Molecular Pathogenesis of Malignant Mesothelioma
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MALIGANT MESSOTHELIOMA

- rare: 2-20 cases / 10^6 / year
- more common with exposure to amphiboles
  chrysotile factories and mines may be
  contaminated with amphiboles
- latency of 15-60 years
- no association with cigarette smoking or asbestosis
- high incidence in shipbuilding and insulation industries
- difficult pathologic diagnosis
- poor response to therapy

Jones, J. S. P. et al., Colour
Atlas of Mesothelioma, MTP

OTHER CAUSES OF MESOTHELIOMA

Human:
- irradiation
- chronic inflammation (tuberculosis)
- fibrous erionite (a hydrated aluminum silicate)

Animals:
- iron chelates* (ferric saccharate)
- potassium bromate*
- fibrous erionite
- silicon carbide fibers
- refractory ceramic fibers
- E glass microfibers

*also cause kidney cancer
Mechanisms of Asbestos Carcinogenesis

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<th>Mechanism</th>
<th>Experimental End-Points</th>
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<td>Genotoxic</td>
<td>Oxidized bases</td>
<td>Chao et al. (1966), Fung et al. (1997)</td>
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<td>DNA breaks</td>
<td>Okayasu et al. (1999)</td>
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<td>Aneuploidy</td>
<td>Reviewed in Jaurand (1996); Jensen et al. (1996)</td>
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<td>Mutations</td>
<td>Park &amp; Aust (1998); Hei et al. (1995)</td>
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<td>Non-genotoxic Mitogenic</td>
<td>Target cell proliferation</td>
<td>Bérubé et al. (1996); Goldberg et al. (1997); Mishra et al. (1997)</td>
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<td></td>
<td>Binding to or activation of surface receptors</td>
<td>Boylan et al. (1995); Pache et al. (1998)</td>
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<td>Growth factor expression</td>
<td>Liu et al. (1996); Brody et al. (1997); Kane et al. (1997)</td>
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<td>Zanella et al. (1996); Fung et al. (1997); Mossman et al. (1997)</td>
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<td>Cytotoxic</td>
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<td>Necrosis</td>
<td>Reviewed in Kane (1996)</td>
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WORKING HYPOTHESIS

- A proposed mechanism for asbestos carcinogenicity is iron-catalyzed generation of free radicals that damage cellular DNA and induce oxidant stress.

- The p53 tumor suppressor gene product is important in mediating cell cycle arrest and DNA repair in response to DNA strand breaks.

- A murine mesothelial cell line with a point mutation in p53 is defective in the G1 cell cycle checkpoint and shows increased sensitivity to asbestos genotoxicity (Cistulli et al., 1996).

- It is hypothesized that p53-deficient mice will show increased susceptibility to mesotheliomas induced by crocidolite asbestos fibers.

Cellular Responses to Oxidant-Induced DNA Damage

oxidants → DNA damage → p53 → apoptosis

ddi pathway → G1 arrest → DNA repair

DDI = DNA damage inducible
A murine model system to study the acute and chronic effects of crocidolite asbestos fibers after direct intraperitoneal injection has been developed. The inflammatory and proliferative reactions to intraperitoneal injection of crocidolite asbestos fibers have been characterized in this model. Focal areas of mesothelial injury are repaired by proliferation of adjacent, uninjured cells after a single intraperitoneal injection of crocidolite asbestos fibers. These proliferating mesothelial cells are potential targets for genetic damage, induced directly by physical interference of fibers with the mitotic apparatus or indirectly by reactive oxygen and nitrogen metabolites released from inflammatory cells. The availability of iron at the surface of fibers is a critical parameter in catalyzing the generation of these highly reactive oxygen and nitrogen radicals. Ferric and ferrous cations are major components of amphibole asbestos fibers; iron may also be present as surface impurities on serpentine asbestos or some man-made fibers. Reactive oxygen and nitrogen metabolites can damage DNA through base mutations, DNA breaks, deletions, rearrangements, insertions, and altered patterns of methylation.
Increased sensitivity of p53-deficient mice to asbestos-induced genotoxicity

Table 1  Induction of Micronuclei in Proliferating Mesothelial Cells In Vivo

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<th>Treatment</th>
<th>p53 +/+</th>
<th>p53 -/-</th>
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<tr>
<td>Saline</td>
<td>0.49 ± 0.49</td>
<td>0.28 ± 0.95</td>
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<tr>
<td>Asbestos</td>
<td>1.35 ± 0.05*</td>
<td>3.48 ± 0.52+</td>
</tr>
<tr>
<td>Wollastonite</td>
<td>0.76 ± 0.09</td>
<td>not tested</td>
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*p < 0.05 asbestos vs. saline-injected controls
+*p < 0.05 p53 +/+ vs. p53 -/- mice

Malignant mesotheliomas induced by asbestos fibers in p53-deficient mice show decreased latency and increased invasion

Figure 2

Figure 3  Papillary growth with spheroids

Figure 4  Local invasion into muscle

Figure 5  Lymphatic invasion
INACTIVATION OF p53 AND TUMOR PROGRESSION

- decreased DNA repair, genetic instability
- decreased apoptosis
- resistance to hypoxia
- increased angiogenesis

CLINICAL RELEVANCE

1. Patients with familial cancer syndromes (Wilms' tumor, Li-Fraumeni syndrome) develop mesothelioma after radiation therapy.
   Antman et al., Austin et al., 1986; Hisada et al., 1998

2. Slightly increased risk of mesothelioma in people exposed to asbestos who have first degree relatives with the Li-Fraumeni syndrome.
   Heineman et al., 1966

3. Point mutations and deletions in p53 are rare in human malignant mesotheliomas.
   Metcalf et al., 1992

4. SV40 DNA sequences and T antigen have been identified in 60% of human malignant mesotheliomas.
   Carbone et al., 1999
MOLECULAR PATHOGENESIS OF HUMAN MALIGNANT MESOTHELIOMA

fibers → ROS → normal cell → DNA damage → preneoplastic lesions → mesothelioma

?SV40

del 1p

inactivation of p53 and Rb

del 9p

p16

p19ARF

NF2

del 22


RETINOBLASTOMA PROTEIN AND p53 TUMOR SUPPRESSOR PATHWAYS

Cell stress → p16INK4a → Oncogene activation

Loss of growth-factor signaling

Transforming growth factor β signaling

D-type cycline - Cyclin-dependent kinases 4 and 6

Human double minute 2

DNA damage

Apoptosis

Simian virus 40 large T antigen

Cell cycle

Asbestos Fibers and SV40 Virus as Co-Factors for Mesothelioma
(Summarized in Science 296: 1012, 2002; INCI 94: 229, 2002)

Evidence Against

Epidemiologic studies show no association between polio vaccine and increased risk of cancer - ? statistical power

What is the route of SV40 transmission in nonvaccinated people?

PCR assays are subject to cross-contamination

Immunoassays cannot distinguish between SV40 virus and other human polyomaviruses – JC and BK

SV40 viral sequences are not found in all human mesotheliomas nor in all cells of the tumor

Evidence For

SV40 virus causes mesothelioma in hamsters

SV40 virus transforms human mesothelial cells in culture

Multiple assays detect SV40 viral sequences in human mesotheliomas – PCR, immunohistochemistry, ISH

Antisense constructs against SV40 T antigen inhibit growth of human mesothelioma cells in culture
Summary

- cDNA microarrays confirm expression of mesothelial cell markers:
  
cytokeratins, vimentin, CD44, N-cadherin

- Gene expression profile of murine malignant mesothelioma cell lines is consistent with molecular or immunohistochemical analysis of human mesotheliomas:
  
N-ras, Bax, c-met, MCP-1, TGF-β, CSF-1, glutathione reductase, and glutathione-S-tranferases

- Multiple functional pathways are altered in mesothelioma cell lines:
  
activation of signaling pathways (PKC-δ, MAPK, p38)
induction of early response genes (c-fos, c-jun, Egr-1)
altered cell-cycle control
resistance to apoptosis and expression of pro-survival genes increased motility
INFLAMMATION IN TUMOR PROGRESSION

- Chronic inflammation predisposes to cancer-liver, stomach, urinary bladder, skin
- NSAIDs decrease risk of colon cancer-inhibition of COX-2 and prostaglandin synthesis
- Mice deficient in macrophages show decreased angiogenesis and slower tumor growth

MACROPHAGES ARE THE INITIAL TARGET CELLS THAT INTERACT WITH FIBERS

The microenvironment of tumors consists of capillaries, fibroblasts, and inflammatory cells. In this murine model of mesothelioma produced by direct intraperitoneal injection of crocidolite asbestos fibers, long fibers are trapped at lymphatic openings on the inferior surface of the diaphragm and provoke focal accumulation of activated macrophages. In response to these biopersistent fibers, activated peritoneal macrophages express inflammatory cytokines (TNFα), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and matrix metalloproteinases (MMP9, MMP12). Fiber clusters are surrounded by multinucleated giant cells and granulation tissue, similar to a healing wound. Diffuse malignant mesotheliomas frequently arise near these fiber clusters and infiltrate the underlying stroma. It is hypothesized that this chronic inflammatory environment facilitates growth and invasion of malignant mesotheliomas.

MACROPHAGES CONTRIBUTE TO PROGRESSION OF MALIGNANT MESOTHELIOMAS