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SHORT REPORT

Ras oncoprotein expression in erionite- and asbestos-induced Turkish malignant pleural mesothelioma patients—a pilot study

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INTRODUCTION

Malignant pleural mesothelioma continues to be a public health problem in Turkey, where, in some villages of rural parts of Anatolia, it is responsible for more than 50% of total deaths (1). Exposure to environmental asbestos or fibrous zeolite (erionite) is the cause of this disease in these villages. The relationship between the exposure to these minerals and the development of malignant mesothelioma has been clearly established by both experimental and epidemiological studies (2). However, the mechanism of mesothelial carcinogenesis in relation to the activation of proto-oncogenes or inactivation of tumour suppressor genes remains unclear. In order to investigate the role of ras expression in malignant pleural mesothelioma (MPM), we examined the expression of p21 ras oncoprotein in 31 environmentally-induced Turkish MPM patients.

MATERIALS AND METHODS

Thirty-one patients with malignant mesothelioma were included in the study. The diagnosis of MPM was based on histological examinations of the tissue samples obtained by percutaneous pleural biopsy, thoracoscopy or thoracotomy. All the patients were clinically followed up. Subjects were divided into two groups according to the mineral type to which the patient was exposed. The environmental exposure to either asbestos or erionite has already been documented in the villages where the patients were living (3). According to this data, the mineral to which the patients were exposed was asbestos for 16 patients and erionite for 15 patient. Ras oncoprotein p21 expression was investigated immunohistochemically in paraffin-embedded tissue sections by using a commercial antibody (ZYMED Laboratories Inc.) and avidin–biotin peroxidase technique. The antibody used in this study detected translational products of the H-, K- and N-ras human genes. The proportion of the positively stained cells was noted and the intensity of staining was scored as mild, moderate and dense. The results in two groups were compared and Fisher's exact test was used for statistical analysis.

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RESULTS

In total five (16%) of the 31 samples showed ras expression: three of 21 epithelial mesothelioma, two of eight mixed type mesothelioma and none of the two fibrous mesothelioma cases. Only focal cytoplasmic staining was observed and the intensity ranged from mild to moderate. Four of 15 erionite (27%) and one of 16 (6%) asbestos induced mesotheliomas were associated with p21 expression; the difference between these groups was not statistically significant ($P=0.17$). The histological type of the tumours with positive p21 expression, the intensity and distribution of immunohistochemical staining and the characteristics of the patients are shown in Table 1.

DISCUSSION

The three ras proteins, H-ras, K-ras and N-ras are 21 kDa oncoproteins. They are tethered to cytoplasmic

Table I. The clinical, histopathological and immunohistochemical features of the erionite- and asbestos-induced mesothelioma cases showing ras expression

Case	Age	Sex	Histological type	Mineral type	Intensity of staining	Proportion of stained cells (%)
1	60	M	Mixed pattern	Erionite	Mild	5
2	55	M	Epithelial	Erionite	Mild	5
3	57	F	Mixed pattern	Erionite	Moderate	25
4	54	M	Epithelial	Erionite	Mild	60
5	60	F	Epithalial	Asbestos	Mild	15

aspect of plasma membrane and flip back and forth between activated signal transmitting form and inactive quiescent state. Several studies indicate that ras plays an important role in the mutagenesis induced by growth factors. Mutation in this oncogene is the single most common abnormality of dominant oncogenes in human tumours and approximately 10–20% of all human tumours contain mutated versions of ras proteins (4). Whether the enhanced p21 ras oncoprotein expression may point to a different mechanism of transformation or may merely reflect differentiation features remains undetermined.

Our results showing focal ras oncoprotein expression in 16% of the mesothelioma cases were consistent with the results of the study by Lee *et al*., which, by using an antibody that reacts with H-, N- and K- ras proteins, RAP-5, found only three of 27 (11%) mesothelioma cases showed focal immunostaining. In that study most of the pulmonary neoplasms but none of the normal tissues showed staining and the frequency and intensity of the expression are distinctly greater in certain tumour types such as squamous, bronchioloalveolar and neuroendocrine neoplasms (5). In contrast to this study, Ramael *et al.* found that N-ras protein was expressed in majority of both neoplastic and non-neoplastic mesothelium, in contrast to H- and K-ras protein, neither of which was found in mesothelium (6).

Our study indicate that ras oncoprotein expression does not seem to be significantly involved (16%) in the pathogenesis of environmentally-induced MPM cases in Turkey. However, more than one quarter of erionite-induced mesothelioma cases showed ras oncoprotein expression, which is a new finding. The contribution of erionite to ras oncogene mutations should be investigated by more specific techniques, such as molecular analysis rather than immunohistochemistry. Determination of ras oncoprotein involved tumors may be important because new chemotherapeutic approaches disabling ras activity by preventing its normal localization are successful in animals and human studies are under investigation (7).

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