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Review

Genetic Factors in the Aetiology of Malignant Mesothelioma

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INTRODUCTION

ALTHOUGH STILL considered a rare tumour, the incidence of malignant mesothelioma is rising [1]. Data from the United States National Cancer Institute's Surveillance End Epidemiological Results (SEER) programme show that, in the U.S.A., the incidence increased from 0.6 cases per 100 000 in 1973 to 1.7/100 000 in 1984 [2]. Similar increases have been recognised in a number of other countries including Australia, Canada, Sweden and the U.K. among others [3, 4]. Much of this increase is due to widespread exposure to asbestos and asbestos-containing materials worldwide.

The aetiologic link between asbestos and malignant mesothelioma is well established [5, 6], with hundreds of studies documenting asbestos cancer risk in a wide variety of occupational and non-occupational settings [7]. In spite of considerable research, the carcinogenic mechanism of asbestos fibres remains obscure. Most of our current konwledge of malignant mesothelioma is derived from epidemiological analyses of asbestos-exposed cohorts. While this has provided important information related to disease occurrence and risk management among defined populations, many questions dealing with the fundamental biology of this tumour remain unanswered.

Malignant mesothelioma is a highly lethal tumour with mean survival times from diagnosis ranging from 2 to 18 months. Clinical management of both the pleural and peritoneal forms of this tumour is suboptimal, with the extensive nature of the tumour at diagnosis often precluding curvative therapy. From the time of initial asbestos exposure, a long latent period is characteristic prior to the onset of disease, often of the order of 3–4 decades. This long latent period suggests that multiple genetic alterations are required for malignant conversion of the mesothelium [8]. Laboratory studies show that asbestos fibres induce cell transformation and chromosomal abnormalities in normal human mesothelial cells [9]. In a number of studies, complex patterns of chromosomal abnormalities have been noted with no specific alteration as yet identified.

The role of other possible genetic mechanisms in the aetiology of this tumour is suggested by work examining oncogene and tumour suppressor gene involvement in human carcinogenesis. An important example of the latter is the *TP53* tumour suppressor gene, now recognised as the most common cancer-related genetic change identified in human tumours. Very little information is available in the current literature regarding the importance of these mechanisms in the development of malignant mesothelioma. Nonetheless, such entities could play a central role in tumorigenesis and disease progression.

Documentation of familial aggregation of this tumour raises additional questions relevant to host susceptibility to cancer. A cancer-prone genotype may act in concert with asbestos exposure as indicated by epidemiological analyses [10]. Unfortunately, host factors and family history have received little attention to date.

This paper will discuss current knowledge of the genetic basis of malignant mesothelioma. An increased understanding of these issues could provide much needed insight into the fundamental biological nature of this tumour, and the development of molecular epidemiological tools with important clinical and public health implications.

FAMILIAL MESOTHELIOMA

The aetiologic link between asbestos exposure and mesothelioma was firmly established in 1960 by Wagner and associates [5]. In this report, the authors described 33 cases of pleural mesothelioma occurring in the Northwestern Cape Province of South Africa. Crocidolite asbestos was implicated as the aetiologic agent among this group, all non-occupationally exposed. Since that time, clustering of malignant mesothelioma within families has been reported in a number of articles. In some instances, cases report no direct occupational asbestos exposure. This phenomenon has led some to suggest that genetic susceptibility may play a role in such disease clustering.

Newhouse and Thompson were the first to report family contact asbestos-associated mesothelioma [11]. In a later report, Anderson and colleagues assessed household asbestos-contact disease risk [12]. At that time, 37 cases of malignant mesothelioma related to domestic asbestos exposure were described in the literature. Of 326 healthy household contacts of amosite asbestos workers examined 2–3 decades after the onset of presumed household contamination, 35% showed X-ray abnormalities characteristic of amosite exposure. At the time of this report, 4 deaths due to mesothelioma had occurred. The investigators concluded that domestic contamination with asbestos from industrial sources commonly occurs. They postulated that a

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small fraction of contacts so exposed could develop asbestos related malignancies.

In 1978, Vianna and Polan [13] published a case–control analysis of occupational histories of 52 females with malignant mesothelioma in order to evaluate the risk of this disease secondary to indirect asbestos exposure. Data on parental cancer history was also obtained for analysis. Having a father or spouse employed in an asbestos-related occupation increased the risk of mesothelioma by a factor of 10. Of particular interest, the frequency of parental cancer, particularly of the gastrointestinal tract, was significantly greater among cases than controls. This finding led authors to hypothesise that a genetic predisposition to mesothelioma may exist.

In the same year, Li and associates [14] described a wife and daughter of an asbestos insulator both developing mesothelioma at ages 50 and 34 years, respectively. No other source of asbestos exposure was noted other than from the husband's (father) work clothes which were laundered at home.

A very unusual description of familial clustering of this tumour is that of Risberg and colleagues [15]. Five cases were reported, i.e. three sons, one daughter and their father. Asbestos exposure may have occurred in 4 of the 5 cases via work in the building trades. With this striking aggregation of mesothelioma, the possible role of genetic predisposition was suggested.

More recently, Hammar and associates reported familial mesothelioma in two families [16]. In the first, three brothers developed pleural mesothelioma more than 25 years from first reported exposure to asbestos. All three were insulators and were diagnosed at ages 54, 58 and 66 years. In the second family, a father developed peritoneal mesothelioma of the epithelial subtype at age 63. During World War II, he worked in a shipyard which was his source of asbestos exposure. This patient's son developed a similar epithelial peritoneal tumour at age 44, 11 years after the death of his father. The son's only known asbestos exposure was from his father's work clothes.

Finally, two other studies provide additional information on 7 cases of this tumour occurring in two separate families. In the report by Otte and associates [17], a Danish family was involved in home manufacturing of an asbestos containing compound called "Rollfix" used to fix screws in drilled holes. Amosite asbestos was used along with gypsum and sand. The mixture was mixed and packaged by hand in the basement of the family home. No protective equipment or special ventilation system was used. Production occurred between 1944 and 1961. The father died in 1984 at age 74 years, the son in 1985 at age 45 years and the mother at age 79 years in 1987. Two other sons and one daughter were unaffected at the time of the report.

Martensson and colleagues described malignant mesothelioma in two pairs of siblings, i.e. a brother and sister and identical twin brothers [18]. The former pair had no direct occupational asbestos exposure, although they reported domestic exposure during childhood from their father. The father worked at a foundry where asbestos insulation was used. His work clothes, which were brought home daily, were often contaminated with asbestos fibres. Both patients had documented pleural plaques as well as pleural mesothelioma. The latter pair of identical twin brothers were employed in a shipyard for approximately 7 years. Both developed pleural mesothelioma 16 years from first exposure resulting in their deaths within 18 months from diagnosis. The occurrence of this rare tumour in a pair of identical twins is intriguing, particulary given the relativley short duration of asbestos exposure.

An additional intriguing aspect of the pathogenesis of familial malignant mesothelioma is the possible role of immune function impairment. At present, little is known about the involvement of the immune system in mesothelioma, although asbestos is capable of interfering with anticancer immune defences [19].

Alterations of both humoral and cellular immunity have been documented in asymptomatic asbestos-exposed individuals as well as in those with asbestos-related diseases [20, 21]. The most consistently observed have been decreased T-cell numbers and function and increased immunoglobulin production [22]. Autoantibodies and rheumatoid factors have also been reported at an increased frequency [22, 23].

Lew and associates examined the primary host immune response or natural killer (NK) cell activity in 118 healthy control subjects, and compared the data to those obtained from 20 patients with malignant mesothelioma and 375 long term asbestos workers without cancer [22]. This analysis revealed similar aberrations in NK cell activity in both study groups, although some quantitative differences were observed. Absolute numbers of total T- and T-helper cells were normal in the asbestos worker group without neoplasia, while they were reduced significantly in those with mesothelioma. The asbestos workers also had significantly elevated T suppressor (T8+) cell levels, while in patients with mesothelioma these were unchanged, resulting in a marked reduction in T-helper/T-suppressor ratios in mesothelioma patients and in asbestos workers at risk. The authors speculated that "biological phenomenon generally categorised as chronic immunosuppression associated with the presence of asbestos fibres in the exposed workers may have caused the eventual breakdown of the host's surveillance system and the onset of neoplasm" [22].

The HLA system has been found to be associated with a number of diseases characterised by immunological abnormalities, such as ankylosing spondylitis which is linked to the W27 antigen [24]. Merchant and colleagues found the W27 antigen to be three times more common among a group of 56 asbestos workers with definite or suspected asbestosis than among normal controls [24]. This antigen also seemed related to the severity of asbestosis. In addition, those positive for W27 tended to have been exposed for a shorter period of time. These data suggest that the W27 antigen may be a marker for increased susceptibility to the pathogenic effects of asbestos. The possibility that specific host factors may play a role in the pathogenesis of mesothelioma remains unclear.

GENETIC ABNORMALITIES

Ploidy and S-phase fraction

Cancer is fundamentally a genetic disease with the normal genetic functions governing cell growth altered by various mechanisms. The various genetic alterations seen in malignancy include numerical chromosomal as well as structural changes. The latter include translocations, deletions, inversions and insertions. Cytogenetic analyses of human tumours has yielded important information of both clinical and epidemiological interest. For instance, the Philadelphia chromosome was the first genetic abnormality found to be characteristic of a human disease, i.e. chronic myelogenous leukaemia. Since then, a wide variety of leukaemias and solid tumours have been studied cytogenetically. As seen in Table 1, some chromosomal changes are now recognised as diagnostic for a specific tumour, whilst others are frequently noted recurrent changes.

Flow cytometric DNA analysis (FCM) is a rapid and reproducible method of chromosomal characterisation. FCM is particularly useful in that paraffin embedded samples as well as fresh

Table 1. *Tumour-specific chromosomal abnormalities*

Tumour	Chromosomal abnormality
Ewing's sarcoma	t(11;22)(q24;q12)
Synovial sarcoma	t(X;18)(p11;q11)
Clear cell sarcoma	t(12;22)(q13;q12 or 13)
Myxoid liposarcoma	t(12;16)(q13;p11)
Meningioma	−22/22q−
Retinoblastoma	13q−

Adapted from: Sandberg A. *CA Cancer J Clin* 1994, **44**, 136–159.

tissue can be used. Several studies have examined ploidy in relation to survival in lung cancer [25, 26]. Patients with diploid tumours appear to have longer survival times than those with aneuploid tumours. Studies characterising the DNA content of mesotheliomas are relatively few. Many of these studies were performed in an attempt to distinguish mesothelioma from other malignant and benign processes.

Croonen and associates in 1988 studied a series of 106 pleural and peritoneal effusions by immunocytochemistry and flow cytometry to determine their contribution to routine cytological diagnosis [27]. Flow cytometry was performed using a double labelling method, with propidium iodide for DNA staining and keratin for labelling epithelial cells. Thirteen effusions from 6 patients with mesothelioma were presented. Eight of the eleven samples analysed showed DNA indices in the euploid range, i.e. 0.88–1.13. This contrasted with indices of pulmonary adenocarcinomas which were largely aneuploid.

Burner and associates used flow cytometric analyses on 46 cases of mesothelioma [28] using fresh tissue and 19 paraffin embedded specimens. 31 cases of non-mesothelial malignant neoplasms of the lung were also examined [28]. 30 cases of mesothelioma were classified as epithelial, 11 were mixed and 5 were sarcomatous. Overall, 65% of mesotheliomas were diploid in DNA content with intermediate to low proliferative rates. Of the 31 non-mesothelial lung neoplasms studied, 85% were aneuploid. Also, the aneuploid neoplasms had a higher mean proliferative rate (S-phase fraction) of 10.6% than diploid non-mesothelial lung neoplasms, i.e. 6.0%. No statistically significant difference in DNA content or proliferative rates were observed among histological subtypes of mesothelioma.

In a much larger analysis, Pyrhonen and colleagues from Helsinki, examined 70 cases of this tumour [29]. Both DNA content and S-phase fraction were analysed by flow cytometry. Sixty of 70 tumours (86%) were found to be diploid or near diploid. DNA indices ranged from 0.81 to 2.52. 3 cases (4%) were tetraploid. The S-phase fraction could be calculated in all but one of the diploid tumours, but in only 9 of 28 aneuploid samples. Median S-phase fraction was 5.6% (range 1.2–19.9) in diploid and 16.0% (range 1.9–35.0) in aneuploid tumours. This difference was statistically significant, i.e. $P = 0.026$.

The authors also found that ploidy was not of prognostic importance, although S-phase fraction was significantly correlated with survival. Patients with tumours having a low S-phase fraction survived almost twice as long as those with high S-phase fractions, i.e. 14 months versus 7.6 months, respectively, for diploid tumours. For aneuploid tumours, the corresponding values were 17.6 and 8.9 months.

The ploidy and S-phase fraction analyses of malignant mesothelioma described above have been further documented by several other research groups [30, 31]. It would appear that, unlike many other solid tumours, mesotheliomas tend to be diploid or near diploid. In almost all other solid tumours, aneuploidy is predominant with DNA indices above approximately 1.3 [27]. For instance, most lung neoplasms show a predominance of aneuploidy ranging from 80 to roughly 95% [32, 33]. Similar correlations between aneuploidy, rapid proliferative rate and shorter survival times have been reported with primary breast adenocarcinomas, renal carcinoma and sarcoma [34, 35].

As with ploidy patterns, S-phase fraction is of prognostic value in solid tumours [36]. Similarly, evidence exists that this parameter may also be of prognostic significance in malignant mesothelioma. Unfortunately, only limited data exist precluding definitive conclusions.

Ironically, the diploid or near diploid DNA content of mesothelioma does not imply genetic stability of this tumour type. As discussed below, multiple chromosomal aberrations have been described, although interpretation of these data remains problematic.

Cytogenetics

As indicated earlier, mesothelioma is characterised by a long latency between asbestos exposure and tumour development; often of the order of 3–4 decades. This delay may indicate that multiple genetic changes are required for the transformation of mesothelial cells. Although the epidemiological nature of mesothelial tumours is generally well documented, the biological effects of asbestos fibres is poorly understood. It is interesting to note that while asbestos fibres act as a co-carcinogen for human tumours other than mesothelioma, they are considered complete carcinogens for mesothelial cells. As Lechner and associates show "Mesothelial cells actively ingest asbestos in a manner analogous to human bronchial epithelial cells, but the resultant effects are markedly more cytotoxic" [9]. That is, mesothelial cells appear highly reactive to fibrous materials. These investigators explored the effects of amosite and chrysotile asbestos on human pleural mesothelial cells using cell cultures established from non-cancerous adult donors. Phagocytosis of asbestos fibres was rapid. Fibre toxicity was expressed as micrograms of fibre per cubic centimetre of culture dish surface area that decreased the colony forming efficiency by 50% (measured using clonal growth dose response assays). Chrysotile fibres were found to be the most toxic (chrysotile 0.06, amosite 0.10, crocidolite 0.40) [9]. The mesothelial cells were substantially more sensitive to asbestos fibres than previously tested human lung cells.

Senescence of asbestos-exposed mesothelial cells undergoing two exposures to amosite asbestos was compared with that of control cultures. Control cultures reached senescence during the fourth subculture, while amosite exposed cells continued to multiply for more than 19 subsequent subculturings (>50 doublings). Karyotypic analysis revealed that the unexposed cultures retained diploid karyotypes until senescence. Cells exposed to amosite were aneuploid or pseudodiploid by the fifth passage. A variety of chromosomal abnormalities were noted "including dicentric chromosomes in about 50% of the hypoploid and 100% of the hyperdiploid cells" [9]. The authors postulated that asbestos fibres may interfere with mesothelial cell cytoskeleton functions which, in turn, may cause chromosomal instability. Oncogene activation and malignant transformation could be the critical subsequent events.

The next question which arises concerns the frequency and type of chromosomal alterations seen in malignant mesotheli-

oma; both asbestos related and "spontaneous". In a recent report, Tammilehto and associates [37] examined 41 cases of mesothelioma in an attempt to correlate clinical characterisics, lung fibre analyses and chromosomal abnormalities. Lung tissue samples for fibre analysis were obtained as biopsy specimens at thoracotomy or at autopsy. Fibres were counted using both scanning and transmission electron microscopy, and fibre identification was performed using X-ray micro-analysis. Mesothelial cells for karyotypic analysis were obtained from 36 patients using fresh tumour tissue and pleural effusion specimens. High lung fibre content was correlated with partial loss of chromosomes 1, 4, 9 and chromosomal rearrangements involving a break point at 1p11-p22. Crocidolite and amosite were consistently associated with partial or total loss of chromosomes 1, 3 and 4 and chromosomal rearrangements involving del (3p). Table 2 shows the association between specific chromosomal alterations and crocidolite and or amosite exposure. This study is consistent with a previous report by the authors which documented a clear association between high lung fibre burden and partial or total loss of chromosomes 1 and 4 and chromosomal rearrangements involving a breakpoint at 1p11-p22 [38].

A recent study from Australia [39] also noted deletions of 9p sequences in malignant mesothelioma. Five malignant mesothelial cell lines derived from pleural effusions showed cytogenetic abnormalities of 9p. Hagemeijer and associates published another series demonstrating a high frequency of both whole chromosome 9 loss (10/30 abnormal karyotypes) and specific 9p loss (23/24 hypodiploid and hypotetraploid cases) [40]. It is interesting that studies of non-small cell lung cancer also indicate that 9p abnormalities are common (85% of cases) [40]. These studies suggest that a gene important in the development of lung tumours may be present in this area of the genome.

Taguchi and colleagues [41] found that the two most frequent chromosomal abnormalities observed in 23 cases of mesothelioma examined were losses of specific regions of 1p (17 cases) and 9p (16 cases). The shortest regions of overlap (SOR) of these losses were at 1p21-p22 and 9p21-p22, respectively. These investigators also noted frequent losses of 3p21 (3 cases) and 6 q15-q21 (9 cases). Numerical losses of chromosomes 14, 16, 18 and 22 were noted (each in 10–13 tumours). The authors point out that "In many of the tumours examined, most or all of these recurrent changes occurred in combination, suggesting the involvement of a pathogenetic cascade in this cancer" [41].

It should be noted that alterations of 1p have been found in malignant melanoma, malignant lymphoma and adenocarcinoma of the breast [42]; specifically deletions and translocations at 1p13-p22. It is unclear at present whether a specific site on chromosome number 1 harbours a tumour suppressor gene involved in the pathogenesis of mesothelioma.

Table 2. Association between chromosomal aberrations and crocidolite/amosite exposure

Chromosomal abnormality	*P*-value
Monosomy 1	0.0017
Monosomy 3	0.0322
Monosomy 4	0.0345
Del (3p)	0.0207
Monosomy 14	0.6624
Monosomy 9	0.1176
Monosomy 22	0.1481
Trisomy 5	0.5843
Polysomy 11	0.2868
Polysomy 12	0.1997

Data obtained from Tiainen *et al. Br J Cancer* 1989, **60**, 618–626.

Chromosome 3 abnormalities have also been frequently observed in this tumour type [43] as well as in all major types of lung cancer. Flejter and associates [44] found deletions and translocations in the 3p14-p25 region in mesotheliomas. The SRO of 3p deletions appeared to be at band 3p21. Lu and colleagues also reported that the common region of chromosomal loss in malignant mesothelioma involving chromosome 3 is within band 3p21 [45]. This region of chromosome 3 is also frequently deleted in small cell lung cancer, renal cell carcinoma, uterine and breast carcinoma [46]. Once again, the possibility is raised that this region of chromosome 3 may contain a tumour suppressor gene playing an important role in the development of mesothelioma and a number of other neoplasms.

One further cytogenetic anomaly seen in mesothelioma is monosomy of chromosome 22. In Flejter and colleagues' analysis, monosomy 22 was noted in 11 of 28 cases examined. The authors pointed out that this chromosomal change has also been consistently noted in meningioma, glioma and acoustic neuroma [44]. 22q has been proposed as a possible site of a tumour suppressor gene [47].

The chromosomal abnormalities documented in the available literature are heterogeneous and complex. Nonetheless, as outlined above, a number of recurring changes exist. The frequent specific chromosomal losses provide evidence for a recessive mechanism of oncogenesis in this tumour [48]. Recurrent loss of genetic material in several chromosomal regions may be consistent with a multistep carcinogenic scheme, similar to that observed in colon cancer. At present, such a scheme for malignant mesothelioma remains theoretical.

ONCOGENES AND TUMOUR SUPPRESSOR GENES

Profound advances in the understanding of the genetic basis of cancer are reflected in the relatively recent elucidation of cellular oncogenes and tumour suppressor genes. The colon cancer model, developed by Aaron and Vogelstein, serves as an important paradigm of the interplay of oncogenes and tumour suppressor genes in multistage tumorigenesis [48].

A number of human tumours are associated with specific alterations of cellular oncogenes e.g. Burkitt's lymphoma, small cell lung cancer and breast cancer [49, 50]. Most others are associated with a number of transcriptionally activated proto-oncogenes [50]. At present, the role of oncogene activation in the pathogenesis of malignant mesothelioma is unknown. Very few studies are available in the current literature examining oncogene involvement in this tumour. Therefore, no coherent model of oncogenesis exists in this context.

One of the few relevant studies is that of Keifer and associates who examined patterns of expression of oncogenes in human non-small cell lung cancer cell lines [51]. Unfortunately, only one mesothelioma cell line was studied. The panel of oncogenes employed included *C-MYC*, *L-MYC*, *V-SRC*, *V-SIS*, *V-ERB*, *C-RAF* 1, *H-RAS*, *KI-RAS* and *N-RAS*. *C-RAF-1*, *Ki-RAS*, *N-RAS*, *H-RAS* and *C-MYC* were expressed in varying amounts in all lung cancer cell lines studied. *C-MYC*, *V-ERBB*, *C-RAF*, *HA-RAS*, *KI-RAS* and *N-RAS* were expressed in low levels in the one mesothelioma cell line. *V-SRC* was expressed in somewhat higher levels. The authors state, "Transcripts of *C-MYB* and *N-MYC* were found exclusively in small cell lung

cancer lines and *V-SIS*, *V-SRC* and *V-ERBB* only in non-small cell cancer (NSCLC) cell lines. A specific pattern of oncogene expression, demonstrable in each cell line examined, was not found nor could an association to a specific NSCLC type be revealed" [51].

The establishment of mesothelioma cell lines has provided a useful modality for the biological study of this tumour. Due to the extensive growth requirements of this cell type in culture, Ke and associates [52] have used SV-40 virus to extend the life-span of mesothelial cells in culture i.e. from 1 month to approximatley 2 years. These cell lines show an increased expression of the *C-SIS* oncogene which has been found in almost all mesothelioma cell lines to date [53]. It is interesting to note that *C-SIS* is located on chromosome 22, which is frequently the site of chromosomal alterations in this tumour, as mentioned above. The *C-SIS* oncogene codes for the B chain of platelet derived growth factor (PDGF). This growth factor is known to be mitogenic for normal mesothelial cells [54], and it has been hypothesised that PDGF represents an autocrine growth factor for malignant mesothelioma development.

Over the course of the last 5 years, another class of gene, important in cell cycle regulation and tumorigenesis i.e. the tumour suppressor genes, has been described. Discovery of tumour suppressor genes was the extension of investigation showing that neoplastic transformation could involve gene products which negatively regulate cell proliferation. Inactivation via deletion or mutation of such genes leads to abnormal proliferation, setting the stage for the cascade of genetic events responsible for tumorigenesis.

The *TP53* tumour suppressor gene, discovered in the late 1970s and positioned on chromosome 17 (17p), has become one of the most intensely studied of such genes, largely due to its apparent involvement in almost half of all human cancers [55]. A possible role for *TP53* in the development of malignant mesothelioma remains unclear. Although a large number of chromosomal abnormalities have been described in this tumour (including deletions in chromosome 17), few studies examine the hypothesis that mutations or loss of *TP53* may be common events in the carcinogenic process leading to mesothelioma. Cote and associates [56] examined four mesothelioma cell lines for *TP53* mutations. Two of the four had abnormalities of chromosome 17 with DNA sequence data, and cytogenetic analysis showing alterations in *TP53*. These alterations consisted of DNA single base pair substitutions resulting in amino acid substitutions. One tumour had an arginine to histidine substitution at position 175 and the other had a glycine to aspartic acid substitution at position 245. Mutations occurring in the coding region of *TP53* associated with tumorigenesis occur largely in a region of the gene showing the greatest cross-species homology i.e. between codons 117 and 286 [57]. Within this region, four "hot-spots" exist in which mutations appear to cluster i.e. codons 132–143, 174–179, 236–248 and 272–281 [58]. The two mutations noted in the mesothelioma cell lines studied by Cote and associates also occurred in the "hot-spot" areas. Similarly, using immunostaining techniques utilising monoclonal antibodies to p53 protein, Kafiri and colleagues [59] showed p53 immunoreactivity in 14 of 20 (70%) cases of mesotheliomas examined. Metcalf and associates [60] examined 20 mesothelial cell lines using direct sequencing of genomic DNA, mRNA expression levels and immunocytochemical analysis. Only three cell lines showed *TP53* abnormalities (point mutations). No demographic information on patients from whom tumour samples were obtained was presented in the above cited studies.

It is possible that some environmental carcinogens cause unique alterations in the *TP53* gene and analysis of the mutational spectra of *TP53* in neoplastic tissue, such as mesothelioma, may provide important information about the origins of these mutations. Associations between specific environmental carcinogens and *TP53* mutations have been suggested for a variety of neoplasms, such as hepatocellular carcinoma (aflatoxin B1 and Hepatitis B virus [HBV]) [61]. Approximately 50% of hepatocellular carcinomas occurring in patients from areas with frequent aflatoxin exposures harbour mutations in the *TP53* tumour suppressor gene, while only 20% of cases in regions with low exposures do so. In addition, the mutational spectrum is quite different between tumours arising in aflatoxin or HBV-exposed versus non-exposed patients. Therefore, specific genetic changes may serve as a "fingerprint" of transformation due to a specific environmental carcinogen.

The retinoblastoma tumour suppressor gene (*RB*), located on chromsome 13, is associated with both familial and sporadic retinoblastomas. A variety of other tumours, such as breast and bladder, are also associated with *RB* inactivation [62]. Shimizu and associates [63] studied 171 non-small cell lung cancers, extrapulmonary small cell and mesothelioma cell lines for RB protein expression. While most small cell lung carcinomas showed absent or mutant RB protein expression (i.e. 88%), five of five mesothelioma cell lines showed only wild type RB.

As mentioned earlier, cytogenetic abnormalities of chromosome 22 are documented in several studies. The neurofibromatosis type 2 tumour suppressor gene (*NF2*) is located on chromosme 22q12 raising the possibility that this gene could be involved in the pathogenesis of mesothelioma. Sekido and associates recently reported an analysis of a series of small cell lung cancer (SCLC), NSCLC and 14 mesotheliomas for mutations in *NF2* [64]. Southern blot and single-strand conformation polymorphism (SSCP) techniques were used to examine mutations in exons 2, 5, 7, 7, 9, 10, 11 and 12. Seven of 17 mesotheliomas (41%) had mutations within the coding region of *NF2*, while none of 75 lung cancer cell lines harboured such mutations. Four mesotheliomas showed deletions of *NF2*, while three cell lines had point mutations or microdeletions. None of the lung cancer lines had mutations of the *NF2* gene.

Deletions in chromosome 9 are recognised as a possible non-random alteration in malignant mesothelioma. Deletions of 9p21-p22 are also common in leukaemia, melanoma and gliomas, suggesting the existence of an important tumour suppressor gene residing in this region [65]. The *P16* gene (*MTSI* gene) has been identified as residing in this region of chromosome 9 and is implicated in the pathogenesis of mesothelioma. Cheng and associates [66] examined 40 mesothelioma cell lines and 23 primary tumours for alterations of the *P16* tumour suppressor gene. Of the 40 cell lines studied, 39 demonstrated alterations, with the most common being homozygous deletions. Only 5 of 23 (22%) primary tumours showed such abnormalities with none having mutations or rearrangements. Although these data suggest an important role for *P16* in malignant mesothelioma, only very limited information is available. Further work is needed to clearly establish the frequency of *P16* alterations in mesothelioma as well as in a variety of other tumours and its function. It is also possible that other tumour suppressor genes could exist within the 9p21-22 regions. This remains to be seen.

At present, although asbestos fibres are known to be the primary aetiologic agent for mesothelioma, the biological mechanism of asbestos carcinogenicity remains unknown. Very little information exists regarding the possible role of oncogenes or

tumour suppressor genes in the development of this tumour. A clearer understanding of the fundamental genetic mechanisms underlying mesothelial transformation may provide important insight into the process of solid state carcinogenesis as well as a clearer understanding of the biological and clinical behaviour of mesothelioma. Recognition of markers of asbestos-induced cancer would also constitute a useful molecular tool for epidemiological analysis. Such a carcinogen-specific genetic marker could have important public health as well as medico-legal implications.

CONCLUSION

Mesothelioma is becoming an increasingly important clinical and epidemiological entity due to its rising incidence worldwide and its poor clinical response to available therapies. Asbestos fibres are clearly the most significant aetiologic factor for this tumour, although its carcinogenic mechanism remains unknown. A large body of literature documents the fact that asbestosis, lung cancer and mesothelioma are the three most frequent causes of morbidity and mortality among asbestos-exposed cohorts. It is unclear which factors dictate the development of any of these maladies. Similarly, fibre carcinogenesis represents a persistent biological enigma.

The clustering of mesothelioma among members of the same family raises a number of questions related to this tumour's aetiology. The possibility exists that cases of mesothelioma in this setting may occur among those with a cancer-prone genotype susceptible to the toxic effects of asbestos. Genetic susceptibility to cancer is known to be genetically determined as an inherited trait in some instances e.g. retinoblastoma, while others arise as a complication of an inherited precursor lesion (e.g. Bloom's syndrome and non-Hodgkin's lymphoma).

The entire issue of genetic susceptibility is, in general, poorly understood in most cancers and has received relatively little attention as an area of research. Clearly, this is the case with mesothelioma. Nonetheless, the sporadic reports discussed in this paper suggest that the issue is one worthy of more intense scrutiny. Further work should be directed towards exploring the potential role of family history and genetic predisposition to this neoplasm. Such work may provide insight into its underlying biology and its clinical management.

As discussed earlier in this paper, the chromosomal abnormalities known to occur in malignant mesathelioma are complex and heterogeneous. Nonetheless, some abnormalities appear to be non-random and, therefore, may represent important events in tumorigenesis. Chromosomes 1, 3, 6, 9 and 22 losses and/or deletions have been documented by various methodologies, and suggest a recessive mechanism of oncogenesis. Recurrent loss of genetic material in several chromosomal regions may be consistent with a multistep carcinogenic cascade as observed in colon cancer. It remains to be seen whether the areas of recurrent chromosomal loss harbour tumour suppressor genes.

Molecular biological tools currently available hold promise for the development of useful epidemiological markers of environmental carcinogenesis. The finding that certain environmetal carcinogens are associated with specific DNA "fingerprints" opens an exciting avenue for further work. Such molecular epidemiolgial methods could lead to a clearer understanding of the biology of mesothelioma as well as provide useful data for epidemiological analyses.

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