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# In Vitro and In Vivo Studies of Mesothelioma

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**Abstract** Pleural mesothelioma is an asbestos-related malignancy characterized by progressive local growth, late metastases, and median survivals between 8 and 18 months. It is only recently that the in vitro and in vivo characteristics of the malignancy have been investigated. These investigations have been aided by the development of cell lines from patients with the disease, as well as lines developed from asbestos-exposed animals. Nude mouse models constructed with subcutaneous, intraabdominal, or intrathoracic innoculation of cultured cell lines or fresh tumor have been used for evaluating response to innovative therapies. Karyotyping has been performed on a number of cell lines and multiple abnormalities involving many chromosomes have been identified. Aneuploidy is commonly seen, along with reported non-random patterns of chromosomal abberations. The role of tumor suppressor genes, including p53 is controversial. Multiple growth factors including PDGF are being investigated for a possible paracrine/autocrine loop, and PDGF receptors seem to be differentially expressed in mesothelioma cells compared to normal mesothelial cells. The role of cytokines in the pathophysiology of the disease, secreted either by the tumor cells themselves or by monocyte/macrophages in the local tumor environment, remains to be defined. 

**Key words:** mesothelioma, cytogenetics, growth factors, oncogenes, asbestos

Mesothelioma is a tumor whose development is linked with asbestos exposure [1–4]. The theories of carcinogenesis from asbestos exposure involve the production of DNA damage, either via production of free radicals or by the direct damage of chromosomes after phagocytosis of fibers [5,6]. Anomalous aggregation of chromosomes may occur due to interference with the spindle apparatus. The consequences of such DNA damage or associated aneupoidy could be the loss of tumor suppressor genes, activation of proto-oncogenes, or unregulated generation of growth factors through paracrine/autocrine mechanisms (see "Oncogene and Growth Factor Regulations," p. 7). Moreover, as discussed later, the asbestos may activate mononuclear cells with further release of mitogenic factors.

This review will touch on only the high points in the in vitro and in vivo investigations of the disease. Much of the emphasis will be placed on (1) the development and analysis of cell lines, either from human tumors or artificially induced asbestos-related animal mesotheliomas, (2) the analysis of chromosomal aberrations, (3) evidence for or against tumor suppressor gene mutations in the pathogenesis of the disease, and (4) oncogene, growth factor, or cytokine changes.

## MESOTHELIOMA CELL LINES

### Human

Cell lines have been derived from primary malignant mesothelioma tumors, pleural effusions, ascites, and metastatic sites [7–11]. We have been able to derive cell lines from 11 of 55 patients with mesothelioma having operations at the NCI/NIH since 1990. The initiation and processing of the lines is similar to that described by Versnel et al. [8]. Briefly, our lines have been derived from primary solid tumor specimens or effusions. Solid tissue is minced into fine pieces and suspended in either serum free Hites, ACL-4, or DFCI media (Table I). Effusions are centrifuged at 1,500 rpm × 5 min, and red blood cells removed with ACK lysis. In certain instances, ficoll separation is employed. Cells are grown to confluency, split as necessary,

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and when colonies of tumor cells appear, the culture is submitted for immunohistochemical analysis for the uniform presence of cytokeratins. Serum is then added in increments of 2% to the media for further expansion and the media switched to serum supplemented RPMI.

All cell lines have been in continuous culture for greater than 15 passages. In general, normal mesothelial cells have failed to grow after 2 months in culture and senesced. Normal fibroblasts have been eliminated by continuous culture in low serum conditions or by the addition of cholera toxin.

The lines have a spindle shape or epithelioid appearance with multiple nucleoli and mitoses. The doubling times have been from 18–72 h and cell size has varied from 59–86 μm.

Mesothelioma cell lines are sensitive in vitro to combination cytokine treatment, including tumor necrosis factor and interferon [12], and despite relative resistance to natural killer cell lysis, they are susceptible to lysis by lymphokine activated killer-cells [13]. In vitro chemosensitivity curves have also been generated using these cell lines and may prove useful for guiding future treatment strategies on an individual patient basis.

### Transformed Immortalized Mesothelial Cells

Other cell lines with mesothelioma type features have been derived from normal mesothelial cells by transforming them with the SV-40 virus [14]. These transformed cells exhibit longer lifetimes in culture, and become tumorigenic when transfected with the ras-oncogene [15].

## NUDE MOUSE MODELS

Human malignant mesothelioma cell lines or freshly harvested tumor preparations have been xenografted into nude mice by the subcutaneous, intraperitoneal, or intrathoracic routes. Arnold et al. are credited with the first report of innoculation of cultured mesothelioma cells into nude mice with subsequent tumor growth, and it appears that the threshold for intraperitoneal tumor establishment occurs with a minimum of $2 \times 10^6$ cells [16]. Chahinian et al. have established six such xenografts by subcutaneous innoculation of fresh tumor specimens into nude mice [17,18], and Reale et al. described the characterization of the commercially available H-MESO-1 line [19]. The resulting xenografts are almost identical to the original tumor with regard to histology, immunochemical markers, and karyotyping even after many passages through the animals. The time to development of the tumor is a function of number of tumor cells innoculated, and with serial passage the doubling time in animals decreases.

These models have been useful in the evaluation of therapeutic modalities which could eventually lead to human trials. Efficacy of treatment, as exhibited by growth delay has been reported in such models using cisplatinum and

**TABLE I. Initiating Media for Mesotheliomas***

| Components | HITES [71] | ACL-4 [71] | DFCI [72] |
|---|---|---|---|
| Basal medium | RPMI 1640 | RPMI 1640 | 2-MEM and Hams-F-12 |
| Insulin | 5 μg/ml | 20 μg/ml | 1 μg/ml |
| Transferrin | 10 μg/ml | 10 μg/ml | 10 μg/ml |
| Selenium | 30 nM | 25 nM | 15 nM |
| Hydrocortisone | 10 nM | 50 nM | .5 μg/ml |
| 17-Beta-estradiol | 10 nM | — | 2 nM |
| Additional supplements | | | |
| Epidermal growth factor | | 1 ng/ml | 12.5 ng/ml |
| Triiodothyronine | | 0.1 nM | 10 nM |
| Hepes | | 10 nM | 10 mM |
| Ascorbic acid | | — | 50 μM |
| P-ethanolamine | | 10 μM | 0.1 mM |
| Cholera toxin | | — | 1 ng/ml |
| Bovine pituitary extract | | — | .035 mgm/ml |
| Ethanolamine | | 10 μM | 0.1 mM |
| Bovine serum albumin | | 0.2% | |
| Sodium pyruvate | | 0.5 mM | |
| Glutamine | | 2 mM | |

*RPMI, Roswell Park Memorial Institute; MEM, minimal essential media.

mitomycin C [18], and combination immunochemotherapy with interferon-α [20]. Other therapies, including TNF with or without actinomycin D and doxorubicin-cyclophosphamide have shown no efficacy [21]. Increased survival of nude mice with malignant mesothelioma ascites after intraperitoneal injection of immunotoxins has been reported [22] and intraperitoneal application of diphtheria toxin has also resulted in long-term cures in nude mouse models [23]. Whether these therapies can be adapted for human use remains to be investigated.

## ASBESTOS INDUCED ANIMAL MODELS OF MESOTHELIOMA

Animal models for mesothelioma have been developed using asbestos fibers since 1962 using amphibole or serpentine asbestos. All varieties of rodents have been used including rats, mice, and hamsters. Generally, doses greater than 25 mg will give consistent intracavitary tumor production [24–27]. Inhalation as well as direct asbestos innoculation will produce pleural and peritoneal mesotheliomas. There have been many similarities between these models of malignant mesothelioma and the human situation in that fiber length, diameter, shape, and durability have been found to be of greater importance than the type of fiber. As in humans, there is a long latency period from the time of inoculation to the development of tumors, approximately 7 months in mice, 12 months in rats, and years in primates [28]. The histological type of mesothelioma which results can be either epithelial, biphasic, or fibrous, although in mice there seems to be a predominance of fibrous tumors. The asbestos-induced tumors have been analyzed in vitro, and have similar properties to human mesothelioma cell lines. Important differences, however, do exist with regard to oncogene analysis and growth factor production (see "Oncogene and Growth Factor Regulation," p.7).

There has also been interest in non-asbestos related mesothelioma, particularly with regard to the contribution of viral particles. Malignant mesotheliomas, immunohistochemically and architecturally identical to those seen in humans, have been induced in chickens when a DNA fragment of the oncogene of the rous sarcoma virus was introduced intraperitoneally [29]. Similar induction of mesothelioma was reported when certain strains of the simian virus (SV40) were injected intraperitoneally or intrathoracically in hamsters [30]. These studies require further verification before one can extrapolate a viral mechanism for human mesothelioma from these animal data, although it is of interest that asbestos, specifically chrysotile, can promote transfection of foreign DNA into cells [31] which could possibly lead to mutation and carcinogenesis due to the inactivation of tumor suppressor genes, activation of protooncogenes from the intruder DNA, promotion of native protooncogene activity, or failure of DNA repair enzymes.

## DNA CONTENT AND KARYOTYPING OF MESOTHELIOMAS

Cytogenetic analysis of mesothelioma has been reported directly from fresh patient samples, short-term cultures (1–5 days), long-term samples (1–8 weeks), and established cell lines. Specifically, mesothelioma DNA content has been examined with both *classical karyotyping* and *flow cytometry*. Since chromosomal karyotyping depends on dividing tumor cells to obtain evaluable metaphases, the analysis may not represent a homogeneous population of cells in freshly cultured mesothelioma tumors. Conversely, flow cytometry will evaluate the whole range of cells in the sample, and may be contaminated by normal cells including fibroblasts, granulocytes, macrophages, and lymphocytes. In addition, due to the varying sizes of chromosomes (chromosome 22 constitutes less than 1% of human DNA content while chromosome 1 is less than 4%), DNA flow cytometry may not accurately reflect absolute changes in total chromosome number.

### Karyotyping Analysis

Classic karyotypic analysis of mesotheliomas using multiple marker chromosomes demonstrates modal chromosome numbers ranging from 34 to 90. Nine of twelve mesotheliomas classified cytogenetically by Gibas demonstrated clonal abnormalities, and the majority were aneuploid (8/9) with modal chromosome numbers between 43 and 85 [32]. Nineteen of 30 mesotheliomas had clonal abnormalities in the Tiainen's report, with chromosomal numbers between 34 and 96 [33]. Successful karyotyping was performed in 39/46 cases by Hagemeijer et al., of which 30 had aneuploid clonal abnormalities with modal chromosome numbers from 38–90 [34]. Malignant pleural effusions can be particularly difficult to classify due to the paucity of malignant cells and mitoses present.

### Flow Cytometry

Diploidy is seen in 39–65% of specimens analyzed by flow cytometry [35–37]. The S-phase fraction (SPF), which characterizes the proliferative activity of the tumor, has ranged from 0.8 to 16.6% with a median of 6% in one study of 168 samples from 70 mesothelioma patients [37]. In a similar study of 70 patients, 60% of the tumors were found to be diploid, and of the 51 specimens in whom it could be calculated, the median SPF value was 5.6% for diploid tumors and 16% for aneuploid tumors [38].

There has been interest in the possible prognostic significance of the SPF in mesothelioma. Although DNA ploidy was not a prognostic determinant in Pyrhonens series [38], patients with low SPF survived twice as long as patients with high SPF for both aneuploid and diploid tumors. Other studies seem to corroborate these findings [37,39].

Despite the absence of a survival advantage for diploid status when measured with flow cytometry, Tianinen et al. found that a normal karyotype and the absence of clonal abnormalities were correlated with survival in a retrospective review of 34 patients [40]. A normal mean chromosome number with no clonal abnormalities was associated with a larger median survival (31 months) compared to patients with mean chromosome numbers either greater than 46 (13 months) or less than 46 (26 months).

## IN VITRO ASBESTOS EFFECTS ON CHROMOSOMES

Since epidemiological studies have associated asbestos exposure with mesothelioma, there has been interest in the cytogenetic effects of asbestos on mesothelial cells. Lechner et al. demonstrated aneuploidy with consistent specific chromosomal losses in mesothelial cells cultured in the presence of asbestos fibres [41]. In 1989, Olofsson and Mark compared the cytogenetic abnormalities of mesothelial cells exposed to asbestos to those associated with mesotheliomas [42] and found few similarities in regards to numerical deviations, e.g., aberrations of chromosomes 22, 4, and 7 were identified. Structural rearrangements in asbestos exposed cultures involved chromosome regions that had been found to be affected in mesotheliomas, e.g., chromosomes 1, 4, 6, 9, 13, and 17.

The presence of asbestos in lung tissue from patients diagnosed with mesothelioma has been examined with respect to the cytogenetic changes found in the mesothelioma. A correlation between high asbestos fibre concentration and chromosome losses of 1, 4, 9 and rearrangements involving a breakpoint at 1p11-p22 was reported by Tammilehto et al. [43] and Tiainen et al. [44]. Tammilehto et al.'s study also found a correlation between the presence of crocidolite/amosite in lung tissue and numerical deviations of chromosome 1, 3, 4 and rearrangements involving del (3p) [43].

## CYTOGENETIC ABNORMALITIES (Table II)

Specific cytogenetic studies of mesotheliomas have revealed complex patterns of chromosomal abnormalities with *no specific aberrations common to all the tumor samples.* However, nonrandom patterns of chromosome aberrations have been reported in many series. These nonrandom karyotyping abnormalities involve changes in chromosomes 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 22 [29,33,34,45–50]. The most common chromosome gains (whole or partial) were found to be with chromosome 7 and chromosomes 5, 8, 11, 12, 15, 20 [33,34,45] in decreasing frequency. The most common chromosome losses (whole or partial) were found to be with chromosomes 4 and 22 and chromosomes 1, 3, 9, 10, 14, 18, and 19 in decreasing frequency [33,49,50]. The loss of chromosome 22 has been identified as the consistent monosonic aberration [50].

Different patterns of structural abnormalities have been identified in almost all chromosomes. The changes in chromosome 1 are partial loss due to deletion or rearrangement frequently involving bands 1p11-pter [34,45,48,50]. The shortest region of overlap occurred at 1p21-p22 in the samples described by Flejter et al. [50].

Chromosome 3 changes involved deletions and rearrangements in the region p14-p25 on the short arm [48,50]. The shortest region of overlap was identified at band 3p21 [34,50]. An abnormality in chromosome 3 was observed in 90% of the 30 mesotheliomas reviewed by Hagemeijer et al. [51].

Monosomy 4 has been observed as a nonrandom event in hypodiploid and hypotetraploid karyotypes [34,43].

Chromosome 6 has been the site of many structural rearrangements that can lead to deletion especially of the long arm, particularly at bands q15-q24 [34,45,52]. Melani et al. have described a mesothelioma cell line whose only

**TABLE II. Cytogenetic Abnormalities**

| Chromosome | Nonrandom aberrations | Reference |
|---|---|---|
| 1 | Deletions/rearrangements involving 1p11-pter | [43, 48, 50] [33, 45, 48] [34, 44, 49] |
| | Breakpoint at 1p11-p22 | [33, 43, 44] |
| | Shortest region of overlap 1p21-p22 | [50] |
| 2 | Occasional deletions/rearrangements involving 9arm | [45] |
| | Breakpoint at 2q11 | [45] |
| 3 | Deletions/rearrangements of 3p usually involving 3p15-p25 | [43, 45, 48] [34, 49, 50] |
| | Shortest region of overlap 3p21 | [34, 50] |
| 4 | Monosomy (partial/total) | [34, 43] |
| 5 | Duplication of the short arm 5p | [11] |
| 6 | Deletion/rearrangement of the long arm | [45, 46] |
| | Loss of 6q15-q24 | [46] |
| 7 | Duplication of 7p arm | [33, 44, 52] |
| | Breakpoints at q11.1-q11.2 | [49] |
| 9 | Various translocations affecting the long arm | [44, 45] |
| | Loss of short arm in hypodiploid and hypotetraploid clones | [34] |
| 11 | Common breakpoint at 11q13 translocations/ rearrangements | [45] |
| 14 | Monosomy (partial or total) | [44, 47, 73] |
| 17 | 17q rearrangements | [45, 50, 49, 33] |
| 22 | Monosomy (partial or total) | [33, 34, 44, 45, 50, 52, 73] |
| | Breakpoint of 22q11 | [45, 50] |

apparent chromosome abnormality was a 6q deletion [46].

Polysomy 7 (partial or complete), especially of the p arm has been associated with mesothelioma [33,44,52,53]. The copy number of chromosome 7 p arm was found to be inversely correlated with survival [44].

Loss of chromosome 9 p arm was noted to be a consistent feature of hypodiploid and hypotetraploid karyotypes [34,44,48]. Various translocations affecting the long arm of chromosome 9 were frequently identified in a series of 12 mesothelioma by Gibas et al. [45].

Rearrangements of chromosome 11 and a common breakpoint at 11q13 with translocation have also been observed [45].

Monosomy 22 (partial or total) was a common finding in many studies [33,44,45,53]. Partial loss or translocation involving the q arm with common breakpoint at 22 q 11 was identified [45,50].

## TUMOR SUPPRESSOR GENE ABNORMALITIES, SPECIFICALLY P53

The discovery of chromosomal abnormalities in mesothelioma, including translocations, has led to the investigation of (1) prootooncogene activation and (2) disruption in the activity of normal genes which control proliferation, i.e., the tumor suppressor genes. Alterations in chromosome 17, specifically 17p, have been described in a variety of tumors, including mesothelioma. This is the locus for the encoding of P53 protein [54–59], which is believed to be the product of a tumor suppressor gene. The importance of p53 to the contribution of carcinogenesis in mesothelioma remains controversial.

### Immunohistochemical Detection of Altered P53

The mutated p53 protein is actually more stable than the wild type and will accumulate in the anaplastic cells. Specific antibodies to p53 protein can then be used for immunohistochemical analysis of paraffin fixed or snap frozen specimens, including CM-1, as well as in tumor cell lines with Pab 1801 (AB-2) or Pab122 (Pharmingen, Inc., San Diego, CA). Kafiri et al. [56] have found that 70% (14/20) paraffin-fixed mesothelioma specimens had increased nuclear expression of p53 on the tumors compared to cases of reactive mesothelial proliferation without tumor. The percentage of tumor cells expressing the antigen was variable, and staining was heterogeneous. This was attributed to altered protein

conformation with prolongation of the protein half-life with accumulation of p53 possibly due to mutations. Other studies using immunohistochemistry staining techniques relate a 43% weak to moderate staining of mesothelioma tissues with Pab 1801 monoclonal antibody [55], with 10–60% of the tumor cell population showing p53 nuclear labeling diffusely or focally. It is noteworthy, however, that p53 immunoreactive activity is detectable without mutation of the p53 gene. As pointed out by Gerwin et al. [see reference 63], p53 protein overexpression can occur in cells with normal gene sequence due to (1) inactivation of degradatory pathways, (2) stabilization of protein through complex formation with a DNA tumor virus protein or cellular oncogene, or (3) overexpression of the myc oncogene product.

### Mutational Analysis of Chromosome 17 and p53

Cell lines derived from murine models of asbestos-induced mesothelioma have reduced or absent expression of p53 messenger RNA compared to the RNA from nontumorgenic cell lines or reactive mesothelial cells [59]. In human cell lines derived from patients with malignant mesothelioma, attention has turned to specific mutations or genetic abnormalities in p53. Cote et al. [58], in a study of four human mesothelioma lines, found that two of the four tumors had cytogenetic abnormalities of chromosome 17, and that two of the tumors had loss of heterozygosity in the 17p13 region as detected by RFLP analysis. Sequence analysis revealed single base substitutions in two of the tumor cell lines. This group has expanded their investigations to 20 cell lines, of which 10 had chromosome 17p abnormalities by cytogenetics or RFLP. Seven of the 20 lines (35%) had immunohistochemical increased expression of p53. This is in sharp contrast to data from Metcalf et al. [57] who found p53 abnormalities in only 3 of 20 mesothelioma cell lines, of which 2 were point mutations. The reported base changes in mesothelioma were in agreement with Cote et al.'s findings of G:C to A:T transitions. Immunohistochemical grading of p53 overexpression was questioned by this group in that normal mesothelial cells, as well as 12 of the mesotheliomas evaluated, expressed low but significant levels.

In our analysis of 10 mesotheliomas (3 cell lines and 7 snap frozen specimens), we have not found evidence for base pair mutations in the evolutionarily conserved exons 5–9 by single strand conformational polymorphism. In 6 evaluable cases, moreover, we have not observed loss of heterozygosity.

Further evidence for the lack of involvement of tumor suppressor genes playing a critical role in mesothelioma is demonstrated by a lack of abnormalities found for the retinoblastoma gene in malignant mesothelioma cell lines [60].

The role of asbestos in producing structural changes to normal mesothelial chromosomes, either through metal-cation based oxyradical production, or physical damage causing deletions is undefined. These structural alterations seem to occur with greater frequency than base-pair mutations. Moreover, the influence of asbestos on macrophages in the local environment to further amplify such changes remains to be elucidated.

## ONCOGENE AND GROWTH FACTOR AMPLIFICATION

Since common nonrandom cytogenetic abnormalities had been found in mesotheliomas, one would expect to identify functional problems with the genes involved in the chromosome rearrangements. The presence of extra copies of chromosomes may represent a mechanism for protooncogene amplification whereas loss of a chromosome may cause a loss of a tumor suppressor gene. Translocation and subsequent gene rearrangement may allow activation of a protooncogene or deactivation of a tumor suppressor gene. The locations of candidate protooncogenes/tumor suppressor genes have been mapped to specific chromosome regions, e.g., N-ras in band 1p13, EGF receptor in band 7p12-p13, PDGF-β in band 22q12.3-q13.1.

For the most part, however, oncogene amplication, has not been a primary characteristic of malignant mesothelioma. Specifically, amplifications seen of N-myc, c-myc, H-ras, Ki-ras, V-myb, IGF-1, Her 2/neu, V-fos, or rb have not been detected [61].

Nevertheless, one of the more intriguing oncogenes in malignant mesothelioma is C-sis, which codes for one of the chains of platelet derived growth factor (PDGF) [62]. It is not surprising that mesothelioma should have associated growth factors, as it has been shown that normal human mesothelial cells will respond to a number of different stimuli, including epidermal growth factor and fibroblast growth factor. Normal human mesothelial cells, however, do not produce either of these factors, but they do

produce a host of other factors including PDGF, TGF-β, both IL-1 α and β, IL-6 and 8, as well as colony stimulating factors.

Platelet derived growth factor has been the factor of greatest interest for autocrine activity. Gerwin et al. described elevation of RNA levels for both the alpha and the beta chain of PDGF in mesothelioma cell lines and correlated the increase with PDGF-like activity secreted by the cells [63]. Data from our laboratory, has confirmed progressive increases in PDGF AB in the conditioned media of growing mesothelioma cell lines. These data corroborate that of Versnel et al. [63A] who described elevation of mRNA for both PDGF chains in malignant mesothelioma cell cultures compared to normal cells, but the elevation was chiefly in the beta chain. Supporting an autocrine mechanism is the finding of receptors for PDGF-β, but not for PDGF-α, in mesothelioma, while normal human mesothelial cells α receptors but few β receptors. Christmas et al. [64] has detected α-receptor by RNAse protection assay, and has, interestingly, reported inhibition of growth of mesothelioma cell lines by addition of antisense oligonucleotides for PDGF A chain but not for the B chain. Of interest is the frequent association of abnormalities of chromosome 22 in malignant mesothelioma which is the site of the C-sis gene; yet, to date, no specific rearrangements of the C-sis gene have been described to explain altered gene activity.

It is crucial to define the model system for mesothelioma that one is working with, for there seem to be some significant species variations, specifically for PDGF. Walker et al. [65] has demonstrated that asbestos-induced mesotheliomas in rats express primarily PDGF-β receptors and not α-receptors, but there was no detectable AA, AB or BB PDGF in the medium conditioned by these cells. This is in distinction to other data [64] in asbestos induced mice tumors where A chain transcripts were detected in 3/3 lines while B chain was detected in only 2 of the three lines. PDGF-α and β receptor transcripts were present in 2 of the three lines, which is also at variance with human data.

Insulin growth factor activity has also been detected in ascitic fluids of rats and humans with primary asbestos induced malignant mesothelioma. Northern blot analysis of 5 mesothelioma lines revealed that only the fibrosarcomatous line had upregulated levels of IGF-1 and IGF binding protein transcripts when compared to the four epithelial lines [66].

### Cytokine Elaboration by Malignant Mesothelioma

Besides growth factors such as PDGF, there may be a role for cytokines in the pathogenesis of mesothelioma. The association between thrombocytosis and mesothelioma has stimulated investigations looking for increased circulating levels of cytokines such as interleukin-3, interleukin-4, granulocyte macrophage colony stimulating factor, erythropoietin, stem cell factor, interleukin-11, and interleukin-6. We have substantiated reports of high levels of interleukin-6 [67,68] and granulocyte macrophage colony stimulating factor [69] from body fluids or cell lines of patients with mesothelioma. Concurrently, we have not seen altered expression in the media of epidermal growth factor or transforming growth factor beta. Other investigators postulate other growth factors apart from EGF, PDGF, and TGF-beta, as yet uncharacterized, may play a role in autocrine or paracrine growth loops in this disease [70].

## MONONUCLEAR CELL INVOLVEMENT

Other cells may be involved in the propagation of events which transform normal mesothelial cells to mesothelioma. These cells could include monocyte/macraphages or other hematopoietic cells. Asbestos fibers themselves can cause chemotaxis with macrophage recruitment. The local environment of the mesothelial cell can then be influenced by the production of reactive oxygen species from respiratory burst, and we have demonstrated that asbestos exposure, specifically crocidolite, will cause release of tumor necrosis factor, IL1-β, and IL-6. Moreover, macrophages are known to produce substances which are, in themselves, mitogenic for mesothelial cells including TGF-α and β, PDGF-A, EGF, and IGF-1. Therefore, future studies attempting to coordinate events relevant to the initiation and promotion of carcinogenesis must link the interconnecting relationships between normal mesothelial cells, monocyte/macrophages, T-cells, and asbestos.

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