

## **Biologic, Cytogenetic and Molecular Factors in Mesothelial Proliferations**

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### **Introduction**

Mesotheliomas tend to be aggressive tumors that arise from the serosal surface cells lining the pleura, peritoneum and pericardium. The majority (80%) of these tumors are associated with exposure to asbestos fibers, either in the environment or work place. Although asbestos has been banned for use in most developed countries and asbestos abatement programs have been in place for the past several decades, over 2,000 cases are diagnosed in the United States each year. This is due to the long latency period from time of exposure to development of mesothelioma (20 to 40 years). Males are at a much higher risk for mesothelioma than females due to occupational exposure (plumbers, pipe fitters, insulation installers, shipyard workers). Although mesothelioma incidence in the United States peaked in the mid-1990's, it is estimated that over 70,000 mesothelioma cases will occur in US males between 2003 and 2054. It must be realized that less than 5% of those exposed to asbestos will develop mesothelioma.

Commercial asbestos fibers are subgrouped as chrysolite and amphibole. Chrysolite is a long curly serpentine fiber. This fiber accounts for 90% of the world's asbestos production. Amphibole is a short rod-like fiber, and includes crocidolite, amosite and tremolite. Amphibole fibers account for the remaining 5 to 10% of asbestos commercial production. The majority of mesotheliomas occur with amphibole fiber exposure. In general, a much smaller fiber burden is associated with mesotheliomas induced by amphibole (1/400<sup>th</sup> asbestos burden) compared with chrysolite. Naturally occurring air-borne fibers of the zeolite mineral erionite and several asbestos minerals account for endemic mesotheliomas in south central Turkey.

Although asbestos has been banned in developed countries, asbestos continues to be used at an alarming rate in Southeast Asia and China. With expansion of industrialization, it is expected that within the next few decades a “mesothelioma epidemic” may be seen in this region.

### **Tumorigenesis and Asbestos**

Asbestos fibers tend to accumulate near the pleural surface and interact with the mesothelial cell layer. It appears that asbestos fibers lead to neoplasia through the generation of reactive oxygen species and the formation of free radicals. These fibers also induce cytokine and growth factor production due to an inflammatory response (Table 1). This results in mesothelial cell proliferation. It has been suggested that the generation of free radicals and cytokines secondary to asbestos fiber accumulation causes DNA damage. Proto-oncogene activation may be induced and this leads to DNA synthesis, cell proliferation and susceptibility to mutations.

The process of tumor formation is a prolonged event with many oncologic steps occurring over many decades. Asbestos is thought to act as a tumor promoter and may facilitate tumorigenesis in synergy with other carcinogens. Aneuploidy with mesothelial cells has been shown to occur due to interference with chromosomal segregation by asbestos. Over time, structural alterations and numerical losses and gains in chromosomes occurs with mesothelial cells exposed to asbestos.

### **Cytogenetics and Mesothelioma**

During the past several decades, cytogenetic studies have been performed in an attempt to identify specific nonrandom alterations that may prove to be of diagnostic value (Table 2). Despite these efforts, karyotyping of mesotheliomas has not provided specific diagnostic anomalies. Monosomy (chromosomes 4, 22) and polysomy (chromosomes 5, 7, 20) of certain chromosomes does occur more frequently with mesotheliomas; however these can not be used as sensitive and specific markers for mesothelioma. Chromosomal losses at specific regions or loci implicate that certain tumor suppressor genes have been altered or lost. Chromosome loss at 1p21-2 and 3p21 are found in a high proportion of mesotheliomas. Many of the tumors have several chromosomal losses that occur in combination.

Fluorescent *in situ* hybridization (FISH) and comparative genomic hybridization (CGH) evaluation of mesotheliomas confirmed the karyotype findings (Table 2). CGH and deletion mapping identified even more chromosomal losses and gains than either conventional cytogenetics or FISH. These methods have defined more specific chromosomal loci that have undergone losses, gains or loss of heterozygosity. With the information from these studies, certain differences between adenocarcinoma of the lung and mesothelioma could be discerned (Table 2).

With the evolution of the human genome project, it has been possible to identify many different oncogenes and tumor suppressor genes that are involved in the multistep process from mesothelial proliferation to mesothelioma development (Table 2). The oncogenes that have been found are not exclusive to mesothelioma, but are shared with many other malignant human tumors. Similarly, tumor suppressor genes that are deleted, altered or inactivated in mesothelioma are those seen in other tumors as well.

The complex cytogenetics and molecular events in mesothelioma development attest to the long latency period and the multistep process from a benign proliferation to a malignant neoplasm. During the past several years, evolving molecular techniques, such as tumor suppressor gene methylation, microarray gene profiling and proteomics, have yielded insight into mesothelioma oncogenesis, diagnosis, prognosis and potential therapy.

### **SV40 and Mesothelioma**

Prior to reviewing recent molecular findings with mesothelioma, it is important to discuss the role of SV40 in mesothelioma (Table 3). SV40 is a DNA tumor virus with transforming ability that contaminated polio and adenovirus vaccines in the 1950's and 1960's. Seroprevalence of SV40 varies from 2 to 20% worldwide. SV40 infection is highest among immune suppressed and compromised individuals. SV40 is found in both adults and children and is thought to be transmitted via maternal-fetal and oral-fecal routes. The SV40 large tumor antigen (T-ag) stimulates host cells to replicate by entering into the S phase of the cell cycle, and is considered the major SV40 transforming protein. This protein binds and inactivates several tumor suppressor genes (p53, Rb) that are responsible for regulation of the cell cycle.

SV40 T-ag is found in a high proportion of mesotheliomas (about 50%), primary brain tumors (21%), non-Hodgkins lymphomas (36%) and osteosarcomas (Table 3). Of particular interest to mesothelioma development, normal mesothelial cell cultures transform readily when infected with SV40. This appears to be related to inactivation of cell regulatory genes by the SV40 T-ag protein (p53, RASSF1A tumor suppressor genes). Other cell signaling and transduction factors are also upregulated (Notch-1, met).

Of interest is the synergy between asbestos and SV40. Asbestos exposure without SV40 leads to mesothelioma in animal models. The combination of asbestos and SV40 results in more rapid development of mesothelioma. With SV40 infection in the absence of asbestos exposure, mesotheliomas in animal models do not occur.

### **Tumor Suppressor Gene Methylation and Mesothelioma**

Gene promoter methylation, along with resultant histone deacetylation, does not alter chromatin structure, but inactivates or silences the methylated gene. Inactivation of tumor suppressor genes by aberrant methylation leads to tumor development and progression. Gene silencing by methylation has been shown to occur in about 20% of mesotheliomas. SV40 virus is a DNA tumorigenic infectious agent that inactivates both p53 and Rb, induces telomerase activity, and induces oncogene activation and growth factor secretion. SV40 utilizes methylation as a means to inactivate tumor suppressor genes and to bypass the regulatory pathways of the cell. Over the past few years, several genes in regulatory and signaling pathways have been discovered to be methylated to a high degree in SV40-infected mesotheliomas.

It is well known that the SV40-Tag protein interacts with p53 and pRb to inactivate their tumor suppressor functions. Other genes that may be inactivated in SV40-infected mesotheliomas via methylation are lesser-known regulators of cell signaling pathways. At least 8 genes have been identified that are methylated in over 20% of mesotheliomas by the "silencing" mechanism (Table 4). DcR1 and DcR2 are anti-apoptotic decoy receptors that bind TRAIL (tumor necrosis factor-related apoptosis-inducing ligand). Both these genes are silenced in some pediatric tumors. Cyclin D2 is a critical cell cycle regulatory gene that is inactivated via methylation in prostate and lung cancer, as well as in several other cancers. HPP1 is silenced in hyperplastic colon

polyps, colorectal carcinoma and lung cancers. HIC1 (hypermethylated in cancer-1) has a p53-binding site that activates this zinc-finger transcription factor gene. It is frequently methylated in several human cancers. NOREA1A is a member of the RAS family of oncogenes, and undergoes inactivation in mesothelioma. CRBP1 (cellular retinol-binding protein 1) carries the alcohol form of vitamin E, participates in the retinoid signaling pathway, and is silenced by methylation in several cancers. RIZ1 (retinoblastoma protein-interacting zinc finger gene) is a nuclear histone protein methyltransferase gene and is commonly methylated in liver and breast cancer. RRAD is a GTPase gene initially identified in skeletal muscle in type II diabetes. Inactivation of RRAD plays a role in tumor growth in breast cancer. DRM/Gremlin is silenced in many types of cancers, and is a homolog to the rat *drm* gene. The silencing of these genes in SV40-infected mesotheliomas is significantly increased, and several of these genes are methylated in over 40% of tumors (Table 5).

Of interest is the finding that SV40-infected mesotheliomas demonstrate progressive methylation of several genes (RASSF1A, HPP1, DcR1, TMS1, CRBP1, HIC-1, RRAD) during serial passage of mesothelial cell lines. With mesotheliomas analyzed from 50 patients with follow-up (range 2 to 68 months, median 14.5 months), it was noted that methylation of TMS1 or HIC1 lead to a significant decrease in survival. Loss of HIC-1 function in medulloblastoma, and lung and breast cancers also correlates with poor prognosis. A novel caspase recruitment domain (CARD) is encoded by TMS1. With silencing of TMS-1, apoptosis mechanisms are inactivated. TMS1 is aberrantly methylated in breast and lung cancers. The ability of SV40 infection to silence genes is noted by mammalian cell cultures infected with SV40. SV40 infection induces expression of methyltransferase enzymes (DNMT1, DNMT3b) that leads to global genomic DNA methylation and tumor suppressor gene inactivation.

### **Gene Profiling and Mesothelioma**

Gene profiling studies are still within their infancy in the investigation of mesotheliomas. There are confusing results with many studies providing a myriad of known, little known and unknown genes that are overexpressed and underexpressed in mesothelioma. For example, one study provides a list of 166 genes that are up-regulated and 26 genes that are down-regulated out of over 4,000 genes studied.

Typical analyses reveal genes that participate in glucose metabolism, mRNA translation, and cytoskeletal remodeling. Perhaps more importantly, these studies are beginning to identify upregulated genes that have potential diagnostic, therapeutic and prognostic implications for patients. Some of these upregulated genes in mesothelioma will be discussed. Adenotin (gp96) is expressed on the cell surface and in the cytoplasm and is closely related to hsp90. This gene is considered to be an important factor in inducing tumor-specific immunity. Lung-related resistance protein gene is up-regulated in mesothelioma and may be partially responsible for chemoresistance. This protein acts as a transporter and removes cytotoxic drugs from the cell (doxorubicin, vincristine, VP-16, taxol, gramicidine-D). Galectin-3 binding protein is a beta-galactoside binding protein that participates in cell growth, differentiation, adhesion and malignant transformation. Increased expression in tumors has been linked to advanced tumor stage, progression, metastases and poor outcome. Laminin receptor (67,000 M<sub>r</sub>) plays a role in tumor development, progression and metastasis. It has been associated with decreased survival in breast, lung and ovarian cancers. Voltage-dependent anion channel genes (VDAC1, VDAC2) provide the primary pathway for metabolite diffusion across mitochondrial outer membranes. VDAC participates in the apoptotic pathway through interactions with the bcl-2 family of proteins. Mesotheliomas express high levels of bax and bcl-xl, and VDAC overexpression may be an attempt to suppress the anti-apoptotic effects of bax and bcl-xl. Ku80 gene participates in DNA double-strand break repair, and its overexpression has been identified in mesothelioma. The protein from this gene opposes anticancer drug-induced apoptosis.

Genes involved in cell signaling pathways have also been reported as up-regulated. The mitogen-activated protein kinase cascade (JNK1, NIK, TRAF2, PAK1, ERK5 genes), notch signaling pathway (JAGGED1, JAGGED2 genes) and Wnt-frizzled signaling pathway (SARP1, FRIZZLED, Dickkopf-1, Disheveled, beta-catenin, n-cadherin genes) are activated in many neoplastic processes. Mesothelioma upregulates these genes and uses these pathways to sustain tumor growth. The cell cycle in mesothelial tumors is also activated via up-regulation of cyclin genes (cyclin D1 11p13, cyclin D3 6q21, CDK phosphatase)

Certain gene profiling studies have compared expression between mesothelioma and lung cancer. Results have been encouraging in differentiating between mesothelioma and lung cancer using such methods. Using 15 ratios between up-regulated genes expressed in mesotheliomas (5 genes, calretinin, VAC-beta, MRX OX-2, PTGIS, KIAA0977) and adenocarcinomas of the lung (TACSTD1, claudin-7, TITF-1), it was possible to accurately categorize the tumors as mesotheliomas or lung cancers in over 90% of cases using just a single expression ratio. When using a two or three gene expression ratio, it was possible to accurately classify mesotheliomas and adenocarcinomas of the lung in 95% and 99% of the cases.

### **Gene Expression Ratio Outcome Prediction in Mesothelioma**

A gene profiling study illustrates the utility of gene expression in predicting outcome with mesotheliomas, regardless of histologic type. A total of 46 genes were identified that were considered to be of prognostic value. From these 46 genes, four upregulated genes that had the highest statistically significant values were chosen for each of the good and poor outcome groups. Genes that were overexpressed in the good outcome tumor group, compared with the poor outcome group, were selenium-binding protein, KIAA0977 protein, EST (similar to L6 tumor antigen), and leukocyte antigen-related protein. The upregulated genes in the poor outcome group, compared with the good outcome group, were cytosolic thyroid hormone-binding protein, calgizzarin, insulin-like growth factor-binding protein-3, and GDP-dissociation inhibitor 1. Five expression ratios (KIAA0977 protein/insulin-like growth factor-binding protein-3; KIAA0977 protein/ GDP-dissociation inhibitor 1; EST (similar to L6 tumor antigen)/ cytosolic thyroid hormone-binding protein; EST (similar to L6 tumor antigen)/ GDP-dissociation inhibitor 1; and leukocyte antigen-related protein/ GDP-dissociation inhibitor 1) each independently correctly placed the mesothelioma cases into the correct good and poor outcome groups. Ratio values greater than 1 predicted good outcome, and ratio values below 1 predicted poor outcome. A test set of an additional 29 patients with mesothelioma was used to validate the gene profiling ratio model. Almost 90% of patients were placed in the correct good and poor outcome groups based upon the gene profiling ratio model. The median survival for those determined to be in the good

outcome group by this model was 35 months vs only 7 months for those placed in the poor outcome group by this model.

### **Stepwise Process to Mesothelioma Development**

As noted previously, the road to mesothelioma development is thought to be a long and winding road. There are many cytogenetic and molecular events that occur along the way (Table 5). The normal mesothelial cell undergoes loss of chromosome 9p, which contains several cell cycle regulation genes. This leads to cell growth and proliferation. Loss of chromosome 22q, which houses NF2, the most frequently lost tumor suppressor gene in mesotheliomas, then occurs. This results in the early phase of mesothelial cell proliferation. Chromosome 11p with several important genes, as well as WT1, is lost, and this leads to further proliferation. Late phase mesothelial cell proliferation is preceded by the loss of 6p (several tumor suppressor genes) and loss of 12p (FHIT tumor suppressor gene). Mesothelioma with its malignant potential develops after loss of chromosome 3p. Loss of 13q (retinoblastoma gene, several other tumor suppressors) and loss of 14q (several tumor suppressor genes) provides an aggressive phenotype to the mesothelioma. Loss of chromosome 1q, 1p and 4q are thought to herald the ability to metastasize to other sites. SV40 virus interacts with asbestos fibers and facilitates chromosomal damage and gene mutations in about 50% of mesotheliomas. The process from increased cell growth to mesothelial proliferation to mesothelioma occurs over many decades.

### **Recent Prognostic Markers for Mesothelioma**

A newly discovered tumor marker, mesothelin, is expressed in normal mesothelial cells, and highly expressed in mesotheliomas, pancreatic cancers, nonmucinous ovarian cancers and certain squamous cell carcinomas. Mesothelin is a 40kDa cell surface glycoprotein that is shed into the serum and can be detected by serologic assays using a monoclonal antibody (K1). This serum marker is found in a high percentage of patients with epithelial and sarcomatoid mesotheliomas (60/69). Non-mesothelial pleural disease and malignant non-pleural lung disease rarely have detectable serum mesothelin levels (1/68). Only 2 of 92 patients with inflammatory non-pleural disease have detectable mesothelin in their serum. In a five-year study, serum mesothelin detection is 84% sensitive and 100% specific for identification of patients

with mesotheliomas. Mesothelin levels were increased for individuals with larger tumors (>3cm). Epithelial mesotheliomas had higher serum concentrations of mesothelin than sarcomatoid types. There was no correlation with the mesothelin level at diagnosis and survival. With surgical debulking, serum mesothelin concentrations decreased by about 40%. Interestingly, 7 of 40 “healthy” individuals with prior exposure to asbestos and no evidence of tumor had elevated serum mesothelin. Three of these seven individuals developed mesotheliomas from 15 to 69 months after the detection of elevated serum mesothelin levels. Mesothelin may act as a longitudinal surrogate serum marker for mesothelioma development in individuals exposed to asbestos. There are current proposals to utilize mesothelin as a target for immunotherapy in mesothelin-expressing tumors, including mesothelioma.

Syndecan-1 is a heparin sulphate proteoglycan family member. This protein binds basic fibroblastic growth factor, modulates neovascularization, upregulates WT1 and plays a role in epithelial differentiation. It has been noted in immunocytochemical studies that epithelial mesotheliomas that express syndecan-1 predict longer survival times. Gene therapy to induce syndecan-1 expression may have a positive effect on survival.

Cyclooxygenase-2 (COX-2) is associated with development of colon polyps, colorectal carcinoma, non-small cell lung cancer and gastric cancer. COX-2 participates in regulation of cell-mediated immunity, promotion of angiogenesis, inhibition of apoptosis and formation of carcinogens. Recently, immunocytochemical studies have shown that high levels of COX-2 expression are associated with decreased survival and more aggressive mesotheliomas. Although recently maligned for a suspected role in myocardial infarctions and strokes, COX-2 inhibitors may provide an additional means to improve survival and prolong life for patients suffering from mesothelioma.

Epidermal growth factor receptor has been found to be present in less than 5% of reactive mesothelial proliferations compared with almost 50% of mesotheliomas. Epidermal growth factor (EGF) receptor is a cell membrane receptor that participates in cell signal transduction and growth. The ligand for this receptor is TGF-alpha, which is often times overexpressed in mesothelioma. Binding of TGF-alpha to the EGF receptor creates an autocrine loop that results in unregulated cell proliferation. Interference with

this autocrine loop may reduce cell proliferation significantly in mesotheliomas. An EGF receptor tyrosine kinase inhibitor (ZD1839) has recently been described. In a murine model, the effect of the combination of this EGF receptor inhibitor and radiation therapy on human mesothelioma cells has been tested. Radiation alone reduced tumor volume by approximately 50%, with complete regression in 4 of 22 tumors. The combination of the EGF receptor inhibitor and radiation resulted in a 98% reduction in volume, with complete regression in 15 of 22 tumors. Assessment of mesotheliomas for EGF receptor expression may become a standard of care in the future in order to determine if the combination of EGF receptor inhibitor administration and radiation therapy would be feasible in individuals with mesothelioma.

### **Factors in Predicting Poor Prognosis with Mesothelioma**

Factors that are predictive of poor outcome in mesothelioma may be divided into host-related, tumor-related, biology-related and environmental-related factors (Table 6) Host-related factors indicative of poor outcome include poor WHO performance status, weight loss and male gender. Tumor-related factors include non-epithelial mesothelioma, local tumor burden, tumor invasion and extension through the diaphragm, lymph node involvement and positive resection margins. Biology-based factors involve proliferation and cell cycle control, promotion of angiogenesis, low antioxidants, high tumor metabolism, presence of SV40, and chromosome 7p gains. The environmental factors in poor prognosis are related to low socioeconomic group, low education level and impaired access to specialized medical centers.

### **Summary**

Although mesothelioma cases may have peaked in the 1990's in developed countries, it is expected that there will be over 70,000 cases diagnosed in the United States over the next 5 decades. With the industrial expansion in Southeast Asia and China and the continued use of asbestos, an epidemic of mesothelioma cases is anticipated over the next several decades. A considerable amount has been learned about the cytogenetic and molecular genetics of mesotheliomas. However, indepth studies are needed to further define specific factors that may provide for early diagnosis, surgical treatment, oncologic management and gene therapy. Serologic markers for surveillance of those with asbestos exposure and at risk for mesothelioma

are needed. Targeted therapy using molecular markers and gene therapy may provide a means to reverse mesothelial proliferations or stabilize tumor growth and allow for surgical resection. The future holds great promise in identifying mesothelioma gene expression profiles (genomics, gene microarrays) and proteins (proteomics) that may produce the key to dealing with this dismal and devastating neoplasm.

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Table 1: Growth Factors and Cytokines in Mesothelial Proliferations

## Growth Factors

PDGF-BB  
HGF  
EGFR  
Telomerase  
TGF-beta

## Angiogenic Factors

VEGF  
FGF-1  
FGF-2  
TGF-alpha  
Fibronectin  
Laminin  
Type IV Collagen  
Tenascin

## Proliferation Factors

Bcl-10  
Bcl-X,  
Mcl-1  
Bax  
P21  
P27  
Syndecan-1

## Cytokines

IL-6  
G-CSF  
GM-CSF  
IL1-beta  
IGF-1  
INF-gamma

Table 2: Cytogenetics and Molecular Genetics of Mesothelioma

## Karyotype

No specific chromosomal anomaly  
 Monosomy Chromosomes 4 and 22  
 Polysomy Chromosomes 5, 7 and 20  
 Chromosome Loss at 1p21-22 (85%), 3p21 (65%), 4q33-34, 4q25-26,  
 4p15.1-15.3, 6q, 6q15-21, 9p21-22, 22q12(possible tumor suppressor loci)  
 Chromosome Losses Occurring in Combination: 1p, 3p, 6q, 9p and 22q

Fluorescence *In Situ* Hybridization

Extra Copies of 1, 3, 6, 7, 11 and 15  
 Loss of 1p21-22  
 Loss of Heterozygosity (LOH) at 1p22

## Comparative Genomic Hybridization

## Losses Detected

9p21 (34%), 22q (32%), 4q31-32 (29%), 4p21-13 (25%), 14q12-24 (23%),  
 1p21 (21%), 13q12-14 (19%), 3p21 (16%), 6q22 (16%), 10p13-pter (16%),  
 17;12-pter (16%), 8p21-pter, 15q11.1-21.1, 3p21

## Gains Detected

8q22-23 (18%), 1q23/1q32 (16%), 7p14-15 (14%), 15q22-25 (14%) 3p12-  
 13, 7q, 5p

## Deletion Mapping

Allelic Loss at 1p21-22 (70%)  
 LOH at 1p36 (45%)  
 Loss of 3p21 (69% at 3p21.3, F3F15S2; 62% at 3p21.2, D3S2)  
 Loss of 6q15-21 (45%)  
 LOH from 6q (61%; 6q14-21, 6q16.6-21, 6q21-23.2, 6q25)  
 Loss of 9p (83%, particularly 9p21-22 [p16/CDKN2A locus])  
 Allelic Loss at 13q (67%)  
 Allelic Loss at 14q (32%, LOH at 14q11.2-13.2, 14q22.3-24.3, 14q32.12)  
 Loss at 15q (15q11.1-15)

## Adenocarcinoma vs. Mesothelioma

## Adenocarcinoma

Gains in X, 1p, 10q and 18q  
 Amplification in 8q  
 N-cadherin negative  
 WT-1 negative

## Mesothelioma

Losses in 10q and 18q  
 Bcl-X, Mcl-1, Bax Overexpressed  
 N-cadherin Expression  
 WT-1 Expression

## Oncogenes

*Myc* (myelocytomatosis virus family)  
*Ras* (rat sarcoma virus)  
*Raf* (ras-activated fragment)  
*Rassf1a* (3p21.3, Ras GTPase family)  
*Met* (N-methyl-N-nitroso-guanidine)  
*Erb-b1* (erythroblastomatosis virus)  
*MDR* (multidrug-resistance gene, p-glycoprotein gene)  
*GPC3* (glypian 3 gene, x-linked recessive overgrowth gene)  
*G5TM-1* (glutathione-5-transferase M1)  
*NAT-2* (N-acetyl transferase 2)  
*HGF* (hepatocyte growth factor/scatter factor)  
*COX-2* (cyclooxygenase-2)  
*NOS2* (nitric oxide synthase)  
*E-cadherin*  
*Beta-Catenin*  
*PDGF-BB* (platelet-derived growth factor-BB)  
*c-fos*  
*c-jun*  
*SV40 Large T-Antigen (Tag)*

## Tumor Suppressor Genes

*CDKN* (9p13-22)  
    P16 (9p21-22, CDKN2A, 70%)  
    P15 (9p21, CDK4, 70%)  
*MTAP* (9p13-22)  
*NF2* (22q12, 41-72%)  
*TP53* (17p, not related to asbestos)  
*WT1* (uncommon, SV40)  
*RB1* (downstream inactivation)  
*MDM2* (12q14.3-q15, overexpression)  
*FHIT* (3p14.2, inactivation)

Table3: Mesothelioma and SV40 Infection

Contamination of polio (1955-63) and adenovirus (1961-65) vaccines with SV40

SV40 Seroprevalence	2 to 20% worldwide
Adult kidney transplant patients	18%
HIV-Infected patients	16%
Non-HIV infected patients	11%
Hospitalized Children	6%
Czech Republic	4%
Hungary	9%
United Kingdom	5%

SV40 Transmission  
 Maternal-Infant  
 Oral-Fecal (fecal shedding)

SV40 Reservoir  
 Tubular epithelium of kidney  
 Lymphocytes

DNA tumor virus with transforming ability (2A carcinogen)

SV40 large tumor antigen (T-ag)  
 Essential replication protein  
 Stimulate infected host cell to enter S phase and undergo DNA synthesis  
 Major transforming protein of SV40  
 Binds cellular tumor suppressor gene proteins (p53, pRb, p107, p130/Rb2)  
 Activation of EF2 to induce expression of growth-stimulatory genes

SV40 T-ag Associated with Tumors in Animals and Humans  
 Mesothelioma  
 Osteosarcoma  
 Brain Tumors  
 Non-Hodgkins Lymphoma

SV40 and Primary Human Mesothelial Cells  
 Highly susceptible to SV40 transformation  
 p53/T-ag complexes high levels  
 Notch-1 and met (hepatocyte growth factor receptor) upregulated  
 RASSF1A tumor suppressor gene inhibited  
 1,000-fold rate of transformation compared with human fibroblasts

SV40 Detection in Human Cancers		
	SV40 Detection	Odds Ratio for Tumor Development
Mesothelioma	49.6%	16.8

Controls	5.5%	1.0
Primary Brain Tumors	21.3%	3.9
Controls	9.9%	1.0
NonHodgkin Lymphoma	35.8%	5.4
Controls	4.7%	1.0

Table 4: Methylation of Regulatory Pathway Genes in Mesotheliomas and SV40 Infection

	All Tumors	Gene Methylation	
		SV40 (+)	SV40 (-)
DcR1 (8q21.2)	65%	74%	56%
DRM/Gremlin (15q13.3)	60%	71%	50%
RRAD (16q22.1)	56%	71%	50%
DcR2 (8p21.2)	41%	48%	34%
Cyclin 2 (12p13.32)	35%	52%	19%
HPP1 (2q32.3)	35%	52%	19%
RASSF1A (3p21.3)	32%	48%	16%
HIC-1 (17p13.3)	22%	23%	22%
RIZ1 (1p36.21)	16%	16%	16%
CRBP1 (3q23)	11%	23%	6%
TMS1 (16p11.2)	6%	13%	0%
NOREA1 (1q32.1)	3%	3%	3%

Table 5: Stepwise Process to Mesothelioma Development: A Several Decades Oncogenesis

Normal Mesothelial Cell

Loss of Chromosome 9p (p15, p16, CDKN2)  
Asbestos Exposure in 80% =>

Increased Cell Growth

Loss of Chromosome 22q (NF2) =>

Early Phase of Mesothelial Proliferation

Loss of Chromosome 11p (WT1) =>

Intermediate Phase of Mesothelial Proliferation

Loss of Chromosome 6p and 12p (FHIT) =>

Later Phase of Mesothelial Proliferation

Loss Chromosome 3p =>

Mesothelioma

Loss of Chromosome 13q and 14q =>

Aggressive Mesothelioma

Loss of Chromosomes 1q, 1p and 4q =>

Metastatic Mesothelioma

|\*

| SV40 T-ag\*\*

\*Malignant transformation.

\*\*SV40 identified in about 50% of mesotheliomas.

Adapted from: Sandberg AA, Bridge JA, Cancer Genet Cytogenet 2001;127:93-110

Table 6: Mesothelioma: Factors in Predicting Poor Prognosis

## Host-Related Factors

- Poor WHO Performance Status
- Weight Loss
- Male Gender
- High Leukocyte and Platelet Counts
- Low Hemoglobin (Anemia)
- Chest Pain
- High Serum Lactate Dehydrogenase (LDH)

## Tumor-Related Factors

- Nonepithelial Cell Type (sarcomatoid, mixed)
- Local Tumor Burden
- Invasion of Visceral Pleura
- Extension Through Diaphragm
- Mediastinal Lymph Node Involvement
- Tumor at Resection Margins

## Biology-Related Factors

- Proliferation and Cell Cycle Control
  - Proliferation Index High (DNA flow cytometry)
  - Aneuploidy
  - Mitotic Index High
  - Apoptotic Count High
  - MIB-1 High
  - p27<sup>kip1</sup> Low
  - p21 Low
  - COX2 High
  - P53 low
  - Mesothelin Serum Levels High

## Angiogenesis

- Basal Lamina Reduplication
- Microvessel Count and Density Increased
- Syndecan-1 Low
- Fibroblast Growth Factor-2 High
- Thrombospondin-1 High

## Anti-Oxidants

- Catalase Low
- Mn SOD Low

## Tumor Metabolism

- High Uptake on PET Scan
- SV40 Sequences Detected
- Serum and Pleural Fluid Markers
  - Cyfra 21-1 High
  - Pleural Hyaluron High
- Chromosome 7p Gains (increased copy numbers)

## Environmental-Related Factors

Socioeconomic Status Low  
Education Level Low  
Long Distance from Medical Centers