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Editorial

Mesothelioma, Mesothelial Proliferations, and Their Mimics: A Multimodal Approach—Society for Ultrastructural Pathology Companion Meeting at United States and Canadian Academy of Pathology Annual Session in San Antonio, February 2005

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Once again, the Society for Ultrastructural Pathology is delighted to publish the proceedings from the 2005 SUP Companion Meeting held at the USCAP session in San Antonio. The topic of the SUP Companion Meeting for 2005 was "Mesothelioma, Mesothelial Proliferations and Their Mimics: A Multimodal Approach." The full spectrum of mesothelial proliferations was presented by internationally recognized pathologists with special expertise in this field. The SUP companion meeting was an exceptional event with integration of an entire array of diagnostic and research techniques. The incorporation of transmission and analytical scanning electron microscopic techniques in diagnostic surgical pathology was thoroughly discussed.

The 2005 SUP Companion Meeting was launched by Samuel Hammar from Diagnostic Specialties Laboratories in Bremerton, Washington. A comprehensive review of the features of mesotheliomas was presented in an informative manner, based on Dr. Hammar's extensive expertise in this arena. It is certain that his paper in the proceedings, entitled "Macroscopic, histologic, histochemical, immunohistochemical, and ultrastructural features of mesothelioma," will be a much-cited reference. The tabular content alone is remarkable and will no doubt serve as a major resource for surgical pathologists in their diagnostic workup of mesotheliomas. Although methods to investigate the biologic, cytogenetic, and molecular events have only recently been developed, there is a great deal of emerging information regarding mesotheliomas. John Hicks from Texas Children's Hospital and Baylor College of Medicine in Houston, Texas put the biologic, cytogenetic, and molecular features associated with mesothelioma into perspective with respect to pathogenesis, clinicopathologic events, and present and future therapy. For those who enjoy learning more about etiopathogenesis and

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potential innovative therapeutic targets, the paper in the proceedings entitled "Biologic, cytogenetic and molecular factors in mesothelial proliferations" will be a must read.

The attendees were particularly fortunate to have the opportunity to learn about the contribution of analytical scanning electron microscopy in establishing the link between asbestos exposure and mesothelioma. Victor Roggli from Duke University Medical Center in Durham, North Carolina is one of only a handful of individuals in the world who routinely evaluate mesotheliomas for asbestos content using SEM analytical techniques. A comprehensive review of over 400 cases of pleural and peritoneal mesotheliomas was presented and is chronicled in Dr. Roggli's paper in the proceedings, entitled "Role of analytical SEM in the determination of causation in malignant mesothelioma." This was followed by an exceptional presentation by Josep Lloreta-Trull from Hospital del Mar-IMAS-IMM, Universtat Pompeu Fabra in Barcelona, Spain. His topic—"Extrathoracic mesothelial proliferations and their mimics"—provided the audience with an appreciation of both reactive and neoplastic mesothelial proliferations, involving a wide variety of extrathoracic sites. Distinguishing among extrathoracic mesotheliomas, reactive mesothelial proliferations, adenomatoid tumors, serosal carcinomas, adenocarcinomas, and Muellerian-derived tumors will be more readily accomplished after digesting Dr. Lloreta-Trull's paper in the proceedings.

For those who were able to attend the 2005 SUP Companion Meeting, the opportunity to have questions answered by the presenters was an added bonus. The ensuing discussion at the end of the session spilled into the lunch hour and continued in the hallway outside the lecture hall. The proceeding papers will allow those unfortunate enough not to attend the 2005 SUP Companion Meeting to benefit from the combined knowledge and expertise of the presenters. It is anticipated that these proceedings papers will prove to be quite useful in the daily practice of surgical pathology. The Society for Ultrastructural Pathology would like to express its gratitude to the presenters for their dedication to the field of ultrastructural pathology and for sharing their expertise in mesothelial proliferations.

The Society for Ultrastructural Pathology is particularly pleased to announce that it has joined forces with the Renal Pathology Society for the 2006 USCAP Companion Meeting. The topic for the 2006 Combined Renal Pathology Society–Society for Ultrastructural Pathology Companion Meeting will be Pathogenesis and Diagnosis of Renal Disease: The Essential Role of Ultrastructural Investigation. The presentations and presenters will be

1. Diagnostic utility of negative-staining electron microscopy for detection of polyomavirus infections in urine: a comparative analysis of electron microscopy, conventional cytology, and PCR. Presented by S. Singh from the University of North Carolina, Chapel Hill, NC
2. Contribution of quantitative techniques, including morphometry, to renal diagnosis. Presented by M. Kashgarian from Yale University, New Haven, CT
3. Plasticity of mesangial cells: a basis for understanding pathological alterations. Presented by G. Herrera, Louisiana State University Health Sciences Center, Shreveport, LA
4. Modulation of autoimmune glomerulonephritis in a murine model: key role of ultrastructural evaluation. Presented by J. Hicks from Texas Children's Hospital, Baylor College of Medicine, Houston, TX
5. Cryo-electron microscopy of organs, cells, and cellular components. Presented by J. Costello from the University of North Carolina, Chapel Hill, NC
6. Three-dimensional structure of the glomerular slit diaphragm as revealed by electron tomography. Presented by K. Tryggvason, Karolinska Institute, Stockholm, Sweden

This joint Renal Pathology Society and Society of Ultrastructural Pathology Companion Meeting has been organized by a combined program committee (Renal Pathology Society: V. Nickeleit [co-chair], C. Alpers, H. J. Groene; Society for Ultrastructural Pathology: J. Lloreta-Trull [co-chair], G. Herrera, J. Hicks). Through the efforts of this program committee, those attending the 2006 Combined Renal Pathology Society–Society for Ultrastructural Pathology Companion Meeting will be updated on the pathogenesis and diagnosis of renal disease by internationally recognized authorities. Perhaps, you should mark your calendar now for the morning of 12 February 2006 in order not to miss this learning opportunity!

John Hicks
President, Society for Ultrastructural Pathology

MACROSCOPIC, HISTOLOGIC, HISTOCHEMICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL FEATURES OF MESOTHELIOMA

Samuel P. Hammar, M.D.

SOCIETY OF ULTRASTRUCTURAL PATHOLOGY COMPANION MEETING
FEBRUARY 27, 2005

INTRODUCTION

The celomic cavity develops early in embryogenesis and is divided by partitioning membranes into the pleural, pericardial and peritoneal cavities. These body cavities are lined by tissue referred to as serosa that have a visceral and parietal layer. The serosal tissue is composed of a layer of epithelial mesothelial cells separated from the underlying connective tissue component by a basement membrane. Mesotheliomas arise from cells forming this serosal membrane. The majority of mesotheliomas (90-95%) arise in the pleural cavity whereas about 5 to 10% arise in the peritoneal cavity. Primary pericardial mesotheliomas are extremely uncommon. Mesotheliomas can arise in the tunica vaginalis which is an invagination of the peritoneum.

Serosal tissue is an extremely reactive type of tissue and shows a prominent reaction to almost any form of injury. Epithelial mesothelial cell hypertrophy and hyperplasia can become extremely severe and be confused with epithelial mesothelioma. Likewise, multipotential subserosal cells proliferate forming a highly cellular invasive appearing type process. One of the most difficult areas in “mesothelioma pathology” is differentiating reactive epithelial mesothelial cell proliferation from an epithelial mesothelioma and from differentiating reactive multipotential subserosal cell proliferation from a sarcomatoid or desmoplastic mesothelioma.

MACROSCOPIC FEATURES OF MESOTHELIOMA

At the time most pleural mesotheliomas are diagnosed, they are composed of multiple small nodules studding the visceral and parietal pleural surface. These nodules range from 1 mm. to occasionally 1 cm. In the majority of cases, this proliferation is associated with a pleural effusion, the pleural fluid usually having the features of an exudate.

As time progresses, the nodules coalesce to form solid tumors that in the case of pleural mesotheliomas encase the lung and obliterate the pleural cavity. Mesotheliomas frequently invade chest wall skeletal muscle and sometimes directly invade skin and subcutaneous tissue. They likewise invade lung parenchyma. It is not uncommon for mesotheliomas to show variability in the thickness of the rind of tumor that encases the lung. In general, the tumor is usually much thicker at the base of the pleural cavity than it is at the apex. Frequently, mesotheliomas have a nodular morphology and if the rind of tumor is relatively thin, these nodules can be confused with primary lung cancers. Occasionally, mesotheliomas metastasize to hilar lymph nodes and produce a hilar mass that is significantly more recognizable radiographically than the thin rind of tumor that encases the lung. Mesotheliomas also frequently

directly invade pericardium and sometimes myocardium. It is not uncommon for pleural mesotheliomas to invade through the hemidiaphragms and extend into the abdominal cavity.

Some epithelioid mesotheliomas produce excess amounts of hyaluronic acid and proteoglycans. Tumors that produce these substances are “slick” and “slimy”. They often have large cystic areas filled with a tannish gelatinous material.

Peritoneal mesotheliomas are similar to pleural mesotheliomas in that they also begin as multiple small nodules that over a period of time coalesce to form a rind of tumor tissue that encase various organs within the abdominal cavity. Sometimes this can be so extensive that the bowel and other organs are compressed to the point of being nonexistent. As with pleural mesotheliomas, most peritoneal mesotheliomas initially are associated with an effusion.

Primary mesotheliomas that arise in the tunica vaginalis often present as a mass in that location. They sometimes remain localized, although not infrequently invade the peritoneal cavity and extensively involve it.

Primary pericardial mesotheliomas are rare. To diagnose a primary pericardial mesothelioma, one has to be certain that the tumor involving the pericardium does not represent an extension of a pleural mesothelioma. Pericardial mesotheliomas are like other mesotheliomas in that they start out as small nodules that coalesce to form a rind of tumor around the heart with obliteration of the pericardial cavity.

Rarely, mesotheliomas occur as localized masses rather than diffusely involving a body cavity. These occur most frequently in the pleural cavity and are called localized malignant mesotheliomas.

Symptoms referable to the site that mesotheliomas begin are often so dominating that metastases are not searched for in mesothelioma. However, metastases are relatively common in mesothelioma, although not as common as one sees in primary lung cancers. The most common site mesotheliomas metastasize to is bronchopulmonary and hilar lymph nodes. The next most common site is to the pleural surface of the lung not involved by tumor. Mesothelioma metastases can involve almost any organ, including adrenal glands, liver, kidneys, etc. There have been about 20 or 25 reported cases of mesotheliomas metastasizing to brain. Desmoplastic mesotheliomas have a propensity to metastasize to bone and can be a diagnostic dilemma because they resemble benign fibrous tissue.

HISTOLOGIC TYPES OF MESOTHELIOMA

Mesotheliomas are subtyped into four major categories:

1. Epithelial
2. Sarcomatoid – fibrous
3. Biphasic – mixed
4. Desmoplastic (this is considered a variant of a sarcomatoid mesothelioma)

This classification scheme is extremely simple compared to what actually exists. There are numerous subtypes of epithelial mesothelioma (Table 1) and there are numerous patterns that one sees with sarcomatoid mesotheliomas and biphasic mesotheliomas. When large tissue samples are available such as a pleural pneumonectomy specimen or an autopsy specimen, it is

common to see variable differentiation. One can often see five or six histologic types of differentiation by the tumor and the more sections one takes, the more likely the tumor is found to be biphasic. Sarcomatoid mesotheliomas can show homologous or heterologous differentiation including osteocartilaginous and lipomatous differentiation. It is debatable whether they show vascular differentiation.

Desmoplastic mesotheliomas are probably the most difficult of all mesotheliomas to diagnose. They should not be diagnosed from a needle core biopsy. The primary differential diagnosis is fibrosing pleuritis. The criteria for diagnosing desmoplastic mesothelioma include:

1. Over 50% of the tumor has to be composed of relatively dense hypocellular fibrous tissue that not infrequently forms vague nodules.
2. Areas of increased cellularity that have the features of a sarcomatoid mesothelioma.
3. Focal areas of stellate necrosis.
4. Invasion of subparietal pleural fat/chest wall or invasion of the lung (most important).

In fibrosing pleuritis, there are more reactive tissue changes with capillary proliferation, inflammation and fibrin deposition. The capillaries that proliferate in the pleura are usually perpendicular to the surface of the pleura which is not seen in desmoplastic mesothelioma.

One has to remember that when desmoplastic mesotheliomas invade or metastasize, they can look extremely bland and can be misdiagnosed as benign fibrous tissue.

HISTOCHEMICAL FEATURES

Histochemistry is infrequently used at this point in time in diagnosing mesotheliomas, although occasionally it can be helpful. Histochemistry is used primarily to differentiate epithelial mesotheliomas from mucin producing adenocarcinoma such as primary pulmonary mucin producing adenocarcinoma. The general rule of thumb is that most epithelial mesotheliomas do not produce mucin and therefore are PAS diastase, mucicarmine and Alcian blue/colloidal iron negative. Epithelial mesotheliomas frequently contain glycogen and are PAS positive with this reaction eradicated with pretreatment with diastase. Likewise, the epithelial mesotheliomas that produce abundant hyaluronic acid or proteoglycans frequently stain strongly positive with Alcian blue/colloidal iron with this reaction often being eradicated by pretreatment of the tissue with hyaluronidase. Approximately 2-5% of all epithelial mesotheliomas stain positive with a mucin stain such as mucicarmine, PAS diastase and Alcian blue/colloidal iron even after pretreatment with hyaluronidase. These mesotheliomas are ones referred to as mucin positive epithelial mesotheliomas. When evaluated ultrastructurally, they frequently show crystalloid material which is discussed below under the heading “Ultrastructural Features”. The mucin positive epithelial mesotheliomas are the ones that often will show focal positive staining for immunohistochemical markers that are often associated with primary pulmonary adenocarcinoma such as CEA, LeuM1, and B72.3.

IMMUNOHISTOCHEMICAL MARKERS

There is extensive literature on the immunohistochemistry of mesothelioma. Immunohistochemistry is most useful in differentiating epithelial mesothelioma from other types of an epithelial neoplasm. Epithelial mesotheliomas characteristically express broad spectrum cytokeratin, cytokeratin 5/6, cytokeratin 7 and about 5 to 10% will show staining for cytokeratin

20. Epithelial mesotheliomas likewise express calretinin in a nuclear and cytoplasmic distribution and show cell membrane staining for HBME-1 and epithelial membrane antigen. About 20% of epithelial mesotheliomas show cell membrane staining for BerEP4 and thus finding a BerEP4 positive tumor does not rule out mesothelioma. Occasional epithelial mesotheliomas show diffuse cell membrane staining for BerEP4. Other antibodies that are used to diagnose epithelial mesothelioma include thrombomodulin, WT-1, mesothelin and N-Cadherin. The antibodies we use in evaluating mesothelioma are shown in tables 3 and 4.

Immunohistochemistry is much less useful in sarcomatoid mesotheliomas, although in the majority of cases, the neoplastic spindle cells coexpress broad spectrum keratin and vimentin. In approximately 30% of the cases, the spindle cells express cytokeratin 7 and only rarely do the neoplastic spindle cells express cytokeratin 5/6. Vimentin staining is seen in essentially 100% of sarcomatoid mesotheliomas. About 30 to 40% of sarcomatoid mesotheliomas express alpha actin. The intensity of the staining can vary from being low intensity to high intensity. Rare sarcomatoid mesotheliomas do not express keratin.

As time has progressed, epithelial and sarcomatoid mesotheliomas have been identified to express other substances including a number of “cluster designation” antigens. Also, epithelial mesotheliomas express neuroendocrine markers. Small cell mesotheliomas are characteristically stated to not express neuroendocrine markers, although I have seen at least one case where the small cell mesothelioma expressed neuroendocrine markers and also expressed typical epithelial markers of mesothelioma, specifically calretinin and CK5/6. Caution is urged in interpreting immunohistochemical markers and it is always better to do a fairly large battery of tests in trying to determine if the tumor is a mesothelioma or some other type of neoplasm.

ULTRASTRUCTURAL FEATURES

As the majority of people attending this conference know, electron microscopy is extremely useful in diagnosing mesothelioma, primarily well to moderately well-differentiated epithelial mesotheliomas. These mesotheliomas characteristically have fairly long sinuous microvilli that are not covered by a glycocalyx. They are not associated with rootlets in the underlying tumor cells and characteristically do not contain mucus granules. Epithelial mesotheliomas frequently show large desmosomes and prominent junctional complexes. They not infrequently show what is referred to as microvillous matrix interaction in which the microvilli directly “penetrate” adjacent collagen fibers. The tonofilaments that are identified in neoplastic epithelial mesothelial cells frequently are in a perinuclear distribution, although sometimes they are distributed throughout the cytoplasm. Remember that some primary pulmonary adenocarcinomas have long microvilli, but these microvilli are invariably covered by a glycocalyx. Epithelial mesotheliomas frequently form intracellular canaliculi that is not a specific finding, but may be more common in epithelial mesothelioma than pulmonary adenocarcinoma. Epithelial mesotheliomas produce excess amounts of hyaluronic acid that appears as a medium electron dense material that covers the microvilli. The proteoglycan granules are not specific for mesothelioma, but are not infrequently seen in glandular lumens of mesothelioma and by electron microscopy have a somewhat stellate appearance and are electron dense.

Mucin positive epithelial mesotheliomas are frequently associated with extracellular and sometimes intraluminal crystalloid structures that in my experience are 100% unique for mucin

positive epithelial mesotheliomas. These crystalloid structures occasionally can be seen in the cytoplasm of the neoplastic mesothelial cells. In cross section, they somewhat resemble chrysotile asbestos fibers in that have a scroll like appearance.

Rare mesotheliomas have a Gauchier-like appearance that ultrastructurally is associated with a unique crystalloid material within the cisternae of the rough endoplasmic reticulum of the neoplastic cells. These often form large scroll-like structures that in my experience are unique for mesotheliomas.

DIFFERENTIAL DIAGNOSIS

Epithelial mesotheliomas have to be differentiated from adenocarcinomas and other epithelial neoplasms. Small cell mesotheliomas have to be differentiated from neuroendocrine neoplasms. There is a type of primary lung cancer called pseudomesothelioma that look identical to mesothelioma macroscopically, but are formed by tumor cells that usually show glandular differentiation and have the characteristic features of an adenocarcinoma. Sometimes, these tumors can be metastatic from sites outside of the chest cavity and can be a difficult diagnostic dilemma. With respect to sarcomatoid mesotheliomas, one has to be aware that sarcomatoid carcinomas of the kidney and pancreas can metastasize to the lung and form a macroscopic pattern characteristic of a mesothelioma (pseudomesotheliomatous metastatic sarcomatoid carcinoma).

Some synovial sarcomas fairly extensively involve the pleura and can be extremely difficult to differentiate from a sarcomatoid mesothelioma or a biphasic mesothelioma. With respect to biphasic mesothelioma, the epithelial component of a synovial sarcoma can have many of the same immunostaining patterns as an epithelial component of a mesothelioma. In cases where this is a question of synovial sarcoma, cytogenetic studies are the only certain way to determine if a tumor is or is not a synovial sarcoma.

A number of other rare sarcomatoid tumors occur in the pleura are pseudomesotheliomatous epithelioid hemangioendotheliomas, primary desmoid tumors of the pleura, calcifying fibrous pseudotumor of the pleura, primary pleural thymomas and pleural pulmonary blastomas.

Lymphomas rarely involve the lung and pleural surface. When they do, they can occasionally be mistaken for a mesothelioma, although with immunohistochemistry and EM, this usually is not a problem.

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TABLE 1

Epithelial Mesothelial Subtypes

1. Tubulopapillary
2. Glandular
3. Histiocytoid
4. Adenoid cystic
5. Microcystic
6. Macrocystic
7. Signet ring
8. Single file
9. Diffuse – NOS
10. Glomeruloid
11. In association with excessive amounts of hyaluronic acid/proteoglycan
12. Small cell
13. Poorly differentiated (Large cell)/Pleomorphic
14. Deciduoid
15. Mucin Positive
16. Gaucher cell-like
17. In-situ

MACROSCOPIC, HISTOLOGIC, HISTOCHEMICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL FEATURES OF MESOTHELIOMA

Samuel P. Hammar, M.D.

18. TABLE 2

ANTIBODY DIRECTED AGAINST	***CLONE***	***CHARACTERISTICS OF ANTIGENS RECOGNIZED***	***IMMUNOGEN***	***MANUFACTURER***	***DILUTION***	***TYPE OF ANTIGEN RETRIEVAL***
Keratin	AE1/AE3	Keratins – Moll numbers 1-5, 6, 8, 9, 10, 14-16, 18	Human Epidermal Keratin	DAKO	1:200	HIER
Keratin	MAK-6	Keratins – Moll numbers 8, 14-16, 18 and 19	Extracellular antigen from MCF-tissue culture and from human sole epidermis	Zymed	1:100	HIER
Keratin	CAM5.2	Keratins – Moll numbers 8 & 18	Colorectal cancer cell line	Becton-Dickinson	1:100	HIER
Keratin	35βH11	Keratin – Moll number 8	Hep3B hepatocellular carcinoma cell line	DAKO	1:50	HIER
Keratin	34BE12	Keratins – Moll numbers 1, 5, 10 and 14	Human stratum corneum keratin	DAKO	1:100	HIER
Cytokeratin 5/6	D5/16B4	Keratins – Moll numbers 5, 6, and to a slight degree, 4	Purified cytokeratin 5	Biocare Medical	1:100	HIER
Cytokeratin 7	OV-TL 12/30	Keratin – Moll number 7	OTN 11 ovarian carcinoma cell line	DAKO	1:100	HIER
Cytokeratin 20	K 20.8	Keratin – Moll number 20	Villi of human duodenal mucosa	DAKO	1:100	HIER
Vimentin	Vim3B4	Intermediate filament 57 kilodaltons	Vimentin from bovine eye lens	DAKO	1:100	HIER
Alpha Actin	1A4	Alpha-smooth muscle isoform of actin	N-terminal decapeptide of human α smooth muscle actin	DAKO	1:100	HIER
Muscle Specific Actin	HHF35	42 kd protein in preparations of purified skeletal muscle actin and extracts of aorta, uterus, diaphragm and heart	SDS extracted protein fraction of human myocardium	DAKO	1:400	HIER
Desmin	D33	53 kd intermediate filament in muscle cells, recognizing 18 kd rod piece of molecule	Desmin purified from porcine stomach	DAKO	1:80	HIER
Calretinin	-----	29 kd calcium-binding protein	Human recombinant calretinin	Zymed	1:50	HIER
Mesothelioma antigen	ABME-1	Antigen present in membrane of mesothelial cells	Suspension of human mesothelial cells from malignant epithelial mesothelioma	DAKO	1:500	HIER
Thrombomodulin	1009	Transmembrane glycoprotein of 75 kd molecular weight containing 6 repeated domains homologous with epidermal growth factor	Recombinant thrombomodulin	DAKO	1:50	HIER
Epithelial Membrane Antigen (EMA)	E29	250-400 kd glycoprotein of milk fat globule protein family	Delipidated extract of human milk fat	DAKO	1:100	HIER
Human Milk Fat Globule Protein-2 (HMFG-2)	115D8	MAM-6 mucus glycoprotein of > 400 kd in glycocalyx of epithelial cells	Purified human milk fat globule protein	BioGenex	1:25	HIER
N-Cadherin	389	Transmembrane glycoprotein involved in calcium dependent cell adhesion	Intracellular domain of chicken N-cadherin	Zymed	1:100	HIER
Polyclonal Carcinoembryonic Antigen (CEA)	-----	CEA and CEA-like proteins including nonspecific cross-reacting substance and biliary glycoprotein	Human CEA isolated from metastatic colonic adenocarcinoma	DAKO	1:16,000	HIER
CD15 (LeuM1)	C3D-1	3-fucosyl-N-acetyl-lactosamine	Purified neutrophils from normal human peripheral blood	DAKO	1:20	HIER
Tumor Associated Glycoprotein	B72.3	Tumor-associated glycoprotein of wide variety of human adenocarcinomas	Membrane-enriched fraction of metastatic breast cancer	BioGenex	1:100	HIER
Human Epithelial Antigen	Ber-EP4	34-& 49 kd glycoproteins on the surface and in	MCF-7 cell line	DAKO	1:100	HIER

		cytoplasm of most epithelial cells, except squamous epithelium, hepatocytes and parietal cells				
Thyroglobulin	-----	Thyroglobulin	Thyroglobulin from human thyroid glands	DAKO	1:16,000	HIER
Thyroid Transcription Factor (TTF-1)	8G7G3/1	40 kd member of NKχ2 family of homeodomain transcription factors	Rat TTF-1 recombinant protein	Biocare Medical	1:200	HIER
Prostate Specific Antigen (PSA)	ER-PR8	33 kd prostate specific antigen	Purified human prostate specific antigen	DAKO	1:100	HIER
Prostatic Acid Phosphatase (PAP)	PASE/4LJ	52 kd human prostatic acid phosphatase	Purified prostatic acid phosphatase from human seminal plasma	DAKO	1:16,000	HIER
Human Epithelial Related Antigen	MOC-31	40 kd transmembrane glycoprotein present on most normal and malignant epithelial cells	Neuraminidase treated cells from small-cell carcinoma cell line	DAKO	1:50	HIER
Lewis Y Antigen	BG8-F3	Difucosylated tetrasaccharide found on type 2 blood group oligosaccharide	SK-LU-3 lung cancer cell line	Signet	1:40	HIER
E-Cadherin	4A2C7	Transmembrane glycoprotein in calcium-dependent cell adhesion	Recombinant protein of human E-cadherin	Zymed	1:100	HIER
Gross Cystic Disease Fluid Protein-15 (BRST-2)	D6	Pathologic secretion of breast composed of several glycoproteins including 15 kd monomer protein	Gross cystic disease fluid protein-15	Signet	1:50	HIER
Estrogen Receptor Protein	1D5	86 kd protein member of nuclear hormone receptor that act as ligand-activated transcription factors	Human recombinant estrogen receptor protein	Biocare Medical	1:200	HIER
c-erbB-2 Oncoprotein	-----	190 kd protein product of c-erbB-2 proto-oncogene	Synthetic human c-erbB-2 oncoprotein peptide	DAKO	1:500	HIER
Human Leukocyte Antigen CD45	DAKO-LCA	Five or more high molecular weight glycoproteins on the surface of the majority of human leukocytes	Human peripheral blood lymphocytes maintained in T-cell growth factor	DAKO	1:200	HIER
CD20 Human B Lymphocyte Antigen	L26	33 kd non-glycosylated membrane spanning protein	Human tonsil B lymphocyte	DAKO	1:800	HIER
CD3 Human T Lymphocyte Antigen	-----	Intracytoplasmic portion of CD3 antigen	Synthetic human CD3 peptide	DAKO	1:100	HIER
CD30 Ki-1 Antigen	Ber-H2	120 kd transmembrane glycoprotein	Co cell lines cells	DAKO	1:20	HIER
bcl-2 Oncoprotein	124	25 kd integral protein localized in mitochondria that inhibits apoptosis	Synthetic peptide sequence amino acids 41-54 of bcl-2 protein	DAKO	1:20	HIER
Neuron-specific Enolase	-----	Gamma subunit of enolase	Neuron-specific enolase isolated from human brain	DAKO	1:400	HIER
Chromogranin-A	DAK-A3	Member of secretogranin/chromogranin class of proteins in secretory granules of endocrine and neuron cells	C-terminal 20 kd fragment of chromogranin-A	DAKO	1:100	HIER
Synaptophysin	-----	38 kd membrane component of neuron synaptic vesicles	Synthetic human synaptophysin peptide coupled to ovalbumin	DAKO	1:100	HIER
S100 Protein	-----	S100 Protein A and B	S100 protein isolated from cow brain	DAKO	1:3000	HIER
Melanoma Antigen	HMB45	Neuraminidase-sensitive oligosaccharide side chain of glycoconjugate in immature melanosomes	Extract of pigmented melanoma metastases from lymph nodes	DAKO	1:200	HIER
CD34	My10	105-120 kd single-chain transmembrane glycoprotein associated with human hematopoietic progenitor cells	CD34 antigen	Becton-Dickinson	1:50	HIER
CD31	JC/70A	100 kd glycoprotein in endothelial cells and 130 kd glycoprotein in platelets	Membrane preparation of spleen from patient with hairy cell leukemia	DAKO	1:40	HIER
Factor VIII Antigen	-----	Human von Willebrand Factor	von Willebrand factor isolated from human plasma	DAKO	1:2000	HIER

TABLE 3

ANTIBODY DIRECTED AGAINST														
TYPE OF NEPLASM	AE1/AE3 Ker	LMWK	HMWK	Ker 7	Ker 5/6	CEA	CD15/ LeuM1	B72.3	BerEP4	TTF-1	Calretinin	HBME-1	EMA	HMFG-2
Well-moderately well differentiated epithelial mesothelioma	+	+	+	+	+/-	R	R	R	-/+	N	+/-	* +/-	* +/-	* +/-
Well-moderately well differentiated pulmonary adenocarcinoma	+	+	+/-	+	R	+	+/-	+/-	+/-	+/-	R	R	** +/-	** +/-

Abbreviations:

LMWK = low molecular weight keratin
HMWK = high molecular weight keratin
CEA = carcinoembryonic antigen
TTF-1 = thyroid transcription factor-1
EMA = epithelial membrane antigen
HMFG-2 = human milk fat globule protein-2

Reactivity:

+ almost always diffuse strong positivity
+/- variable staining, mostly positive
-/+ variable staining, mostly negative
R rare cells positive
N almost always negative

Note:

*** Cell membrane distribution**
**** Cytoplasmic distribution**

TABLE 4

CYTOKERATIN MOLL NUMBER, MOLECULAR WEIGHT AND ISOELECTRIC pH																				
TYPE OF NEPLASM	1 68 kd 7.8	2 65.5 kd 7.8	3 63 kd 7.5	4 59 kd 7.3	5 58 kd 7.4	6 56 kd 7.8	7 54 kd 6.0	8 52.5 kd 6.1	9 64 kd 5.4	10 56.5 kd 5.3	11 56 kd 5.3	12 55 kd 4.9	13 54 kd 5.1	14 50 kd 5.3	15 50 kd 4.9	16 48 kd 5.1	17 46 kd 5.1	18 45 kd 5.1	19 40 kd 5.2	20 46 kd
Primary Pulmonary Adenocarcinoma	N	N	N	N	N	N	+/-	+/-	N	N	N	N	N	N	N	N	N	+/-	+/-	R
Epithelial Mesothelioma	N	N	N	N	+/-	+/-	+/-	+/-	N	N	N	N	N	+/-	N	N	+/-	+/-	+/-	R
Primary Pulmonary Squamous Cell Carcinoma	N	N	N	+/-	+/-	+/-	R	-/+	N	N	N	N	N	+/-	-/+	-/+	+/-	-/+	+/-	R

Reactivity Designation:

+	**almost always diffuse strong positivity**
+/-	**variable staining, mostly positive**
-/+	**variable staining, mostly negative**
R	**rare cells positive**
N	**almost always negative**

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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. Chyrsolite 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 crocridolite, 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 (1/400th 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, RASSFIA 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 inactives 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 inactive 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 (RASSFIA, 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_r) 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 place 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 proceeded 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 anti-oxidants, 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 an d18q
  - 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 synthease)
- *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

	Gene Methylation		
	All Tumors	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)	=>	SV40 T-ag**
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		

*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
    - $p27^{kip1}$ Low
    - p21 Low
    - C0X2 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

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Biologic, Cytogenetic, and Molecular Factors in Mesothelial Proliferations

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ABSTRACT Although mesothelioma cases may have peaked in the 1990s 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, in-depth 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.

KEYWORDS biology, cell cycle, cytogenetics, epidemiology, growth factors, mesothelioma, molecular pathology, SV40, tumor suppressor genes

Mesotheliomas tend to be aggressive tumors that arise from the serosal surface cells lining the pleura, peritoneum, and pericardium [1–6]. The majority (80%) of these tumors are associated with exposure to asbestos fibers, in either the environment or the workplace. 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 2000 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 (20–40 years). Males are at a much higher risk for mesothelioma than females due to occupational exposure (plumbers, pipefitters, insulation installers, shipyard workers). Although mesothelioma incidence in the United States peaked in the mid-1990s, it is estimated that over 70,000 mesothelioma cases will occur in U.S. 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 [1–10]. Chrysolite is a long, curly, serpentine fiber. This fiber accounts for

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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 5–10% 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 (1/400th asbestos burden) compared with chrysolite. Naturally occurring airborne 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, it continues to be used at an alarming rate in Southeast Asia and China [1, 3–5, 9]. 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 [3–10]. 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 [3–5]. Asbestos is thought to act as a tumor promoter and may facilitate tumorigenesis in synergy with other carcinogens [6–10]. 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) [3–32]. 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 do occur more

TABLE 1 **Cellular and Extracellular Factors in Mesothelial Proliferations**

Growth factors and cytokines
PDGF-BB
HGF
EGFR
Telomerase
G-CSF
GM-CSF
IGF-1
INF-γ
TNF-α
TGF-α and -β
IL1-β
IL-6
IL-8
MIP-1α
MIP-2α
Plasminogen activator
MMP-1
KGF
Angiogenic factors
VEGF
FGF-1
FGF-2
TGF-alpha
Fibronectin
Laminin
Type IV collagen
Tenascin
Proliferation and apoptosis factors
Bcl-10
Bcl-X
Bax
Bcl-2
Caspases
P53
Mcl-1
P21
P27
Syndecan-1
Antioxidants
MN-SOD
Cu/ZN-SOD
Catalase
GPx

Source. Compiled from references [3–10].

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 6q 15-21 (45%)
  - LOH from 6q (61%; 6q 14-21, 6q16.6-21, 6q21-23.2, 6q25)
  - Loss of 9p (83%, particularly 9p21-22 [p 16/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 an d 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, 1p33, 2q24, 8q24)
  - *Ras* (rat sarcoma virus, 8q24)
  - *Raf* (ras-activated fragment, 11p15)
  - *Rassf1a* (3p21.3, Ras GTPase family, 3p21)
  - *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, 7q21)
  - *COX-2* (cyclooxygenase-2)
  - *NOS2* (nitric oxide synthease)
  - *E-cadherin* (16q22)
  - *β-Catenin* (3q22)
  - *PDGF-BB* (platelet-derived growth factor-BB, 22q12)

(*Continued*)

TABLE 2 Continued

c-fos (14q24)
c-jun
MLH1 (8q24)
TSP1 (15q15)
TEP1 (14q11)
SDCCAG1 (14q22)
SV40 large T-antigen (Tag)
Tumor suppressor genes
CDKN (9p 13-22)
P16 (9p21-22, CDKN2A, 70%)
P15 (9p21, CDK4, 70%)
MTAP (9p 13-22)
NF2 (22q12, 41-72%)
TP53 (17p13, not related to asbestos)
WT1 (11p13, uncommon, SV40)
RB1 (13q14downstream inactivation)
MDM2 (12q14.3-q15, overexpression)
FHIT (3p14.2, inactivation)
Cyclin D1 (11q13)
TERT (5p15.3)

Source. Compiled from references [3–32].

frequently with mesotheliomas, but these cannot 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) [3–32]. 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) [3–6, 11, 14–32]. 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) [1–5, 33–38]. SV40 is a DNA tumor virus with transforming ability that contaminated polio and adenovirus vaccines in the 1950s and 1960s. 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 (Tag) stimulates host cells to replicate by entering into the S phase of the cell cycle, and is considered the

TABLE 3 Mesothelioma and SV40 Infection

Contamination of polio (1955–63) and adenovirus (1961–65) vaccines with SV40		
SV40 seroprevalence	2–20% 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 Tag associated with tumors in animals and humans		
Mesothelioma		
Osteosarcoma		
Brain tumors		
Non-Hodgkin 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		
1000-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
Non-Hodgkin lymphoma	35.8%	5.4
Controls	4.7%	1.0

Source. Compiled from references [1–5, 33–38].

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 Tag is found in a high proportion of mesotheliomas (about 50%), primary brain tumors (21%), non-Hodgkin lymphomas (36%), and osteosarcomas (Table 3) [1–5, 33–38]. 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 Tag protein (p53, RASSFIA tumor suppressor genes). Other cell signaling and transduction factors are also upregulated (Notch-1, met).

Of interest is the synergy between asbestos and SV40 [1–5, 7–10, 33–38]. 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 these animal models do not occur. However, a recent Syrian hamster model has shown that SV40 induces diffuse malignant mesothelioma and sarcomas in over 35% of animals with intraperitoneal inoculation with SV40. The presence of SV40 within the tumor cells was confirmed with immunocytochemistry and direct immunofluorescence studies. Both epithelial and biphasic mesotheliomas were created with the following immunophenotypes: HBME1 (100%), AE1/AE3 (88%), mesothelin (75%), CAM 5.2 (50%), calretinin (25%), EMA (13%), CK 5/6 (0%), and CEA (0%). This animal model without asbestos exposure may serve as a means for understanding oncogenesis and therapeutic management of mesotheliomas. None of the controls developed tumors.

A recent investigation comparing SV40 Tag protein expression using molecular and immunocytochemical means provides interesting results [37]. Comparison with mesotheliomas from the United States with "environmental" mesotheliomas from South Central Turkey were carried out. Immunocytochemistry demonstrated SV40 Tag protein in 92% of United States mesotheliomas, 82% from Middle Anatolia, and 15% from Southern Anatolia. By polymerase chain reaction (PCR), SV40 DNA sequences were detected in 50% of United States cases, 18% of Middle Anatolia, and only 7.5% of Southern Anatolia. SV40 was detected in mesotheliomas to a variable degree from all 3 geographic locations. It was concluded that geographical conditions, type of asbestos fiber inhaled, and genetic characteristic of the population may play a role in mesothelioma development. SV40 contaminated polio vaccine explains the viral source in the United States [1–5, 33–36, 38], but does not provide a reason for the virus in Turkey and the discrepancy between Middle and Southern Anatolia [37].

TUMOR SUPPRESSOR GENE METHYLATION AND MESOTHELIOMA

Gene promoter methylation, along with resultant histone deacetylation, does not alter chromatin structure, but inactives or silences the methylated gene [33–38]. 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 inactive 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 [3–38]. 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

TABLE 4 **Methylation of Regulatory Pathway Genes in Mesotheliomas and SV40 Infection**

	Gene methylation (%)		
	All tumors	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

Source. Compiled from references [33–38].

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 (RASSFIA, HPP1, DcR1, TMS1, CRBP1, HIC-1, RRAD) during serial passage of mesothelial cell lines [33–36, 38]. With mesotheliomas analyzed from 50 patients with follow-up (range 2–68 months, median 14.5 months), it was noted that methylation of TMS1 or HIC1 leads 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), which 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 [3–10, 14–18, 24, 26, 28]. 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 upregulated and 26 genes that are downregulated out of over 4000 genes studied [14].

Typical analyses reveal genes that participate in glucose metabolism, mRNA translation, and cytoskeletal remodeling [3–10, 14–18, 24, 26, 28]. Perhaps more importantly, these studies are beginning

TABLE 5 **Stepwise Process to Mesothelioma Development: A Several Decades' Oncogenesis**

Normal mesothelial cell		
Loss of chromosome 9p (p14, 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 (AP2, MDM2)	=>	SV40 T-ag**
Later phase of mesothelial proliferation		
Loss of chromosome 3p (Rassf1a, FHIT)	=>	
Mesothelioma		
Loss of chromosome 13q and 14q (Rb, FOS, TEP1)	=>	
Aggressive mesothelioma		
Loss of Chromosomes 1q, 1p and 4q	=>	
(N-Ras, Rho-C, bcl 10, COX2, VEGF, FGF2, FAT, BSP)		
Metastatic mesothelioma		

*Malignant transformation.
**SV40 identified in about 50% of mesotheliomas.
Source. Compiled from references [3, 4, 6, 14–18].

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 upregulated 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 β-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_r) 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 upregulated [3–10, 14–18, 24, 26, 28, 30–32]. The mitogen-activated protein kinase cascade (JNK1, NIK, TRAF2, PAK1, ERK5 genes), notch signaling pathway (JAGGED1, JAGGED2 genes), ras signaling pathway (RAS, RASSF1A, RAF, NORE1, CYCLIN D1, AKT), and Wnt-frizzled signaling pathway (SARP1, FRIZZLED, Dickkopf-1, Disheveled, β-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 upregulation of cyclin genes (cyclin D1 at 11p13, cyclin D3 at 6q21, CDK phosphatase). Several genes are altered in mesothelioma and affect cell proliferation via the cell cycle pathway (p53, pRb, MDM2, TGF-alpha, EGF-receptor, HGF, PDGF, p14, p16, as well as SV40 Tag).

Certain gene profiling studies have compared expression between mesothelioma and lung cancer [16]. Results have been encouraging in differentiating between mesothelioma and lung cancer using such methods. Using 15 ratios between upregulated 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

Gene profiling studies [14–16] illustrate 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, 4 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 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 on 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 path to mesothelioma development is thought to be a long and winding road. Many cytogenetic and molecular events occur along the way (Table 5) [3, 4, 14–18]. 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 proceeded 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 chromosomes 1q, 1p, and 4q is 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 [39–41]. Mesothelin is a 40-kDa 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). Nonmesothelial pleural disease and malignant nonpleural lung disease rarely have detectable serum mesothelin levels (1/68). Only 2 of 92 patients with inflammatory nonpleural disease have detectable mesothelin in their serum. In a 5-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 (>3 cm). 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 7 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 sulfate proteoglycan family member [12, 13]. 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 [42]. 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 [43–45]. 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 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 (ZD 1839) 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. More recently, it has been shown that EGF receptor is amplified in 38% of epithelial, mixed, and sarcomatoid mesotheliomas. The EGF receptor copy number in tumor cells averaged 6 to 7 in amplified tumors. It was noted that the rate of gene amplification appears to be lower than EGF receptor protein overexpression in mesotheliomas. This suggests that in addition to gene amplification other mechanisms of EGF receptor protein upregulation are involved. Immunocytochemical detection of protein expression would be preferred in selection of patients to be treated with EGF receptor antibody therapy. Assessment of mesotheliomas for EGF receptor expression may become a standard of care in the future to determine if the combination of EGF receptor inhibitor administration and radiation therapy would be feasible in individuals with mesothelioma.

Although not proven to be of prognostic significance yet, new markers may help to distinguish mesotheliomas from nonsmall cell lung carcinomas [46]. A recently introduced antibody, D2-40, which is a novel monoclonal antibody that reacts with lymphatic endothelium, has now been shown to immunoreact with epithelial mesotheliomas (71% of cases). These mesotheliomas have a moderate to strong granular membranous and cytoplasmic pattern of staining in 70–100% of tumor cells. Only

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
$p27^{kip1}$ low
p21 low
C0X2 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
Antioxidants
Catalase low
Mn SOD low
Tumor metabolism
High uptake on PET scan
SV40 sequences deteceted
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

Source. Compiled from references [1, 3, 5, 6, 14, 15, 17].

squamous cell carcinomas of the lung reacted with D2-40 in a weak pattern. Squamous cell carcinoma may be distinguished from mesothelioma by utilizing the p63 antibody, a p53 family member [32]. P63 is reported as positive in 97% of squamous cell carcinomas of the lung, while mesotheliomas are uniformly negative. This antibody may be particularly helpful in distinguishing squamous cell carcinomas from calretinin negative mesotheliomas. Steroid and androgen targets for hormonal and pharmacologic treatment may be of importance and prove to be of prognostic significance. DAX1, a nuclear receptor and steroidogenesis regulator, is expressed in all mesotheliomas in a considerable proportion of tumor cells [47]. Likewise, androgen receptors were identified in a large percentage of tumor cells in over 85% of mesotheliomas. The high frequency of DAX-1 and androgen receptors introduces the concept of hormonal therapy as an adjunct in the management of mesotheliomas.

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) [1, 3, 5, 6, 14, 15, 17]. Host-related factors indicative of poor outcome include poor WHO performance status, weight loss and male gender. Tumor-related factors include nonepithelial 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 1990s 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, in-depth 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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Extrathoracic Mesothelial Proliferations and Their Mimics

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INTRODUCTION

Mesothelial proliferations occurring in extrathoracic sites share many epidemiological and morphological features with their thoracic counterparts. However, they also have important clinical and pathological differences, relating to their anatomical location, the organs and tissues they may involve, and the spectrum of non-mesothelial tumors that may arise in these regions. The application of electron microscopy (EM) and immunohistochemistry (IHC) to their differential diagnosis must, therefore, take these peculiarities into consideration. This review is focused on mesothelial neoplasms arising in the peritoneum and in the testis and paratesticular structures, as well as on the main tumors that may be confused with them.

EM AND IHC IN THE IDENTIFICATION OF MESOTHELIAL DIFFERENTIATION: ADVANTAGES, PITFALLS, AND LIMITATIONS

EM has been the classic, gold standard tool used to confirm or identify mesothelial differentiation. Highly sensitive and specific diagnostic clues include the characteristically long and tortuous, occasionally dichotomized microvilli, with scanty filamentous cores, well-developed desmosomes, and tonofilament bundles [1-5]. In contrast, adenocarcinomas have much shorter, slender microvilli, with denser filamentous cores, forming core rootlets in the apical cytoplasm. These features are characteristic of gastrointestinal and other mucinous adenocarcinomas, but they can be less prominent in other sites, such as the breast, thyroid, urinary bladder, prostate or endometrium. Two well known disclaimers of EM are that it does not allow the identification of cells as neoplastic, nor does it distinguish between benign and malignant tumor cells. In addition, careful correlation with light microscopy is essential, because reactive mesothelial cells, admixed with metastatic tumor cells, may be erroneously identified as the neoplastic population. Keeping all this in mind, distinguishing between mesothelioma and adenocarcinoma by EM is a relatively easy task. However, prospective studies, which address the diagnostic accuracy of EM through a long series of unselected, clinically well-documented mesotheliomas, are lacking. Specific diagnostic, ultrastructural features are gradually lost in

less-differentiated, solid, or sarcomatoid varieties; thus, they require careful sampling and an extensive search. Nevertheless, and in spite of the lack of quantitative data, it can be stated that EM allows the identification of mesothelial differentiation, either focal or widespread, in most cases [1,4,6].

There is a plethora of reports dealing with the application of IHC to the differential diagnosis of mesothelial proliferations, however the ideal antibody or combination of antibodies has not yet been found [7-23]. Due to the great variation from one study to another, it is difficult to obtain precise figures on the sensitivity and specificity of the antibodies used in the differential diagnosis between mesothelioma and adenocarcinoma. A recently developed website, contains information on the application and sensitivity of many antibodies, pooled and averaged from many references (www.immunoquery.com). Table 1 is the result of a search on this website, regarding the overall sensitivity of the antibodies most commonly used in the diagnosis of mesothelioma. Some antibodies show a high sensitivity, but then their specificity tends to be less optimal. Thus, while AMAD-2 has 100% sensitivity, according to the laboratory where this antibody was originally synthesized, it also stains around 10% of the pleural metastases [24]. Currently, calretinin, thrombomodulin, WT1 gene product, and keratin 5/6 are considered the best antibodies for the identification of mesothelial differentiation [16]. It is generally advised that, in addition to positive “markers”, the panel should also include antibodies which should be negative in mesothelial cells and which may be positive in adenocarcinoma, particularly carcinoembryonic antigen (CEA), Ber EP4, Leu M1, MOC31 or B72.3 [13-15]. Many other antibodies have been used in the past, and new antibodies are being tested, but the definitive optimal markers are yet to be identified. Thus, D2-40, a recently developed antibody for germ cell neoplasia and lymphatic endothelium, has been shown to be positive in around 96% of the mesotheliomas and reactive pleural lesions, but it is also positive in 65% of the tested ovarian serous carcinomas [25]. Most studies, dealing with the diagnostic value of putative mesothelial markers, fail to include EM as their gold standard [26]. Thus, the diagnostic accuracy of new antibodies is compared with that of the old ones without an external control method to confirm the diagnosis. EM is not a generally available technique but it should be included, whenever

possible, in the work-up of peritoneal and testicular tumors, particularly in uncommon tumors or in cases with negative or paradoxical immunohistochemical results. As a general approach for the diagnosis of most peritoneal mesotheliomas, Ordóñez has suggested a panel of two positive (calretinin and cytokeratin 5/6) and two negative (CA19-9 and MOC-31) antibodies. When this panel fails to solve the case, he prefers EM, rather than a second panel of antibodies, to reach the correct diagnosis [16].

These general remarks apply to all mesothelial proliferations, but the situation is further complicated in peritoneal and testicular sites, due to the presence of embryologically related, but distinct, epithelial components. Thus, the so-called secondary Müllerian system and the epithelium covering the testicular appendages form a continuum with the respective mesothelial linings. This explains the occurrence of tumors with non-mesothelial features in these serosal surfaces. Some of the main antibodies used to identify mesothelial differentiation in the pleura may be positive in tumors derived from abdominal organs or the testis (Table 2), and therefore specific immunohistochemical strategies should be designed to address the differential diagnosis of extrathoracic mesotheliomas. In many of these cases, contribution of EM may be crucial to attain the correct diagnosis.

TABLE 1. SENSITIVITY OF ANTIBODIES COMMONLY USED FOR IDENTIFYING MESOTHELIAL DIFFERENTIATION

AMAD-2	100%
CD44H	91%
Mesothelin	90%
Cytokeratin 5/6	85%
Calretinin	84%
N-Cadherin	81%
HBME-1	80%
WT1	67%
Thrombomodulin	64%
EMA	56%

(Retrieved from www.immunoquery.com)

TABLE 2. MESOTHELIAL ANTIBODIES THAT MAY BE POSITIVE IN NON-MESOTHELIAL ABDOMINAL AND TESTICULAR TUMORS.

Calretinin	Steroid secreting tumors (adrenal, sex cord)
WT1	Serous carcinoma (ovary)
Thrombomodulin	Urothelial tumors
HBME-1	Serous tumors (ovary, peritoneum, testis)
CD10	Renal cell carcinoma. Endometrial tumors
D2-40	Serous carcinoma (ovary)

NORMAL MESOTHELIUM AND OTHER PERITONEAL AND TESTICULAR LINING CELLS

Normal Mesothelium

During embryonic development, cells of mesodermal origin line the celomic cavity. Similar to epithelial cells, they cover surfaces, contain keratins, and are joined by desmosomes and tight junctions. However, these are truly hybrid cells because, in addition, they secrete hyaluronic acid, an important component of the intercellular substance of connective tissue also produced by fibroblasts, and their cytoskeleton contains vimentin. The celomic cavity will later be divided into a pleural and pericardial cavity, a peritoneal cavity, and, in the male, a derivative of the latter that will give rise to the tunica vaginalis, upon migration of the testis to the scrotum.

In all of these serosal areas, there are two different populations: mesothelial and sub-mesothelial cells. The former show all of the characteristic features, the long, tortuous, often branching microvilli, well-developed desmosomes and tight junctions, tonofilaments, abundant cytoplasmic glycogen, and basal lamina. They express several keratin antibodies (5/6, 7, AE1-AE3), vimentin, HBME-1, calretinin, and thrombomodulin, among many others. Interestingly, normal and reactive mesothelial cells may also express desmin [27]. The sub-mesothelial connective tissue contains a mixture of fibroblasts (vimentin, CD34) and mesothelial spindle cells (keratin and vimentin).

Secondary Müllerian System

The female reproductive system arises from the müllerian or paramesonephric duct. Under the influence of the wolffian or mesonephric duct, the paramesonephric duct develops in both sexes from a placode-like thickening and deepening of the celomic epithelium. This celomic epithelium gives rise to the surface epithelium of the ovary and to the so-called extraovarian peritoneum [28-30]. Thus, the concept of the secondary müllerian system implies that a certain potential towards müllerian differentiation is retained by the peritoneum in the adult. This potential may be revealed under endogenous or exogenous

hormonal stimuli, mostly in women, but also, exceptionally, in men [29,30]. This notion has been considered a histogenetic link between the normal peritoneum and the peritoneal surface tumors, showing clinical and morphological features similar to their ovarian counterparts.

Testicular Appendages

The appendix testis or hydatid of Morgagni, the appendix epididymis, the vas aberrans, and the paradidymis constitute the so-called testicular appendages [31,32]. The appendix testis is a remnant of the müllerian duct found in more than 90% of normal individuals, and as such, it is covered by a cylindrical or cubic epithelium that may contain some ciliated cells. The appendix epididymis is present in around 25% of all testes, and it almost always has a cystic structure and a columnar epithelial lining. The paradidymis and the vas aberrans are related structures, also with a columnar lining around a cystic space. Out of all of these structures, the hydatid of Morgagni represents the normal counterpart that explains the occurrence of müllerian tumors in this area.

REACTIVE MESOTHELIAL HYPERPLASIA

Irritation of serosal surfaces, with or without a subsequent effusion, induces the proliferation of mesothelial, and often sub-mesothelial cells, giving rise to a variety of hyperplastic appearances. In some instances, the degree of hyperplasia may be so severe as to suggest a malignant process, particularly when examining small biopsies [28]. Criteria for this differential diagnosis have been dealt with extensively in the literature, and are mostly based on light microscopic features [33]. The invasion of intraperitoneal organs or fat is the most reliable indicator for malignancy, but this may be mimicked by mesothelial cells entrapped in organizing granulation tissue or between fat lobules. These benign cells usually arrange themselves in a more orderly fashion and parallel to the peritoneal surface, and they are associated with perpendicular blood vessels and abundant histiocytes, but all manner of exceptions can occur. The clustering of mesothelial cells on the peritoneal surface is usually observed in mesothelial hyperplasia, but when it is organized in papillary structures or cohesive, grossly apparent nodules, malignancy should be suspected. On the other hand, mesothelial cell clusters within the stroma, particularly if arranged in cords, nodules, papillae or gland-like structures, are highly suspicious for malignancy, but reactive, entrapped mesothelial cells may also focally present with this appearance. Necrosis and nuclear atypia are more often seen in mesothelioma, but atypical cells are quite common in reactive mesothelium, and necrosis of these benign cells may occasionally occur [33,34].

IHC or EM is of little help in this differential diagnosis. Although desmin expression is more often observed in reactive mesothelial cells, it may also, very rarely, be found in mesothelioma [27,35]. An admixture of mesothelial cells with CD68-positive macrophages is characteristic of reactive mesothelial hyperplasia, but the presence of macrophages cannot be taken as evidence for the benign nature of a mesothelial proliferation [36]. p53 overexpression and EMA positivity are more often found in malignant, rather than in reactive mesothelium, but they are not helpful in individual cases [28]. As stated above, EM is an excellent tool for the study of cell differentiation, but it cannot be used to determine the neoplastic, either benign or malignant, nature of cells [34].

MESOTHELIAL TUMORS AND TUMOR-LIKE CONDITIONS

Nodular Mesothelial Hyperplasia

This is a reactive condition presenting mostly, but not exclusively, in male children, as one or several nodules that may be grossly identified in hernia sacs [37]. The nodules are composed of polygonal cells, which may be moderately pleomorphic, showing low mitotic activity and accompanied by hyperplasia of the surface mesothelium. These lesions have been shown to be mostly made up of histiocytes, with a minor component of entrapped mesothelial cells. Due to this fact, it has been suggested that the term nodular histiocytic hyperplasia is more adequate [36]. The occasional presence of multinucleated and occasionally elongated cells may lead to confusion with embryonal rhabdomyosarcoma in pediatric cases. On the other hand, these nodules may contain a variable proportion of vacuolated histiocytic cells, which may be wrongly interpreted as signet-ring cell carcinoma.

Adenomatoid Tumor

Adenomatoid tumor is a benign, usually well-circumscribed proliferation of mesothelial cells, arranged in tubules, cords and gland-like spaces. It is the most common neoplasm in the epididymis [38-40]; it is also relatively common in the Fallopian tube and uterus [41], and has been reported in other locations, including the spermatic cord, ejaculatory duct, ovary, adrenal gland [42], pancreas [43], pleura[44], and even the heart [45].

Initially considered to be of mesothelial origin by Pierre Masson, it was later the subject of some histogenetic controversy. The presence of gland-like spaces with very thin linings, mimicking lymphatic channels or early vascular structures, lead to the suggestion of a possible endothelial nature. EM and later IHC confirmed the mesothelial phenotype [46]. Considered a reactive lesion by some, there is now a general consensus on its benign, neoplastic nature.

Adenomatoid tumors present as relatively small, well-demarcated, non-encapsulated nodules with a mean diameter of 2 cm [38]. The cut surface is firm and solid with variable cystic spaces. Microscopically, cords, channels, and

microcystic spaces are lined by cuboidal or flattened cells, with vesicular nuclei and occasional nucleoli. Intracellular lumina may predominate, imparting a signet-ring appearance to the tumor, or may coalesce into larger spaces. Quite often, tumor stroma contains a prominent smooth muscle component and abundant elastic fibers [38-39]. This smooth muscle component may be particularly abundant in tubaric and uterine adenomatoid tumors [41]. In the uterus, they may be easily mistaken for leiomyomas with a peculiar, prominent blood vessel component. Although most uterine adenomatoid tumors are subserosal, some of them are totally intramural and even submucosal, and they often coexist with leiomyomas.

Ultrastructurally, the typical mesothelial features are recognized, with abundant, irregular microvilli projecting into the gland-like spaces, well-developed cell junctions, and tonofilaments [40,46]. As the correlate of these EM features, careful light microscopic examination of the luminal aspect of these cells reveals a prominent fuzzy border, often associated with the accumulation of a faint basophilic material. This may be shown to be hyaluronic acid by Alcian Blue stain with hyaluronidase digestion. The cells are positive with antibodies for cytokeratin 5/6, calretinin, EMA, AE1-AE3, and for most of the antibodies used for mesothelial cells. Antibodies for CEA, MOC-31, Ber-EP4, B72.3, and Leu M1 are usually negative. The differential diagnosis between adenomatoid tumors and endothelial cell neoplasms, particularly epithelioid hemangioendothelioma, is easily accomplished with EM, and also by showing its negative staining with antibodies for Factor VIII, CD31, and CD34 [38,39].

Benign Mesothelioma

Both in the peritoneum and in paratesticular locations, isolated papillary or multicystic proliferations of mesothelial cells are usually classified as benign mesotheliomas. The cystic cases must be distinguished from hydrocele in the testis or from cystic lymphangiomas in both the testis and the peritoneal cavity [15,38,47,50-52]. There is question regarding their true neoplastic nature. On the other hand, the benign papillary mesotheliomas must be single and relatively small lesions to be confidently classified as benign. Multifocality, the combination with solid areas, and atypia must raise the suspicion of a potentially malignant tumor. Actually, even some apparently benign lesions have evolved towards a more aggressive course [28,38,50,53].

Malignant Mesothelioma

It is defined as a proliferation of mesothelial cells with malignant morphological features and aggressive growth, arising in the peritoneal cavity, or in the tunica vaginalis and the tunica albuginea [28,38,50-53]. Patients with mesothelioma originating in one of these two sites may subsequently develop a tumor in the other. The latter are usually considered an extension of the primary lesion, rather than synchronous or metachronous tumors. When malignant mesothelioma simultaneously involves the scrotum and the peritoneal cavity, it is impossible to determine the origin of the process, although it is usually assumed to most probably be a primary peritoneal tumor. Association with asbestos exposure has been estimated in around 50% of the reported peritoneal tumors, and it is considered to be similar for the testicular cases [1,54,55].

Peritoneal mesotheliomas occur most often in men, usually over 40 years in age. Fibrous plaques are found in cases of peritoneal mesothelioma more often than in pleural mesothelioma. Clinical presentation depends on the areas and organs involved, and it is usually accompanied by ascites. Grossly, peritoneal mesotheliomas form multiple nodules or plaques, and they only occasionally present as isolated lesions. Again, microscopically, there is a wide range of appearances. The usual epithelioid mesothelioma may grow in tubules, papillae with variable psammomatous components, and solid areas. Nuclei may be

pleomorphic but are more often monotonous, either with dispersed or condensed chromatin [3,28,56-58]. Cell cytoplasms may be vacuolated, due to degenerative changes. They may also contain numerous lipid vacuoles, sometimes mimicking liposarcoma (lipid-rich mesothelioma) [59,60], or large glycogen pools, resulting in the clear-cell variety that may be confused with renal cell carcinoma [61]. Sarcomatoid mesothelioma, made up of diffusely growing spindle cells with variable degrees of pleomorphism, may predominate or occur as a component in a biphasic tumor [62,63].

Several clinicopathological varieties of peritoneal mesothelioma have been recognized. Well-differentiated papillary mesothelioma is mostly a superficial, multifocal tumor, most often found in women [28]. Thus, the main differential diagnosis will be made with peritoneal and ovarian serous carcinomas. A morphological variant of peritoneal mesothelioma that may also occur in the testis is the so-called deciduoid mesothelioma [64,65]. As the name implies, it is made up of cells with a decidualized appearance. In women, this may result in confusion with benign, sub-mesothelial decidualized nodules, and thus, the malignant nature of the lesion may be overlooked. In contrast with true decidualized cells that contain large amounts of glycogen, the appearance of these cells has been shown by EM to be the result of the prominent accumulation of a variety of organelles, including intermediate filaments, either dispersed or arranged in bundles, mitochondria, and rough and focally smooth endoplasmic reticulum [64,65]. Another variety, linfohistiocytoid mesothelioma, is characterized by the combination of tumor cells along with a variable component of lymphocytes and histiocytes [66]. A rare variety, leiomioid mesothelioma, shows the co-expression of mesothelial markers with desmin and actin [67]. All of these varieties share the same ultrastructural and immunophenotypical profile as conventional mesotheliomas.

Testicular mesotheliomas present in a wide age range, from children to elderly men, with a mean age around 50 years. Although testicular mesothelioma is rare, it is the second most common malignancy in this location, after soft tissue tumors. It usually presents either as an incidental finding during hernia repair, in association with hydrocele, with or without a clinically

detectable mass, or primarily as a palpable tumor. Grossly numerous, small papillary lesions, multiple nodules or diffuse thickening of the vaginalis or albuginea are most often found. There may be obvious signs of infiltration into adjacent structures. Microscopically, 75% of the testicular mesotheliomas are epithelial and show combinations of tubular, papillary and solid areas. In the remaining cases, a variable proportion of sarcomatoid elements are found, resulting either in a biphasic or even purely spindle-cell tumor [38,51,53,68].

Clinically, peritoneal and testicular mesotheliomas are aggressive tumors. For those arising in the peritoneum, prognosis is extremely poor, and the majority of patients die two years after diagnosis. Progression is associated with extensive infiltration to adjacent organs, and with metastases to pelvic and retroperitoneal lymph nodes [69,70]. The exception to this rule would be the well-differentiated papillary mesotheliomas arising in women, which tend to show a more protracted course. Testicular mesotheliomas tend to recur after two years of diagnosis, infiltrating other testicular and paratesticular structures, the spermatic cord, and disseminating into inguinal and retroperitoneal lymph nodes, the peritoneum, mediastinum, lungs and pleura, bones, and the brain.

DIFFERENTIAL DIAGNOSIS OF PERITONEAL AND TESTICULAR MESOTHELIOMA

Common Epithelial Tumors of the Ovary and Uterus and Their Testicular Counterpart

This is an important differential diagnosis, with remarkable therapeutic and prognostic implications. Out of all the varieties, the main difficulties may be encountered with serous papillary carcinomas of the ovary. These tumors present with variable combinations of papillary and solid areas and may extend along the peritoneal surface, forming nodules and plaques, and making it difficult to establish their ovarian origin [28,71]. Similarly, serous carcinomas of the endometrium often disseminate to the peritoneum, with relatively little or no ovarian involvement [72]. Another finding that can be misleading is the presence of mesothelial hyperplasia, in association with some serous borderline

tumors [71]. To further complicate the situation, mesotheliomas may preferentially involve the ovarian surface [58]. When topographic data are not contributory, the differential diagnosis must be based on a combination of light microscopic, immunohistochemical, and ultrastructural features. Histologically, cells in mesothelioma tend to have more monomorphous and less atypical nuclei than those of carcinomas, and they may display a more prominent tubulopapillary pattern. Mucin stains such as mucicarmin are of little use in serous carcinomas, and, on the other hand, mucicarmin-positive mesotheliomas have been reported [3]. In the rare instances in which the differential diagnosis is a mucinous ovarian neoplasm, Alcian Blue stain, with and without hyaluronidase digestion, may be a more useful technique.

The immunohistochemical study of these tumors is even more problematic than the study of those in other sites (Table 2). Out of all of the mesothelial antibodies, calretinin shows the best sensitivity and specificity for mesothelioma in this setting. However, sex cord-stromal tumors (i.e., retiform Sertoli-Leydig cell tumor) may present with peculiar papillary areas, and calretinin is typically positive in most of these cases [71,73]. This may lead to an erroneous diagnosis, if calretinin is not used in combination with other antibodies. WT1 gene product is of little practical value, as it is typically expressed by most ovarian and surface serous carcinomas, and also by over 50% of the endometrial serous carcinomas [74,75]. This finding is related to the fact that the Wilms tumor gene plays complex roles in the development of the genitourinary tract and mesothelium, thus providing additional evidence for the histogenetic relationship between müllerian epithelia and mesothelial cells. Other antibodies used for mesothelioma or carcinoma are of limited value here: thrombomodulin is reported to be positive in 56% of the mesotheliomas and 30% of the serous carcinomas, cytokeratin 5/6 in 53% of the mesotheliomas and 25% of the carcinomas, and CD44H in 47% of the mesotheliomas and 25% of carcinomas [76]. HBME-1 is also expressed in serous carcinomas, although the membranous pattern of positivity is apical, compared to the more extensive apical and lateral pattern seen in mesothelioma. In addition, only 35% of the serous carcinomas express Leu M1, 10% polyclonal CEA and 5% monoclonal

CEA [13,16,77,78]. In fact, according to Ordóñez, CA19-9 is preferable to CEA in this setting [16].

In serous carcinomas, EM reveals the presence of shorter and straight microvilli, frequent ciliated cells, and junctional complexes, instead of the isolated, large desmosomes or tight junctions seen in mesothelioma [79,80]. Junctions between mesothelioma cells are most often located closer to the basal domain, resulting in a wide circumferential area covered by the characteristic microvilli [81]. In contrast, junctional complexes tend to be located closer to the apical domain in serous carcinomas, except in cells with a prominent hobnail appearance. This explains the different patterns of staining with antibodies against membranous or glycocaliceal components of microvilli, such as HBME-1. Although EM may easily lead to the diagnosis, it is crucial to adequately select the areas of interest, in which all of the diagnostic features may be found, avoiding to erroneoulsy identify entrapped or hyperplastic mesothelial cells as being part of the tumor. Both ovarian and mesothelial tumors tend to be large, and, in poorly differentiated cases, the diagnostic areas may be focal and, therefore, missed in the initial sampling for EM. However, they may be selected and retrieved from the paraffin block, without losing the essential information, provided that the previous fixation and paraffin embedding were adequate. In summary, the differential diagnosis between peritoneal mesothelioma and ovarian tumors is clinically relevant, and it may be achieved, in most cases, by paying close attention to morphology. Difficult cases will benefit from IHC and, if available, EM confirmation.

Tumors identical to those arising on the ovarian surface, benign, borderline or malignant, may arise in the testis and paratesticular structures. These are very uncommon neoplasms with a müllerian phenotype, mostly serous, but also of mucinous, endometrioid or Brenner types, and show an immunohistochemical profile and ultrastructural features similar to their ovarian counterparts. It is assumed that these tumors have a better prognosis than mesotheliomas in this location, but this is only based on single case studies or small series [38,51,53,82].

Peritoneal Serous Tumors

There is a group of serous papillary neoplasms, benign, borderline or malignant, that present in the absence of ovarian involvement. They can be considered the equivalent of serous ovarian tumors in an extraovarian location [83,84]. Their histological features and ultrastructural appearance are identical, including cilia, short microvilli and junctional complexes [30]. The vast majority of cases occur in women, although there are isolated reports in men. It has been hypothesized that their origin could be traced through serous metaplasia of the mesothelium or endosalpingiosis, in the context of the so-called secondary müllerian system [29,30,83,84]. It is important to distinguish between a primary peritoneal serous tumor and an implant or metastasis from an ovarian primary lesion, because of obvious differences in staging. Apparently these tumors behave in a similar fashion and are susceptible to similar treatment strategies with their ovarian equivalents, and therefore, it is even more important to differentiate between a primary serous peritoneal lesion and a malignant mesothelioma. Again, a judicious combination of clinical and histological features, IHC and EM will allow the diagnosis. Also, a special note of caution applies to these tumors, as many of the antibodies that show moderate to low positivity in the ovarian tumors tend to be completely negative, or only focally and faintly positive, in many of the peritoneal ones. This is the case with CEA, either mono or polyclonal, Leu M1, or B72.3 [13,29,74,76]. In spite of their serous phenotype, many of these tumors are reported to be positive with stains for epithelial mucin, but, in the absence of other supporting features, this is not enough evidence for the diagnosis, since mucin stains may be positive in mesothelioma [3].

Rare Epithelial Testicular and Epididymal Tumors

Testicular mesotheliomas must be distinguished from a group of very uncommon neoplasms, arising in the epididymis or rete testis. Some of them are benign, and others tend to be more aggressive than conventional testicular mesothelioma. Because of their rarity, the diagnosis may be easily missed.

Papillary cystadenoma of the epididymis

This is a benign cystic tumor, arising in the epididymis and showing variable, aborizing papillary structures, that are made up of glycogen-rich, clear cells, in

which light or EM shows occasional cilia. These tumors are bilateral in about half of the cases, and particularly in patients with the Von Hippel-Lindau syndrome, in which they are much more prevalent. The main differential diagnoses for these tumors are the malignant counterpart, clear-cell carcinoma of the epididymis, and clear-cell renal cell carcinoma, which may rarely metastasize to the testis and paratesticular structures. Some areas in papillary cystadenoma of the epididymis may show features strongly reminiscent of mesothelioma, and, on the other hand, mesotheliomas may on occasion show a prominent accumulation of glycogen [61]. Therefore, all of the previously discussed immunohistochemical and ultrastructural features may be helpful in this differential diagnosis, but it is obviously essential to be aware of the entity in order to recognize it [38,48,51,53].

Adenocarcinoma of the epididymis

This is an uncommon, malignant glandular proliferation, arising in the epididymis, which presents as an occasionally painful mass, associated with hydrocele in about half of the cases. It occurs between the third and ninth decade, and may attain a variable size. It shows a tubular, tubulopapillary, or cystic growth pattern, and is made up of cuboidal or columnar cells, often containing cytoplasmic glycogen. Usually, there is no associated, desmoplastic stromal response. The main differential diagnoses include, in addition to the benign epididymal variety already discussed, adenomatoid tumor, mesothelioma, serous carcinoma, and tumors of the rete testis [38,48,51,53]. For rete testis and serous tumors, topography is important, as rete testis tumors must be seated in this structure to assume that they originate within it, and serous tumors tend to arise in the testiculo-epididymal groove. However, when the tumors are large, topography may be difficult to assess. Relatively, the easiest differential is for mesothelial neoplasms, as they may be excluded or confirmed by histological features, plus the usual ancillary techniques. Again, IHC must be used with care. Epididymal adenocarcinoma shows strong luminal staining with antibodies for EMA, and CEA has shown contradictory results, according to different authors; also, Leu M1, B72.3, Ber-EP4, as well as alpha-fetoprotein, prostatic acid phosphatase, prostatic specific antigen, and vimentin are reported to be negative in most cases [38,51]. EM reveals the classic

features of adenocarcinoma, with occasional cilia [51]. The long, branching microvilli of normal epididymis, also known as stereocilia, have not been reported in these tumors. In addition to anatomical location, the distinction between epididymal carcinoma and serous and rete testis tumors will mainly rely upon subtle histological features. There is little information on prognosis, due to the small number of published cases, but around 50% of the patients with epididymal carcinoma are reported to die with disseminated tumors, in spite of several methods of treatment.

Adenoma of the rete testis

This is also a rare tumor, with a solid, cystic or mixed macroscopic appearance, arising in the hylum of the testis. Characteristically, nodular aggregates of tumor cells project into cystic spaces. The cells form papillae, slit-like spaces, and tubules reminiscent of Sertoli cell tumors. The term sertoliform cystadenoma has been applied to those cases in which the latter component predominates. In addition, when there is stromal proliferation, the term adenofibroma is preferred [52,53,85].

Adenocarcinoma of the rete testis

This is a tumor reported exclusively in older Caucasian men, and, mainly due to its location in the posterior aspect of the testis, it is often missed in its initial stages. Related in part to this fact, adenocarcinoma of the rete testis has a poor prognosis, with frequent local and lymphatic spread, and with an average survival of 8 months after diagnosis [85,86]. Strict requirements for the diagnosis of adenocarcinoma of the rete testis, proposed by Nochomovitz and Orenstein, include the absence of an extrascrotal tumor with a similar morphology, a location centered in the testicular hylus, a morphology different from any other testicular or paratesticular tumor, microscopic evidence of transition between the rete testis and the tumor, and a predominantly solid growth, although focal cystic change is allowed [52,85,86]. Tumor cells arrange themselves in nodules and form slit-like spaces, combined with papillary, tubular, and solid areas. Cells are relatively small, lack overt pleomorphism, and have molded or grooved nuclei. Serous tumors and mesothelioma are the main differential diagnostic considerations [52,85].

EMA is characteristically positive in rete testis adenocarcinoma, Leu M1 is negative, and CEA gives contradictory results. Positivity with HBME-1 and thrombomodulin antibodies has been reported in one case that was classified as an adenocarcinoma of the rete testis [87]. As stated above, HBME-1 is known to be positive in many adenocarcinomas, and it is the pattern of positivity that helps in its distinction from mesothelioma. On the other hand, thrombomodulin is not exclusive for mesothelioma. Similar to the ovary, calretinin must be used with great caution in the differential diagnosis between mesothelioma and these testicular neoplasms, which may mimic one of the many histological patterns of sex cord tumors, in which calretinin is also positive. Ultrastructurally, rete testis adenocarcinoma is characterized by variable proportions of short microvilli, devoid of core rootlets, complex lateral interdigitations with abundant desmosomes, and characteristically indented nuclei. The cytoplasm contains variable amounts of lipid and glycogen, but lacks secretory granules. These features are similar to those of normal rete testis epithelium [88].

In summary, the use of IHC in the diagnosis of mesothelioma is a difficult and unsettled issue. In both the peritoneum and the testis and paratestis, it is further complicated by the sometimes paradoxical or unexpressive immunohistochemical phenotypes of the many tumors which enter the differential diagnosis. There is no single clue or magic marker, and therefore EM may be particularly helpful in this setting. As in many other areas of Pathology, the careful evaluation of clinical and histopathological data, along with the judicious application of IHC and EM, are required to reach the correct diagnosis.

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THE ROLE OF ANALYTICAL SEM IN THE DETERMINATION OF CAUSATION IN MALIGNANT MESOTHELIOMA

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Asbestos is a group of mineral fibers that share certain characteristics making them useful in the manufacture of a variety of products. There are two large groups of asbestos fibers: serpentine, of which chrysotile is the only asbestiform member, and amphiboles, which include five distinct mineral species. Amosite and crocidolite are amphibole fibers that were used commercially, whereas tremolite, actinolite, and anthophyllite have had limited commercial usage. These noncommercial amphiboles, however, are important as contaminants of other mineral species. A variety of non-asbestos mineral fibers may also be identified within human lung samples. The analysis of the mineral fiber content of lung tissue by means of scanning electron microscopy (SEM) has provided useful information regarding the causation and pathogenesis of malignant mesothelioma. The following is an abbreviated summary of the author's experience in this regard during the past 24 years.

Tissue Selection. In most circumstances, formalin-fixed lung tissue is utilized, although fresh specimens work just as well. In some instances, paraffin-embedded tissue is all that is available. Such samples can be deparaffinized and rehydrated. A correction factor must be applied to equate the values obtained from paraffin blocks to those obtained from formalin-fixed tissue. In the author's laboratory, the correction factor has been determined to be approximately 0.7. Areas of consolidation, congestion, or tumor should be avoided as much as possible. Since there is some site to site variation of mineral fiber content within the lung, the more tissue that is available for analysis the better. Ideal specimens include autopsy or pneumonectomy specimens, with analysis of multiple sites. In the authors' laboratory, two or three samples are typically analyzed from a pneumonectomy specimen, whereas four sites (upper and lower lobes of each lung) are sampled when both lungs are available at autopsy. Samples usually include lung parenchyma abutting against the visceral pleura, with each sample typically weighing 0.25-0.35 gm (wet weight). However, analyses may be performed on as little as 0.1 gm of wet tissue. Transbronchial biopsy specimens are unlikely to be representative.

Digestion Technique. Techniques for mineral fiber analysis generally involve three basic steps: dissolution and removal of the organic matrix material in which the fibers are embedded, recovery and concentration of the mineral fibers, and analysis of fiber content by some form of microscopy.[1] Digestion is accomplished with sodium hypochlorite solution (commercial bleach). Once digestion is complete, the inorganic residue may then be collected on a polycarbonate filter, with a pore size of 0.2 to 0.45 μ m. Use of a pore size which is too large in relation to the size of the fibers to be analyzed can result in significant loss of fibers and underestimation of the mineral fiber content of the sample.

Fiber Identification and Quantification. Conventional light microscopy is ideal for the quantification of asbestos bodies, which are counted at a magnification of 200-400x. The results are reported as numbers per gram of wet lung tissue. Alternatively, a piece of lung tissue adjacent to the one actually analyzed can be dried to constant weight to obtain a wet-to-dry weight ratio, and the results reported as asbestos bodies per gram of dry weight. As a rule of thumb, one fiber/gm wet lung ≈ one fiber/cm^3 ≈ ten fibers/gm dry lung. SEM has utility for the

quantification of fibers within the lung and identification of fiber types. The latter can be accomplished by coupling the SEM with energy dispersive x-ray analysis (EDXA) to determine the elemental composition of individual fibers. This information can be used to classify a fiber as asbestos or non-asbestos and to determine the specific asbestos fiber type. Sample preparation for SEM is relatively simple, requiring only that the filter be mounted on a suitable substrate and then coated with an appropriate conducting material.

Variability of Results. Interlaboratory comparison trials demonstrate that striking differences can occur among laboratories even when the same sample is analyzed. Some asbestos bodies and fibers may be lost during the preparation process, and some of the smallest fibers are difficult to recognize and count in a reproducible fashion. Nonetheless, there is evidence for internal consistency within individual laboratories, with similar ranking of samples among different laboratories from the lowest to the highest tissue fiber concentration. Still, one must use caution in comparing results between laboratories, bearing in mind any differences in the analytical procedures employed.[1]

Intralaboratory variation can occur due to variation in fiber content from one site to another within the lung. In the author's experience, paired samples have asbestos body and fiber concentration values ranging from identical to within a factor of two or three. Rarely, two samples from the same patient may differ by as much as a factor of 10 or more. There is a growing consensus that the fiber burden that persists in the lung is the primary determinant of subsequent disease.[1]

Malignant Pleural Mesothelioma. The author has analyzed the asbestos content of the lung in 396 patients with malignant mesothelioma. The median asbestos body count for pleural mesothelioma cases that also had asbestosis is much higher than cases that had parietal pleural plaques (PPP) without asbestosis (12,400 AB/gm vs. 845 AB/gm), which in turn is much higher than cases that had neither plaques nor asbestosis (105 AB/gm). A similar trend is observed for uncoated fibers as measured by SEM. The asbestos body content was within our normal range of 0-20 AB/gm in 74 cases, or 20% of the total. In 27 of these 74 cases, the fiber content was found to be elevated by SEM. Hence, the asbestos content was indistinguishable from that of a background population in 13% of cases. Approximately 87% of pleural mesotheliomas that we have analyzed have elevated fiber content and thus appear to be related to prior asbestos exposure.

Malignant Peritoneal Mesothelioma. The median asbestos body count is higher for patients with peritoneal as compared to pleural mesotheliomas. For cases that also had asbestosis, the median values were 132,000 AB/gm for peritoneal as compared to 12,400 per gram for pleural cases. For PPP only, the values were 350 vs. 845 AB/gm. For peritoneal cases with neither PPP nor asbestosis, the median asbestos body count was only 3.9 AB/gm. These findings are consistent with the observation that, on average, greater exposure to asbestos is necessary for the development of peritoneal mesothelioma than is needed to develop pleural mesothelioma.

Malignant Mesothelioma in Women. The median asbestos body count for 48 women with mesothelioma was 15.5 AB/gm. Twenty-seven cases had asbestos body counts within background range, and eight of these had an elevated fiber count by SEM. Hence, about 40% of mesotheliomas in women had an asbestos content indistinguishable from background. Approximately 64% or pleural mesotheliomas in women that we have studied appear to be

asbestos related. Most of these are secondary to exposure as a household contact of an asbestos worker. The majority of peritoneal mesotheliomas in women are not related to asbestos.

Mesothelioma and Fiber Type. The predominant fiber type identified in patients with mesothelioma is commercial amphibole (amosite or crocidolite). In a study of 94 cases from the United States, Roggli et al.[2] found that 58% of more than 1500 fibers analyzed were amosite, whereas only 3% were crocidolite. When cases were grouped by exposure category, more than 94% of 1445 cases fit into one or more of 12 different industrial, six different occupational, or one non-occupational categories.[3] The one non-occupational category, that of a household contact of an asbestos worker, accounted for 6% of all cases and more than half of mesotheliomas among women. Most cases with a normal-range asbestos body count and elevated fiber content by SEM had predominantly non-commercial amphiboles (mostly tremolite). These fibers typically are in the size range between 5 and 20 μ m. Asbestos bodies usually form on fibers that are greater than 20 μ m in length. Chrysotile is a much less potent inducer of mesothelioma in humans, and there is no convincing evidence that chrysotile causes or contributes to the development of peritoneal mesothelioma.[4]

Mesothelioma and Fiber Size. In view of the experimental observations that fibers 8.0 μ m or greater in length and 0.25 μ m or less in diameter are the most efficient at producing mesotheliomas, it is of interest to examine fiber dimension data in studies of human cases of malignant mesothelioma. In a study of amphibole asbestos-induced mesotheliomas, Churg and Wiggs[5] reported that 39% of amosite fibers and 23% of crocidolite fibers exceeded 5 μ m in length. In contrast, a study of chrysotile-related mesotheliomas showed that only 11% of chrysotile fibers and 13% of tremolite fibers were 5 μ m or greater in length.[6] The vast majority of fibers in both studies were less than 0.25 μ m in diameter. McDonald et al. found that amphibole fibers 8 μm or greater in length and 0.25 μm or less in diameter accounted for essentially all of the risk of mesothelioma, with no additional information provided by short fibers, chrysotile fibers, or non-asbestos mineral fibers.[7] The bio-persistence of relatively long amphibole fibers in lung tissues is the likely reason for the greater potency of amosite and crocidolite fibers in the production of mesothelioma as compared to chrysotile. The latter tends to fragment into shorter fibers and has a much shorter half-life within the lung.

Analysis of Pleural Samples. It should be noted that most of the studies of fiber burdens in mesothelioma patients have examined lung parenchyma. It is reasonable to assume that fibers actually reaching the pleura are the ones responsible for pleural disease, and the dimensions and types of fibers accumulating in the pleura are of interest in this regard. Sebastien et al. reported that in individuals exposed to mixtures of fibers, short chrysotile fibers (<5 μ m) tended to accumulate in the pleura whereas longer amphibole fibers accumulated in the lung parenchyma.[8] Suzuki and Yuen[9] also reported primarily short chrysotile fibers in the pleura and in mesothelial tissues. Dodson et al.[10] found some long commercial amphibole fibers in samples of pleural plaque from asbestos workers, and Gibbs et al.[11] also identified similar fibers in pleural samples of patients with diffuse visceral pleural thickening. Boutin et al.[12] found a preferential concentration of long commercial amphibole fibers in black spots on the parietal pleura. Clearly, fibers of the type and size known to be associated with the greatest risk of mesothelioma do in fact migrate to pleural tissues. The identification of short chrysotile fibers in these tissues is of questionable relevance, since there is no convincing data that these fibers are pathogenic.

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TABLE 1. ASBESTOS CONTENT OF LUNG IN 368 PLEURAL MESOTHELIMA CASES

	N	AB/gm	N	UF/gm
Asbestosis	**44**	**12,400**	**44**	**77,800**
Plaques	**113**	**845**	**106**	**21,800**
Other[a]	**207**	**105**	**201**	**15,200**

a. Neither plaques nor asbestosis

TABLE 2. ASBESTOS CONTENT OF LUNG IN 28 PERITONEAL MESOTHELIMA CASES

	N	AB/gm	N	UF/gm
Asbestosis	**12**	**132,000**	**12**	**328,000**
Plaques	**5**	**350**	**5**	**23,300**
Other[a]	**11**	**3.9**	**11**	**6120**

a. Neither plaques nor asbestosis