Oncogenes and Tumor-Suppressor Genes in Mesothelioma—A Synopsis

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Invariably mesothelioma is diagnosed late in the development of the disease when treatment is no longer effective. Therefore, a key to reducing the mortality rate of this neoplasm is knowledge of the general sequence of genetic events between initiation of mesothelial cells and the emergence of the metastatic tumor cells. Unfortunately, relatively little is known about the early changes in the genesis of this disease. Of the known changes, the most frequent are in the tumor-suppressor genes p16INK4a and NF2 and possibly the SV40 virus large T-antigen onc gene. The molecular nature of the changes in these genes as well as other alterations are discussed in this overview. — Environ Health Perspect 105(Suppl 5):1061–1067 (1997)

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Delineating the genetic changes that produce a cell with uncontrolled and often unlimited growth potential is important to the understanding of carcinogenesis mechanisms. Knowledge of these molecular processes also enhances the design of early detection and therapeutic protocols. Unfortunately, relatively minimal information is available on cancers associated with fiber exposure. This overview summarizes these observations, with primary emphasis on the alterations that have been described in human mesotheliomas.

Molecular Pathways of Growth Control

Progression of eukaryotic cells through their cell cycle is regulated by the sequential formation, activation, and inactivation of a series of cyclin/cyclin-dependent kinase (CDK) complexes. In addition to positive regulation by the activation of cyclin/CDK complexes, negative regulation of the cell cycle occurs through cyclin-dependent kinase inhibitors (CKIs) to prevent premature entry into the next phase of the cycle before completion of necessary macromolecular reactions (1–3).

Cell replication begins at the restriction point (R; Figure 1), where growth and arrest signals from the extracellular environment are integrated to determine whether the cell divides or remains quiescent (1–3). For example, elevated expression of platelet-derived growth factor (PDGF), which is a hallmark of mesothelioma, can elevate the concentration of c-myc in the nucleus. c-myc then induces the expression of cyclin Ds, and the balance between cyclin D/CDK4 and its CKI is tipped toward more rapid cell division (3). Owing to a growth-positive balance of these factors that has developed, the cell is committed to traverse the cell cycle.

In the first phase (G1), different species of cyclin D (D1, D2, D3) are expressed and form complexes with CDK4 and CDK6 (1,2,4). The activated cyclin/CDK complexes phosphorylate the retinoblastoma (RB) gene product and its related proteins (p107, p130). When these proteins are hypophosphorylated, they are complexes with transcription factors, e.g., E2F; hyperphosphorylation of the RB protein releases these transcription factors (1,2,4–6), which then stimulate the transcription of mRNAs that encode proteins required for the cell to progress further through the cell cycle (1,2,4,5). The next complex, cyclin E/CDK2, further phosphorylates RB family proteins, and the cell begins to synthesize DNA. Once the cell enters S phase, i.e., begins to synthesize DNA, a complex between cyclin A and CDK2 kinase forms. This complex, whose role is unclear, is degraded as the cell enters the G2 phase. Finally, the cyclin B/CDK2 complex phosphorylates proteins involved in chromosome condensation and the progression of the cell through mitosis, e.g., H1 histone, nuclear lamin, nucleolin, and intermediate filaments (1,2).

Two checkpoints in the cell cycle provide the cell with opportunities to govern its rate of cycling. The first, which is at the G1/S border, employs proteins denoted as CKIs. Currently, two structurally defined classes are known. The first, exemplified by p16INK4a and p15INK4b and including p19 and p18, primarily regulates CDK4 and CDK6 (7,8) by binding to the associated cyclins (9). The second family, characterized by p21CIP1 and including p27KIP1 and p57KIP2, regulates the activities of the CDK2 and CDK4/6 complexes (1,2,7). As will be discussed later, aberrations in the G1/S checkpoint function are associated with mesothelioma. Inhibitor proteins that prevent the activation of the cyclin B/CDK2 complex at the G2/M checkpoint also have been described (1,2), but no correlations between these proteins and fiber-caused carcinogenesis have been reported. However, because disruption of mitotic processes is a salient feature of cell–fiber interactions (10–14), examining mesothelioma cells for aberrations of the G2/M checkpoint genes may identify one or more that are highly correlated with mesothelioma.

Expression of CKI genes is stimulated by stress factors. For example, damaged DNA initiates events that cause the p53 gene product to accumulate within the cell nucleus (15,16). This protein induces the transcription of certain genes while inhibiting others. Two major regulatory proteins induced by the accumulation of p53 are the kinase inhibitor p21CIP1 and the protooncogene mdm2 (15). The ability of
This decrease of inhibitor falls (1). During the arrest period, the DNA damage is repaired. p53 also initiates transcription of the protooncogene mdm2, which then inactivates the p53 protein. This decrease in p53 activity leads to the fall in concentration of p21<sup>cip1</sup> protein, and the cell resumes its progress through the cell cycle (17).

The cell cycle is permanently stopped by programmed cell death, e.g., apoptosis. Overabundance of the Bax protein promotes apoptosis; on the other hand, excess Bcl-2 protein can extend cell survival by preventing apoptosis. Excessive damage to DNA elevates the concentration of the p53 gene product, which transactivates expression of the Bax protein (18). This mechanism is responsible for apoptotic death of a potentially mutant cell. However, if p53 is dysfunctional or is inactivated by binding with excess mdm2 protein, Bax levels remain low and the mutated cell can survive. Alternatively, inappropriate expression of various growth factors or growth factor intermediates can cause overexpression of Bcl-2, which prevents programmed cell death (18), in part by inhibiting expression of p21<sup>cip1</sup>. Consequently, mutated cells continue to replicate.

### Tumor-Suppressor Genes in Mesotheliomas

Tumor-suppressor genes check and regulate cell division. In general, these genes can become aberrant by point mutation, partial deletion, inappropriate expression, epigenetic silencing, gene amplification, gene rearrangement, complete gene loss, or combinations of these mechanisms. All could be involved in the genesis of a mesothelioma from exposure to asbestos. However, a hallmark of human (10–14, 20–34) and rodent (35–37) mesotheliomas is the large number of their nonrandom cytogenetic alterations. For human tumors, these include monosomy or partial monosomy of chromosomes 1, 3, 4, 6, 9, 14, 15, 18, 19, and 22, and trisomies and polysomies of chromosomes 1, 5, 7, 11, 12, and 20. This array of nonrandom chromosome deletions in human mesotheliomas suggests that many tumor-suppressor genes and oncogenes are probably involved in the genesis of the disease. The tumor-suppressor genes located at bands 1p21–22, 3p21, 6q15–21, and 22q will be identified eventually because these cytogenetic regions are involved in the genesis of other human cancers (21, 26, 28, 30–34, 38). However, current information confirms the involvement of only the following five tumor suppressor genes, i.e., p16<sup>INK4a</sup>, p15<sup>INK4b</sup>, p53, NF2, and WT1.

Cytogenetic analyses have shown that chromosome bands 9p13 to p22 are deleted in 50% of mesotheliomas. Two known tumor suppressors have been mapped to this region: the CDK4,6 inhibitors p16<sup>INK4a</sup> and p15<sup>INK4b</sup> (39). Cheng et al. (40) showed that 85% of the mesothelial cell lines they investigated had a homozygous deletion of p16<sup>INK4a</sup>. However, only 22% of the primary tumors showed this deletion. In contrast, Xiao and co-workers (39) reported that both of these genes were deleted in more than 70% of primary mesotheliomas. A similar loss of p16<sup>INK4a</sup> was recently reported by Kratzke et al. (41).

In addition, these authors showed that when p16<sup>INK4a</sup> was transfected into mesothelioma cell lines, their growth rates were inhibited 5- to 10-fold. In parallel, Spillare et al. (42) showed a 50% inhibition of colony-forming efficiency when p16<sup>INK4a</sup> was transfected into cultured mesothelioma cells. Several primary mesotheliomas exhibit cell heterogeneity for expression of p16<sup>INK4a</sup> and p15<sup>INK4b</sup>, implying that the loss of these genes was a late event (41). On the other hand, a primary consequence of asbestos exposure is extensive chromosomal alterations (11,24). Thus, it is also possible that these genes were deleted as a direct result of a fiber interacting with chromatin or spindle proteins (43). In human lung tumor cells, loss of p16<sup>INK4a</sup> is inversely related to retaining the Rb tumor-suppressor gene, and vice versa (41). Mesotheliomas follow this rule as well; three studies have shown that Rb is not deleted in these tumors (44–46).

The p53 tumor-suppressor gene, which resides in band p13 of chromosome 17, shows the most frequent rate of genetic alteration in human cancers, especially of the lung (15). In support of this statement, Cote et al. (47) found that three of four mesothelioma cell lines exhibited an abnormal p53 gene. Specifically, two had point mutations, and the third did not transcribe p53 mRNA. One of the cell lines with a point mutation, as well as the nontranscribing line, also showed loss of heterozygosity for 17p. However, Metcalf et al. (48) analyzed 20 mesothelioma cell lines and found that only 2 had point mutations, and 1 failed to transcribe p53. These latter data are in accord with the fact that mesotheliomas with deletions of chromosome 17p have been reported only occasionally (22,24). These data on human mesothelioma contrast with murine data where frequent (76%) rearrangement of the gene was found and p53 mRNA expression was frequently reduced or absent (49).

p53 overexpression in the nucleus often reflects abnormal p53 function (15). Metcalf et al. (48) reported that three of the lines without mutations exhibited strong staining of p53 protein, while most of the others showed some positive cells. Further, Mayall et al. (50), Kafiri et al. (51), and Segers et al. (52) reported strong immunostaining of p53 protein in tumor sections. As noted by Metcalf et al. (48), p53 expression without gene mutation suggests upregulation of p53 because of overexpression of c-myc, or perhaps the stabilization of the protein because of complexing with an
oncogene. The latter mechanism is supported by Segers et al. (52); 60% of the p53 immunopositive but nonmutated tumors they evaluated also overexpressed the p53-inactivating oncogene mdm2. However, Ungar et al. (53) recently reported that mdm2 was not overexpressed in the 17 mesothelioma cell lines they analyzed.

A second p53-inactivating mechanism could be the expression of the T-antigen (T-ag) of the SV40 virus. In cells that have been infected, the p53 protein apparently is overexpressed because it is complexed with large T-ag. This virus causes mesotheliomas when injected into the pleural space of hamsters (54). Further, Carbone et al. (55) recovered fragments of SV40-like DNA sequences from 60% of the mesotheliomas they examined by polymerase chain reaction (PCR). In addition, 79% of the tumors exhibited SV40 T-ag when assessed using immunohistochemistry, and there was a high correlation between T-ag immunoreactivity and the presence of SV40 sequences. Subsequently, Cristaudo et al. (56) found the same SV40-like DNA sequences in 72% of the mesotheliomas they investigated. In contrast, Metcalf et al. (48) found no evidence of SV40 T-ag in any of the 20 mesothelioma cell lines they investigated using immunohistochemistry. Further conflicting information, however, is in the recent report by Galateau-Salle et al. (57). These authors showed that SV40-like sequences are found not only in some mesotheliomas but also in pleural plaques, lung tumors, parenchyma distal to the tumors, and in parenchyma of individuals without cancer.

The Nf2 autosomal dominant tumor-suppressor gene resides on chromosome 22 (58). As noted above, this chromosome frequently is abnormal in mesotheliomas (24). Consequently, this gene has been evaluated in several cell lines. The results show that frameshift and nonsense mutations, and deletions of small portions of the Nf2 gene were often present in cell lines (58,59). These alterations also have been found frequently in primary mesotheliomas (59), but they are rare in human lung carcinomas (59). Nf2 codes for a protein called merlin, which may play a role in cell surface dynamics and structure by linking the cytoskeleton to the plasma membrane (58). Asbestos fibers are known to be more efficient in deforming the cytoskeleton in mesothelial cells than in airway epithelial cells (70). Thus, loss of stabilizing function supplied by a normal Nf2 gene product may be involved in the mechanism of fiber-caused transformation of the mesothelial cell.

A small percentage of mesotheliomas exhibit a cytogenic aberration—an interstitial deletion of bands 11p11 to 11p13 (24), where the transcription factor tumor suppressor gene WT1 resides (60). For example, Park et al. (61) reported a homozygous mutation of this gene in a peritoneal mesothelioma. Normal mesothelial tissue of both humans and rats abundantly expresses this gene (60–63). Amin et al. (62) found that WT1 mRNA was undetectable in 3 of 19 mesothelioma cell lines and in 3 of 8 mesothelioma tumors. In contrast, Walker et al. (63) reported that the WT1 was ubiquitously expressed by the human and rat mesothelioma cell lines they studied, although there was a relatively lower expression of the gene in the lines that gave rise to sarcomatous tumors in nude mice. This latter relationship could not be confirmed by Langerak et al. (60). It is possible, however, that an aberration in WT1 expression could stabilize and sequester p53 protein (64), thereby explaining the frequent immunohistochemical detection of p53 in mesotheliomas.

Oncogenes in Mesotheliomas

Protooncogenes are genes that promote cell division, and alterations in protooncogenes may contribute to uncontrolled cell growth. As noted above, nonrandom rearrangements and polynucleosomes of chromosomes 1, 7, and 22 are also hallmark, in human mesotheliomas. These structural aberrations can generate growth-promoting oncoproteins by rearrangements and amplification. Using Southern blot analyses, Tiainen et al. (65) examined 23 mesotheliomas and found no rearrangement and amplifications of several oncogenes located on these chromosomes, i.e., N-ras, epidermal growth factor receptor (EGFR), Met, and PDGF-B chain. Further, Kishimoto (66) could find no immunohistochemical evidence of overexpression of EGFR. On the other hand, Ramael et al. (67) detected amplifications or rearrangements of EGFR in all three of the mesotheliomas they examined using a more sensitive PCR assay. Thus, reevaluation of the known oncogenes on chromosomes 1, 7, and 22 using PCR approaches may reveal additional loci that are frequently rearranged or amplified in mesotheliomas.

Metcalf et al. (48) found no activating mutations of the K-ras oncogene in the 20 mesothelioma cell lines they examined. Further, Lee et al. (68) reported that overexpression of ras protein is a rare event for mesotheliomas. More recently, however, Kishimoto (66) did find frequent immunostaining of N-ras, and Ramael et al. (69) found that more than 50% of the cells within 78% of the mesotheliomas they evaluated had immunoreactivity to N-ras antibody. Notably, N-ras is located on chromosome 1 which is often polyomasic in mesotheliomas. Finally, sustained expressions of c-jun and c-fos is induced by fibers (70), and c-myc, along with c-neu were found to be overexpressed in the majority of the mesotheliomas of Japanese patients (66).

Many exogenous peptides increase the growth rate of mesothelial cells (71–74), and mesothelioma cells produce several growth factors and cytokines (71,75–79). Oncogenes often cause inappropriate expression of growth factors and/or their receptors. As a consequence, normal growth control mechanisms are abrogated. Oncogenes causing the production of growth factors that simulate autoreplication are referred to as autocrine (80). Autocrine activity, shown not to be PDGF, TGF-β, or EGF, has been detected in the conditioned medium of a mesothelioma cell line; however, the actual factor has not been identified (81). Nonetheless, repeatedly observed alterations in the expression of PDGF proteins and the associated PDGF receptors by mesotheliomas have suggested that this ligand and receptor produce an autocrine loop in many mesotheliomas (77,82). Several laboratories have examined this possibility, with somewhat conflicting and puzzling results.

PDGF-A protein and PDGF-B protein chains dimerize to form three species of PDGF that are each mitogenic for mesothelioma cells. One is A-chain homodimers and one is B-chain homodimers; the third is an A/B-chain heterodimer. The PDGF-A and PDGF-B chains are coded by separate genes, as are the two receptor proteins, α and β (83). The A/B-chain heterodimer and the B-chain homodimer are recognized by the β receptor. All three forms of PDGF bind the α receptor, although with differing affinities. Therefore, alterations in any of four different genes could affect growth of mesothelial cells. Normal mesothelial cells express the α and β receptors (82–89). Mesotheliomas often overproduce PDGF A-chain and B-chain proteins and low levels, if any, of the β receptor. Therefore, the PDGF-B-chain protein produced by the mesotheliomas may serve as an autocrine for continuous
replication of the tumor cells (82,85,89). However, mesothelioma cells also express the PDGF-A-chain growth factor, and the α receptor has been detected using RNase protection assays and recently by run-off analyses (83). In addition, transfection experiments with the immortal but nontumorigenic human mesothelial cell MeT-5A have shown that overexpression of the PDGF-A chain will transform these cells to the tumorigenic phenotype (90,91). Further, it has been shown using antisense oligonucleotides that the PDGF-A chain, but not the PDGF-B chain, will inhibit the growth of mesothelioma cell lines but not the MeT-5A cells (84). Thus, even though the PDGF growth factors and their receptors are differentially expressed in human mesothelioma cells compared to their normal cell counterparts, the role of these changes in the transformation of mesothelial cells is unclear. Parenthetically, rat mesotheliomas do not produce any form of PDGF, which suggests that fiber exposures can produce dissimilar results in different species (92,93).

The other growth factors and cytokines that are produced by at least some human mesothelioma cells include TGF-α, TGF-β1, TGF-β2, IGF-1, IL-1α, IL-1β, G-CSF, GM-CSF, M-CSF, IL-8, MCP-1, LIF, TNF-α, IL-6, and IL-8 (71,75,78). Both normal mesothelial cells and mesotheliomas produce IGF-1 and express the receptor for this growth factor (94). Further, a hamster mesothelioma cell line that was transfected with an IGF-1 receptor antisense plasmid exhibited both decreased growth in vitro and tumorigenicity (95). These observations suggest that IGF-1 may function as an autocrine for both the normal and transformed cells. Peritoneal mesothelial cells secrete detectable amounts of IL-1 and express the IL-1 receptor. However, an in vivo role for IL-1 as an autocrine for these cells is unclear because exogenously added IL-1 is required for long-term growth of the cells in culture (79). IL-6 has been shown to be an autocrine for normal human mesothelial cells (76,80); it remains to be determined if IL-6 is an autocrine factor for mesotheliomas, as well. IL-8, on the other hand, is only produced by mesothelioma cells (75); it is not known whether the receptor for this factor resides on the surface of mesothelioma cells. However, because IL-8 is a major angiogenesis factor (75), it may play an important role in the growth of the mesothelioma tumor.

Figure 2. Possible steps in the genesis of a mesothelioma.

Conclusions

Although information is minimal on the molecular changes that produce fiber-associated malignant mesotheliomas, the available data do show that point mutations, partial deletions, inappropriate expression, gene rearrangement and complete gene loss, and possibly epigenetic silencing and gene amplification probably are all involved. Many of these aberrations occur in the genes regulating the initiation of cell division, checking cell division rate, maintaining the cytoskeleton, regulating transcription, and inducing angiogenesis. It is also known that these aberrations are attributed to carcinogens other than asbestos fibers. Thus, the molecular biology underlying the genesis of these tumors is not significantly different from other neoplasms attributed to nonfibrous carcinogens; only the actual battery of genetic alterations seems to differ. The observations of Meloni et al. (33) and Knuttila et al. (24) along with the recent report from Hansteen et al. (23) do, however, permit a hypothesized ordering of genetic changes in the genesis of human mesothelioma (Figure 2). Their models disagree, but in general, they propose that losses of chromosomes 6q, 1p, and 22q may be among the earliest events. Other areas of data inconsistency include the specific form of PDGF and PDGF receptor, the status of p53 and WTI, and the possible role of SV40 viral infection. Plausible reasons for these discrepancies include inherent species differences, dissimilar routes of fiber exposure, and the creation of new alterations that manifested themselves during the development of cell lines. Some discrepancies might be resolved by the exchange of tumor specimens and cell lines as well as protocols and reagents.

Mesothelioma invariably is diagnosed late in the development of the disease when treatment is no longer effective (24). Therefore, a key to reducing the mortality rate of this disease is knowledge of the general sequence of genetic events that transpire between initiation of mesothelial cells and the emergence of the metastatic tumor cells. As with all tumor systems, but especially for mesothelioma, considerable work remains to be done to clarify which genetic changes are the most important in transformation of the normal cell. The ultimate resolution of these changes will permit the designing of anti-tumor treatment approaches that could reverse the effects of the altered genes. Future work will require confirmation of the proposed model (Figure 2), clarifying the role of the above discussed genetic changes, and elucidating the importance of as yet uninvestigated mechanisms, e.g., the induction of genomic instability (96,97), reduced DNA repair capacity (96), disruption of programmed cell death (98), and inherent susceptibility (99).

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