason for the present clinical outcome among the p53-negative patients is unknown. In this respect, it was recently reported that the MDM2 gene encodes a number of alternatively spliced mRNAs that give rise to proteins ranging in size from 40 kDa to 90 kDa. Several investigators have described not only a p90 protein, the original form, but also the representative forms, p57-58, p74, p76 and p85 proteins as the MDM2 gene products in various types of tumours (Olson et al, 1993; Haines et al, 1994; Landers et al, 1994; Bueso-Ramos et al, 1995; Gudas et al, 1995). In particular, it is noteworthy that the variant forms p74 and p76, lacking the N-terminal protein domain of p90 protein, do not inhibit p53 protein (Olson et al, 1993; Haines et al, 1994). The antibody IF2, used in the present study, enables detection of such forms as p90, p74, p76 and p57-58 (Haines et al, 1994; Gudas et al, 1995). Therefore, some immunohistochemically detected MDM2-positive patients may have been included as showing a different function of variant MDM2 protein, e.g. p74 or p75, from the original MDM2 protein, p90. Secondly, p53 abnormalities examined by immunohistochemistry are not always consistent with those examined by analysis of its gene. In fact, several patients even with strongly positive p53 accumulation showed no p53 mutation by the PCR-FS2SSP method (Table 1). Conversely, it is possible that some p53-negative patients may have been included as those with some p53 abnormalities at the gene level. The present prognostic analysis was firmly based on immunohistochemistry for tumour phenotype and, therefore, further study is needed to elucidate the mechanism underlying the apparently contradictory effects of MDM2 protein on prognosis.

In other tumours, the prognostic value of MDM2 abnormalities remains controversial. In breast cancers, MDM2 overexpression is strongly associated with oestrogen receptor expression, suggesting that MDM2 expression status may also be a favourable prognostic factor (Sheik et al, 1993; Takami et al, 1994; Marchetti et al, 1995a; McCann et al, 1995; Gudas et al, 1995). In bladder cancer, Lianes et al (1994) reported that MDM2 overexpression was observed in patients with relatively early-staged and low-grade tumours, suggesting that MDM2 overexpression may be an early event or possibly a favourable factor associated with low-grade malignancy, although there have been no reports clearly describing its association with prognosis. On the other hand, in oesophageal cancer, MDM2 gene amplification has been described as being a rather unfavourable prognostic factor (Schibagaki et al, 1995). MDM2 overexpression in leukaemias also appears to be associated with unfavourable chromosomal abnormalities (Bueso-Ramos et al, 1993). Thus, the influence of MDM2 gene abnormalities on tumour malignancy may appear to be different when studied with tumour tissues. Further study is needed considering p53 abnormalities in these tumours to determine the prognostic value of MDM2 abnormalities.

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