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p53, p21 and metallothionein immunoreactivities in patients with malignant pleural mesothelioma: correlations with the epidemiological features and prognosis of mesotheliomas with environmental asbestos exposure

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The aim of this study is to investigate immunoreactivity for p53, p21 and metallothionein in diffuse malignant pleural mesothelioma (DMPM) and to determine the relationships between the age, sex, asbestos exposure time, survival of DMPM patients with environmental asbestos exposure and immunoreactivity to p53, p21 and metallothionein.

Sixty-seven histopathologically-confirmed DMPMs, 38 of whom had environmental and 29 had occupational asbestos exposure, were included. The tumour tissue samples were immunostained with antibodies against p53, p21 and metallothionein. Epidemiological data and the survival times for the DMPM patients with environmental asbestos exposures were obtained from hospital records. Thirty-three per cent of the DMPMs were positive for p53, 35% for p21 and 52% for metallothionein. There was no statistical difference between the histological subtypes of DMPM in terms of immunoreactivity for p53, p21 and metallothionein. For p21 and metallothionein there was a statistically significant difference between the exposure characteristics: patients with environmental asbestos exposure had shown more immunopositivity. There were statistically significant differences between age groups and between asbestos exposure times for metallothionein, and between asbestos exposure times and p21. The patients with positive immunostaining had longer exposure times and were older than those having negative immunostaining. The differences between survival of the patients were not statistically significant in terms of the immunohistochemical results for p53, p21 and metallothionein.

Key words: mesothelioma; p53; p21; metallothionein, survival.

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Introduction

Malignant mesothelioma is a rare tumour which can affect the pleura, peritoneum and pericardium (1,2). The majority of cases are linked to asbestos exposure, particularly the amphibole forms, and in Turkey's erionite (3–5). There is a long latent period (several decades) from the initial time of asbestos exposure to the onset of disease. After phagocytosis of inhaled fibers by macrophages, reactive oxygen species, lysosomal enzymes, arachidonic acids, cytokines and growth factors are released. It is thought that all of these enzymes and metobilities have roles in the pathogenesis of malignant mesothelioma (4–6) and that multiple genetic alterations take place during the process of tumour development (7). In recent years, some chromosomal abnormalities have been shown (7–9). These chromosomal alterations may activate oncogenes, cause mutations or suppress tumour-suppressor genes, such as p53, p16, Rb, NF2 and p21 (7). However, to date, the exact pathogenetic mechanism has not been clearly defined and no specific

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mutations in these oncogenes nor any specific variations in tumour suppressor genes have been described in malignant mesothelioma.

Asbestos fibres have different physical and chemical properties: the response of host to inhaled asbestos fibres is widely affected by various parameters including the physical, chemical, or biological properties of fibres, cumulative fibre dose, latency, and individual factors of the host. Environmental asbestos exposure has some important differences from occupational settings in terms, for example, of fibre type, exposure dose level, and latency (10).

In this study, our aim was to investigate immunoreactivity for p53, p21 and methallothionein, a low molecular protein released in response to any kind of biological and physicochemical stress, in diffuse malignant pleural mesothelioma (DMPM) and to determine the relations between the age, sex, asbestos exposure time and survival of DMPM patients with environmental asbestos exposure, and immunoreactivity to p53, p21 and metallothionein.

Materials and methods

PATIENTS

Paraffin-embedded samples from the 67 DMPMs were obtained from the Department of Pathology, Medical Faculty of Osmangazi University, Eskisehir, Turkey and the Department of Histopathology, Llandough Hospital, Cardiff, U.K. Thirty-eight of the 67 patients had been diagnosed and followed-up in the Department of Chest Diseases, Medical Faculty of Osmangazi University. All of those 38 patients had environmental asbestos exposure from birth and the epidemiological data of only these 38 malignant mesothelioma cases was obtained from the hospital records of the patients. The other 29 patients had been diagnosed in the Department of Histopathology, Llandough Hospital and had occupational asbestos exposure.

HISTOPATHOLOGICAL DIAGNOSIS

All patients had histologically-confirmed DMPM. Histopathologic examination of biopsy samples obtained by thoracoscopy, thoractomy or computer tomography-guided pleural needle biopsy in all patients was initially performed in the Department of Pathology, Medical Faculty of Osmangazi University and later by Dr A.R. Gibbs. The samples were histochemically stained with haematoxylin–eosin, alcian blue and mucicarmine. Immunohistologic procedures for carcinoembryonic antigen (CEA) and Leu-M1 were performed.

IMMUNOHISTOCHEMISTRY

Sixty-six DMPM patients were stained with p53 and metallothionein antibodies and 65 patients with p21. Histological sections (4 μm) were obtained from paraffin-embedded blocks. Histological sections were dewaxed in xylene and hydrated in absolute and 70% alcohol and then treated with 3% H_2O_2 solution in phosphate buffer for 5 min, in order to block endogeneous peroxidase activity. Retrieval procedures were carried out using solution in a microwave, after which the slides were treated with normal rabbit serum for 20 min and with primary antibody for 1 h, respectively. Primary antibodies were p53 (diluted as 1/1000) (Clone DO-7, DAKO), p21 (diluted as 1/100) [WAF(Ab-1), Clone EA10] and monoclonal mouse anti-metallothionein antibody (Clone E9, DAKO). After application of the primary antibody, the slides were treated with biotinylated rabbit antimouse immunglobulins for 30 min and with streptavidin HRP for 30 min, respectively. The slides were then washed with phosphate buffer. Peroxidase activity was demonstrated with DAB peroxidase substrate. The slides were then treated with Mayers haematoxylin. For the last step the slides were dehydrated with absolute alcohol and xylene.

STATISTICAL ANALYSIS

Data were evaluated by an SPSS computer programme and *t*-tests and chi square tests were used for comparison of the parameters. Duration of survival and median and mean event times were estimated according to the Kaplan–Meier method, with 95% confidence intervals (CI). Differences in time distributions between the groups were tested for statistical significance using the log-rank test. *P*-values less than 0·05 were considered as statistically significant. The duration of survival was calculated from the time of diagnosis.

Results

Sixty-seven DMPM patients were included in the study. Thirty-eight of the DMPM patients were from Eskisehir, Turkey. All of those had been environmentally exposed to asbestos from birth in rural areas of the Eskisehir province, due to the use of white-soil in the whitewashing of their houses and for insulation on the roofs (10). Mineral analysis of these white-soil samples identified contamination with tremolite, tremolite–chrysotile, or, at a lower rate, various combinations of actinolite, anthophyllite or chrysotile (10). The other 29 patients were from Cardiff and had occupational exposure to commercial amphiboles.

Of the 67 DMPMs, 25 were epithelial cell type, 20 mixed, 20 sarcomatoid and two transitional. None of the DMPM showed positive immunostaining for CEA or Leu M_1 and none showed positive staining with mucicarmine.

Immonohistochemical results for p53, p21 and metallothionein of these patients are shown in Table 1. Occasional results are missing because these were small biopsies and occasionally the tissue sections did not contain enough tumour for adequate assessment.

There was no statistical difference between the histological subtypes of DMPM in terms of immunoreactivity for p21, p53 and metallothionein ($P > 0{\cdot}05$). There was no statistical difference between the exposure characteristics,

TABLE 1. The results of immunoreactivity for p53, p21 and metallothionein according to the cell types and exposure characteristics in patients with malignant mesothelioma

Cases	p53$^+$	p21$^+$	Metallothionein$^+$
Malignant mesothelioma	22/66 (33%)	23/65 (35%)	34/66 (52%)
Epithelial	5 (20%)	12 (48%)	13 (52%)
Sarcomatoid	10 (50%)	5 (25%)	11 (55%)
Mixed	7 (35%)	6 (30%)	10 (50%)
Occupational exposure	9/28 (32%)	4/28 (14%)	10/28 (36%)
Environmental exposure	13/38 (34%)	19/37 (51%)	24/38 (63%)

occupational or environmental, of DMPM patients, in term of immunoreactivity for p53 ($P>0{\cdot}05$). However, for p21 and metallothionein, there were statistically significant differences ($P<0{\cdot}05$). The patients who had environmental asbestos exposure presented more immunopositivity for p21 and metallothionein.

Of the 38 DMPMs who had environmental exposure to asbestos and had data available for epidemiologic analysis, half were male and half were female. The mean and median ages of the 38 patients were $56{\cdot}6\pm2{\cdot}1$ years and 56·5 years, respectively. The mean and median asbestos exposure times of these patients were $34{\cdot}9\pm3{\cdot}4$ years and 31·0 years, respectively.

Table 2 shows the epidemiological data of 38 DMPM patients in relation to the immunohistochemical results for p53, p21 and metallothionein.

There were no statistically significant differences between the age, sex and asbestos exposure times in terms of the immunohistochemical results for p53 ($P>0{\cdot}05$). For p21, there were no statistically significant differences between the age and sex ($P>0{\cdot}05$), but we detected a significant difference between asbestos exposure times ($P<0{\cdot}05$): immunopositive cases had longer exposure times. There was no significant difference between the sexes in terms of immunohistochemicals result for metallothionein ($P>0{\cdot}05$). There were statistically significant differences between age groups ($P<0{\cdot}05$), immunopositive cases were older and between asbestos exposure times ($P<0{\cdot}001$), immunopositive cases had longer exposure times.

Of the 38 DMPM patients with environmental asbestos exposure, the survival times were available in 36 patients for p53 and metallothionein, and in 35 patients for p21. The median survival time was 9 month, and mean was 13·7 months for 36 patients. The mean and median survival times according to the immunoreactivity to p53, p21 and metallothionein are shown in Table 3.

TABLE 2. The epidemiological data of 38 DMPM patients with environmental asbestos exposure in relation to the immunohistochemical results for p53, p21 and metallothionein

	p53		p21		Metallothionein	
Characteristics	+	−	+	−	+	−
n	13	25	19	18	24	14
Male	8 (42%)	11 (58%)	12 (63%)	7 (37%)	10 (53%)	9 (47%)
Female	5 (26%)	14 (74%)	7 (39%)	11 (61%)	14 (74%)	5 (26%)
Mean age (years)	54·1-±4·0	57·9-±2·6	56·6-±2·9	56·7-±3·6	59·9-±2·2	50·2-±4·2
Mean asbestos exposure time (years)	34·7±4·8	34·9±4·6	39·4±4·4	31·4±5·7	41·2-±4·0	22·7±5·0

TABLE 3. The mean and median survival times of DMPM patients with environmental asbestos exposure according to the immunoreactivity to p53, p21 and metallothionein

Cases	Mean*	Median* (CI)	Log-rank
p53$^+$	9·6	8·0 (5·01–10·99)	1·15; P=0·28
p53$^-$	15·8	12·0 (1·72–22·28)	
p21$^+$	11·5	8·0 (3·66–12·34)	0·91; P=0·34
p21$^-$	15·6	12·0 (1·61–22·39)	
Metallothionein$^+$	12·6	8·0 (2·41–13·59)	0·50; P=0·48
Metallothionein$^-$	15·2	9·0 (0·00–18·17)	

*Survival time (months).

Although the survival times of the patients who had p53, p21 or metallothionein immunonegativity were longer than those who had positive immunostaining, the differences were not statistically significant.

Discussion

p53 is a nuclear protein of a tumour-suppressor gene localized on the short arm of chromosome 17 (8,11,12) and controls the cell cycle in the G1/S period (13). Normally, when any damage has been detected in DNA, p53 stops the cell cycle at G1 phase (14–16). Since p53 is localized on the short arm of chromosome 17 and deletion of chromosome 17 has been found in malignant mesothelioma (7), mutations or suppression of tumour-suppressor genes are to be expected (8). We found a 33% rate of immunopositivity in DMPM cases, compared to four other studies where the rates were 44, 48, 25 and 70% (11,17–20). In another study where mesothelial cell lines were used, genetic abnormalities were detected in only two cell lines out of four (8). Mayall *et al.* studied the immunohistochemical staining of p53 in cases of reactive mesothelial hyperplasia and malignant mesothelioma tissue and found no immunopositively in reactive mesothelium but a 45% rate of immunopositivity in malignant mesothelioma (11). A positive result appeared to indicate malignancy but a negative result could not exclude a diagnosis of malignant mesothelioma. However, although immunoexpression for p53 is common, p53 mutations in mesothelioma are uncommon (21).

p21, which is coded by ras genes, is functionally related to p53 and has structural similarities to G proteins. G proteins are localized on the inner side of the cell membrance and participate in signal transduction. p21 prevents inactivation of the Rb gene dependent on p53. In this way p21 controls the cell cycle at G1 and G2 phases (6). This procedure can also develop independently of p53 when there is damage to DNA. If point mutations develop on 12th, 13th and 61st codons, a transforming potential of ras genes may occur (6,13). Ramael *et al.* demonstrated immunoreactivity for N-ras in malignant and benign mesothelial tissues (78% and 50%, respectively). They did not find any immunopositivity for K- and H-ras. N-ras is localized on chromosome 1 and this chromosome could have polysomy in malignant mesothelioma (22). In another limited study only two of the 11 malignant mesothelioma cases had immunopositivity for p21 (23). In our study, the rate of the immunopositivity for p21 was 35% in DMPM. The cause of the high immunopositivity in Ramael's study may be related to the sensitivity of antibodies and different immunohistochemical methods used.

Metallothionein is a low molecular protein which has been described in most vertebrates. Metallothionein comprises four types (metallothionein I, II, III and IV). In normal cells it is expressed at basal levels, but its expression can be induced by metals, inflammatory cytokines and harmones (24). Metallothionein has important roles in living cells, such as protecting cells from biological and physicochemical stresses, detoxification of heavy metals, homeoestasis of zinc and copper and protecting cells from the toxic effects of free radicals (25,26).

Metallothionein may be important during the course of malignant diseases, but the nature and extent of its activity is not well understood, due to the limited research camed out so far. In cases of metallothionein deficiency spontaneous mutagenesis and sensitivity to the carcinogenic and anti-carcinogenic effects of cadmium as well as to anticancer drugs has been shown to be enhanced (24). Induced metallothionein synthesis has been shown to increase defensive activity against the toxic effects of cisplatin, adriamycin, bleomycin, cyclophosphamide, TNF and radiation (25). On the other hand, after an increase in metallothionein expression, development of resistance to anticancer treatment, for instance to adriamycin, which destroys tumour cells by forming free radicals, has been demonstrated (24,25). Analyses have been done recently by immunohistochemical methods on the expression of metallothionein in several malignant tumours. In high-grade malignancies like skin and ductal breast cancers, melanomas, cervical and pancreatic cancers and a cute lymphoblastic leukaemia, its expression is started to be increased (24). In ductal mammarian carcinoma an increase of metallothionein expression indicates poor prognosis (25). Further, it has also been demonstrated that metallothionein expression can increase in low-grade malignancies such as colon, bladder and fibroblastic skin tumours (27,28). Its expression has not yet been sufficiently evaluated for ovary, testis, thyroid and lung tumours (24). Up to the time of this study, we could not find a published study of metallothionein expression in mesotheliomas. In the present study we have found that the rate of immunopositivity for metallothionein was 52% in DMPM.

There were no statistically significant differences in rates of immunopositivity for p53, p21 and metallothionein between the various histological subtypes of DMPM.

The patients with environmental asbestos exposure had a higher rate of immunoreactivity for p21 and metallothionein and we found significant differences for both p21 and metallothionein between the asbestos exposure durations of these patients. There was also a statistical significance between immunopositive and negative cases for metallothionein in terms of age. Immunopositive cases had longer asbestos exposure times and were older than negative cases. As mentioned before, one of the hypotheses about the role of asbestos in pathogenesis of malignant mesothelioma is that asbestos exposure causes release of various cytokines and free radicals which have mutagenic and fibrogenic effects. As exposure time increases for mesothelial cells, meetings with those substances increase such that metallothionein expression may increase in order to protect cells from the toxic effects of those. However, this statement is speculative, because of both limited knowledge of mesothelioma pathogenesis and the limited number of patients in the present study. However, we think that all of these features may be treated to the different fibre types and cumulative exposure dose of environmental exposure, due to white-soil usage in rural areas (10).

In rural areas there may be important differences not only in terms of the nature of the exposure between those with environmental and those with occupational exposure but in individual characteristics. The response of the host to inhaled asbestos fibres is affected by various parameters including the physical, chemical or biological characteristics of fibres, cumulative fibre dose, latency and individual factors of the host.

It was commonly accepted that occupational fibre exposure levels are much higher, on average, than indoor and outdoor environmental exposure levels. We found that air fibre concentrations in villages using asbestos-contaminated white-soil were between 0·004 and 0·28 fibres ml^{-1} (unpublished data). In another study from Turkey, indoor air fibre concentrations in villages using asbestos-contaminated white-soil before and after the floor was swept were 0·14 and 0·94 fibres ml^{-1}, respectively (29). Although the fibre exposure levels of environmental asbestos exposure are lower than occupational settings, the cumulative asbestos exposure levels may be as much as those from occupational settings. Dumortier *et al*. suggest that the occupational exposure duration is limited to about 2000 h $year^{-1}$ and begins with employment, whereas the exposure duration may be nearly 8700 h $year^{-1}$ for a villager who spends most of his time in his village and begins at birth (30). For a 40-year-old man the cumulative asbestos exposure duration will be more than 340 000 h whereas the same duration is nearly 80 000 h in occupational setting for a 40 years working period. We have not been able to explain precisely the relation and differences between immunoreactivities of p21 and metallothionein, and some epidemiological features, but further studies are warranted.

Although the survival times of the patients who had negative staining were longer than those who had positive staining in terms of immunoreactivity for p53, p21 or metallothionein (Table 3). We were unable to detect any significant differences, possibly because of the limited number of cases studied. To the best of our knowledge these relationships have not been previously studied and suggest the need for further investigations.

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References

1. Bignon J. Overview of current issues with respect to mesothelioma. *Eur Respir Rev* 1993; **3:** 12–17.
2. Light RW. Tumours of the pleura. In: Murray JF, Nadel JA, eds. *Testbook of Respiratory Medicine*. 2nd edn. Vol 2. Philedelphia: W.B. Saunders, 1994; 2222–2234.
3. Tammilehto L, Tuomi T, Tiainen M, *et al*. Malignant mesothelioma: clinical characteristics, asbestos minerology and chromosomal absormalities of 42 patients. *Eur J Cancer* 1992; **28:** 1373–1379.
4. Bielefeldt-Ohmann H, Jarnicki AG, Fitzpatrick DR. Molecular pathobiology and immunology of malignant mesothelioma. *J Pathol* 1996; **178:** 369–378.
5. Craighead JE, Kane AB. The pathogenesis of malignant and nonmalignant serosal lesions in body cavities cosequent to asbestos exposure. In: Jauren MC, Bignon J, eds. *Mesothelial Cell and Mesothelioma*. New York: Marcel Dekker, 1994; 79–101.
6. Kern JA, McLennan G. Genetic and molecular changes of human lung cancer. In: Fishman AP, ed. *Fisherman's Pulmonary Diseases and Disorders*. 3rd edn. Vol 2. New York: McGraw Hill 1998; 1695–1705.
7. Hunchareck M. Genetic factors in the aetiology of malignant mesothelioma. *Eur J Cancer* 1995; **31:** 1741–1747.
8. Cote RJ, Jhanwar SC, Novick S, *et al*. Genetic alterations of the p53 gene are a feature of malignant mesothelioma. *Cancer Res* 1991; **51:** 5410–5416.
9. Knuutila S, Tammilehto L, Mattson K. Chromosomal abnormalities in human malignant mesothelioma. In: Jauren MC, Bignon J, eds. *Mesothelial Cell and Mesothelioma*. New York: Marcel Dekker, 1994; 245–253.
10. Metintas M, Özdemir N, Hillerdal G, *et al*. Environmental asbestos exposure and malignant pleural mesothelioma. *Respir Med* 1999; **93:** 349–355.
11. Mayall FG, Goddard H, Gibbs AR. p53 immunostaining in the distinction between benign and malignant mesothelial proliferation using formalin-fixed paraffin sections. *J Pathol* 1992; **168:** 377–381.
12. Levine AJ, Momand J, Finlay CA. The p53 tumour supressor gene. *Nature* 1991; **351:** 453–456.
13. Smit EF, Groen HJM, Splinter TAW, *et al*. Prognostic factors in resectable non-small cell lung cancer. *Thorax* 1996; **51:** 638–646.
14. Nuorva K, Mäkitaro R, Huhti E, *et al*. p53 protein accumulation in lung carcinomas of patients exposed to asbestos and tobacco smoke. *Am J Respir Crit Care Med* 1994; **150:** 528–533.
15. McAllen R, Kudzu I, Dunhill M, *et al*. The relationship of p53 immunostaining to survival in carcinoma of the lung. *Br J Cancer* 1992; **66:** 735–738.
16. Harris CC, Hollstein M. Clinical implications of the p53 tumour-supressor gene. *N Eng J Med* 1993; **329:** 1319–1327.
17. Mayall FG, Goddard H, Gibbs AR. The frequency of p53 immunostaining in asbestos-associated mesotheliomas and non-asbestos associated mesotheliomas. *Histopathology* 1993; **22:** 383–386.
18. Cagle PT, Brown RW, Lebovitz RM. P53 immunostaining in the differentiation of reactive processes from malignancy in pleural biopsy specimens. *Hum Pathol* 1993; **25:** 443–448.
19. Ramael M, Lemmens G, Eerdekens C, *et al*. Immunoreactivity for p53 protein in malignant mesothelioma and nonneoplastic mesothelium. *J Pathol* 1992; **168:** 371–375.
20. Kafiri G, Thomas DM, Shepard NA, *et al*. p53 expression in malignant mesothelioma. *Histopathology* 1992; **21:** 331–334.
21. Carbone M, Rizzo P, Grimley PM, *et al*. Simian virus-40 large T antigen binds p53 in human mesotheliomas. *Nat Med* 1997; **3:** 908–912.
22. Ramael M, Deblier I, Eerdekens C, *et al*. Immunohistochemical staining of ras oncogene product in neoplastic and non neoplastic mesothelial tissues: immunoreactivity for N-ras and lack of

immunohistochemical staining for Ha-ras and K-ras. *J. Pathol* 1993; **169:** 421–424.
23. Cristaudo A, Vivaldi A, Sensales G, *et al*. Molecular biology studies on mesothelioma tumour samples: preliminary data on H-ras, p21 and SV40. *J Environ Pathol Tox Oncol* 1995: **14:** 29–34.
24. Jasani B, Schmid KW. Significance of metallothionein overexpression in human tumors. *Histopathology* 1997; **31:** 211–214.
25. Satoh M, Cherian MG, Imura N, *et al*. Modulation of residence to anticancer drugs by inhibition of metallothionein synthesis. *Cancer Res* 1994; **54:** 5255–5257.
26. Osmak M. Multifactorial molecular mechanisms are involved in resistance of preradiated human cervix carcinoma cells to cis-dichlorodiamminoplatinum (II) and vincristine. *Neoplasma* 1993; **40:** 97–101.
27. Satoh M, Kloth DM, Kadhim SA, *et al*. Modulation of both cisplatin nephrotoxicity and drug resistance in murine bladder tumor by controlling metallothionein synthesis. *Cancer Res*. 1993; **53:** 1829–1832.
28. Giuffre G, Barrest G, Sturniolo GC, *et al*. Immunohistochemical expression of metallothionein in normal human colorectal mucosa, in adenocarcinomas and their associated metastates. *Histopathology* 1996; **29:** 347–354.
29. Çöplü L, Dumortier P, Demir AU, *et al*. An epidemiological study in an Anatolian village in Turkey environmentally exposed to tremolite asbestos. *J Environ Pathol Toxic Oncol* 1996; **15:** 177–182.
30. Dumortier P, Çöplü L, De Maertelaer V, Emri S, Bary I, De Vuyst P. Assessment of environmental asbestos exposure in Turkey by bronchoalveolar lavage. *Am J Respir Crit Care Med* 1998; **158:** 1815–1824.