Molecular Biology of Mesothelioma: Implications for the development of new targeted therapies

Marc Ladanyi, M.D.

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Memorial Sloan-Kettering Cancer Center
New York, NY, USA
Malignant Pleural Mesothelioma

• **Major somatic mutations are in tumor suppressors:**
  – **P16:** 75-80%: homozygous deletion of gene $p16/CDKN2A$ (9p21)
    • Testa 1994; Fletcher 1995
    • ~100% cell lines
  – **NF2:** 60%: loss of heterozygosity of $NF2$ (22q12); inactivating mutations
    • Testa 1995; Minna 1995
  • **BAP1:** 20-25% inactivating mutations; 30% genomic loss
    • 42% cases with either or both
    • Ladanyi 2011; Testa 2011
  – **LATS2:** approx. 5-15% inactivating mutations
    • Sekido 2011
    • Higher in cell lines (up to 35%)
Major mesothelioma tumor suppressors: *P16, NF2, BAP1*

Statistically significant recurrent copy number aberrations in pleural mesotheliomas

Malignant Pleural Mesothelioma

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  - P16/CDKN2A, NF2, BAP1: correlates

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<td>Yes, more in sarcomatous</td>
<td>Poor</td>
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</table>
Kaplan-Meier plots of overall survival after surgery according to p16/CDKN2A homozygous deletion status.

Prognostic significance of p16/cdkn2a loss in pleural malignant mesotheliomas

Sanja Dacic · Hannelore Kothmaier · Stephanie Land · Yongli Shuai · Iris Halbwedl · Patrizia Morbini · Bruno Murer · Camilla Comin · Françoise Galateau-Salle · Funda Demirag · Handan Zeren · Richard Attanoos · Alan Gibbs · Philip Cagle · Helmut Popper
Frequent p16 inactivation by homozygous deletion or methylation is associated with a poor prognosis in Japanese patients with pleural mesothelioma

Naruyuki Kobayashi, Shinichi Toyooka, Hiroyuki Yanai, Junichi Soh, Nobukazu Fujimoto, Hiromasa Yamamoto, Shuji Ichihara, Kentaro Kimura, Kouichi Ichimura, Yoshifumi Sano, Takumi Kishimoto, Hiroshi Date
# P16/CDKN2A correlation with histology

Summary of FISH data on CDKN2A Homozygous deletion in 95 cases of pleural mesothelioma

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelioid</td>
<td>49/71</td>
<td>69%</td>
</tr>
<tr>
<td>Biphasic</td>
<td>16/19</td>
<td>84%</td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>5/5</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>70/95</strong></td>
<td><strong>74%</strong></td>
</tr>
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<tr>
<td>NF2</td>
<td>No correlation</td>
<td>No correlation</td>
<td>MTOR inhibitor, CRL inhibitor, FAK inhibitor?</td>
<td>FISH, IHC</td>
</tr>
</tbody>
</table>
Genomic copy number data in 53 mesotheliomas
Hierarchical clustering

Minimal Common Region analysis – Chromosome 22

Gain/Loss Image (53 Tumors) -- Chr 22
### NF2 mutations in 11/53 tumor samples

<table>
<thead>
<tr>
<th>Case</th>
<th>cDNA pos</th>
<th>Mutation</th>
<th>Type</th>
<th>Previously described (COSMIC)</th>
<th>Previously mutated in:</th>
<th>Relevance</th>
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<tbody>
<tr>
<td>32</td>
<td>655 G&gt;A</td>
<td>V219M</td>
<td>Missense</td>
<td>Yes</td>
<td>Schwann</td>
<td>In 4.1 domain</td>
</tr>
<tr>
<td>99</td>
<td>331 C&gt;T</td>
<td>Q111*</td>
<td>Nonsense</td>
<td>Yes</td>
<td>Schwann</td>
<td>Stop at exon 3</td>
</tr>
<tr>
<td>30</td>
<td>1333 G&gt;T</td>
<td>E445*</td>
<td>Nonsense</td>
<td>No</td>
<td></td>
<td>Stop at exon 12</td>
</tr>
<tr>
<td>46</td>
<td>172 G&gt;T</td>
<td>E58*</td>
<td>Nonsense</td>
<td>Yes</td>
<td>Meso</td>
<td>Stop at exon 2</td>
</tr>
<tr>
<td>97</td>
<td>599 +1</td>
<td>Abnl splice</td>
<td></td>
<td>Yes</td>
<td>Mening</td>
<td>Abnormal splice</td>
</tr>
<tr>
<td>102</td>
<td>363 +1</td>
<td>Abnl splice</td>
<td></td>
<td>No</td>
<td></td>
<td>Abnormal splice</td>
</tr>
<tr>
<td>43</td>
<td>36-42</td>
<td>S13fs*35</td>
<td>2 bp del</td>
<td>Yes</td>
<td>Schwann</td>
<td>Truncate exon 1</td>
</tr>
<tr>
<td>3</td>
<td>271-272</td>
<td>P91fs*32</td>
<td>1 bp del</td>
<td>Yes</td>
<td>Meso</td>
<td>Truncate exon 12</td>
</tr>
<tr>
<td>69</td>
<td>1324-1340</td>
<td>S444fs*47</td>
<td>11 bp del</td>
<td>No</td>
<td></td>
<td>Truncate exon 12</td>
</tr>
<tr>
<td>7</td>
<td>1641-1642</td>
<td>E547fs*2</td>
<td>1 bp del</td>
<td>No</td>
<td></td>
<td>Truncate exon 15</td>
</tr>
<tr>
<td>73</td>
<td>1458-1459</td>
<td>P486fs*8</td>
<td>1 bp insert</td>
<td>No</td>
<td></td>
<td>Truncate exon 14</td>
</tr>
</tbody>
</table>

NF2 Inactivation in MM: Results

• *NF2* genomic loss in 66% of tumor samples

• Samples with *NF2* loss have 40% decrease in *NF2* mRNA expression (p = 0.005) and lower protein expression by IHC (p=0.007).

• *NF2* genomic loss in 44% of cell lines

• Integrated data in 53 tumors:
  – 7 loss + mutation (13%)
  – 32 either loss or mutation (60%)
  – 14 neither (26%)

NF2
Inactivation in Mesothelioma

mTORC1
CRL4-DCAF1
Hippo pathway
MST1/2 (STK3/4)

- Loss of NF2 correlates with activation of mTORC1 signaling and sensitivity to mTOR inhibition in MPM cell lines

- A phase I study of the dual PI3K/mTOR inhibitor GDC0980 showed a 15% partial response rate in MPM.
- A similar phase I study with the dual PI3K/mTOR inhibitor LY3023414 in MPM is ongoing at MSKCC.
NF2 inactivation increases phospho-mTOR

NF2 loss is associated with increased p-mTOR expression (p=0.002)

→ IHC for NF2 and phospho-mTOR could be useful in selecting MM patients for trials targeting mTOR pathway activation due to NF2 loss
Mechanisms of NF2(Merlin)-mediated inhibition of proliferation

- NF2/Merlin exists in an open, inactive form and a closed, active form. Matrix adhesion and activation of integrin RTK signaling activate the PAK kinase. PAK in turn phosphorylates the C-terminus of NF2/Merlin, disrupting the intramolecular association that maintains the protein in a closed conformation. The resulting inactivation of NF2/Merlin removes a block to cell-cycle progression in normal cells.
- When not inhibited by NF2/Merlin, CRL4/DCAF1 positively regulates a broad oncogenic program of gene expression, which includes mitogenic signaling components, antiapoptotic proteins, and Hippo pathway target genes.

Rationale for CRL inhibitors in MPM

• The closed, active form of Merlin accumulates in the nucleus and interacts with DCAF1, the receptor component of the E3 ubiquitin ligase CRL4-DCAF1, inhibiting ubiquitination of nuclear proteins.

• De-repressed CRL4\textsuperscript{DCAF1} functions in the nucleus to suppress the output of Hippo signaling. The mechanism of this suppression of Hippo signaling by CRL4\textsuperscript{DCAF1} has recently been elucidated.
  – F. Giancotti lab, Cancer Cell, in press

• This provides a rationale to assess the pharmacological targeting of CRL4 for the treatment for MPM and other NF2 mutant tumors.

• The potential efficacy of the CRL inhibitor MLN4924 (Millenium-Takeda) in NF2 mutant MPM will be assessed in patients with previously treated MPM with NF2 loss in a single institution phase II study at MSKCC.
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3p21 deletions in mesothelioma centered on \textit{BAP1}.

- Overall:
  - 20-25\% inactivating mutations
  - 30\% genomic loss
  - 42\% cases with either or both
  - 3\textsuperscript{rd} most frequently altered gene in mesothelioma (after \textit{p16} and \textit{NF2})

16/53 tumors show genomic loss of \textit{BAP1} (30\%).

Neoplasms with frequent somatic mutations of BAP1

- Mesothelioma
- Uveal Melanoma
- Clear cell renal carcinoma
- Intrahepatic cholangiocarcinoma
- Special benign cutaneous melanocytic lesions
  - “melanocytic BAP1-mutated atypical intradermal tumors” (MBAITs) / epithelioid atypical Spitz tumours / “Wiesner nevus”
BAP1 protein function: which one is relevant to MPM?

Clinical features of patients with *BAP1* mutant versus *BAP1* normal mesothelioma

→ patients whose tumors contained *BAP1* mutations were more likely to have smoked.

<table>
<thead>
<tr>
<th></th>
<th>BAP1 mutant % (N=24)</th>
<th>BAP1 normal % (N=97)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>79/21</td>
<td>68/32</td>
<td>0.33</td>
</tr>
<tr>
<td>Median age</td>
<td>65</td>
<td>63</td>
<td>0.31</td>
</tr>
<tr>
<td>Asbestos exposure</td>
<td>54</td>
<td>46</td>
<td>1</td>
</tr>
<tr>
<td>Former and current smoking</td>
<td>75</td>
<td>42</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>Family history of mesothelioma</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Family history of cancer</td>
<td>38</td>
<td>47</td>
<td>0.49</td>
</tr>
<tr>
<td>Personal history of cancer</td>
<td>8</td>
<td>8</td>
<td>0.46</td>
</tr>
</tbody>
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Zauderer et al., J Thor Oncol 2013
Clinical Characteristics of Patients with BAP1-mutated mesothelioma

- **Age Analysis:**

<table>
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<th>Group</th>
<th>Mean/Median (Range) Age at Diagnosis</th>
</tr>
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<tr>
<td>+ BAP1 Mutations, Bott et al. Population (n=24)</td>
<td>65 (42-74)</td>
</tr>
<tr>
<td>No BAP1 Mutation, Bott et al. Population (n=97)</td>
<td>63 (33-81)</td>
</tr>
<tr>
<td>+ Germline BAP1 Mutation (n=22), Carbone et al.</td>
<td>55 (37-85)</td>
</tr>
<tr>
<td>National Median Age at Diagnosis</td>
<td>74</td>
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- **BAP1 mutations were associated with Tobacco Use:**
  - 75% of the BAP1 Mutation Positive patients were Current or Former smokers, compared to 42% of the BAP1 WT Population ($\chi^2$ p-value=0.006)
  - 2/24 mutations are point mutations classically associated with smoking
  - Possible mechanism: BAP1 mutation occurs independent of smoking, and then increases sensitivity to carcinogenic effects of cigarette smoke

Zauderer M et al., J Thor Oncol 2013
Distribution of mesothelioma histologic subtype by somatic BAP1 mutation status

Zauderer M et al., J Thor Oncol 2013
BAP1 gene mutations found in:
  - 13/16 epithelioid vs 1/7 biphasic or sarcomatous
  - P = 0.005
Impact of *BAP1* mutation status on survival?

→ No in mesothelioma vs Yes in other cancers

MSKCC Pleural Mesothelioma patients
Zauderer et al., J Thor Oncol 2013

MSKCC Clear Cell Renal Carcinoma patients
Hakimi et al., European Urology 2012
Correlation with \textit{BAP1} expression:

- \textbf{mRNA}: Tumors with \textit{BAP1} genomic losses have lower \textit{BAP1} mRNA expression than those without ($p < 0.0001$) in Affy expression data.
- \textbf{protein}: Tumors with loss and/or mutation also show loss of \textit{BAP1} staining in tumor cells by IHC ($p = 0.002$).

- \textit{BAP1 wild type} vs. \textit{BAP1 mutant}.

- \textit{..but some cases can have point mutations in ubiquitin hydrolase domain of \textit{BAP1} and express non-functional protein}.
- \textit{? use \textit{BAP1} IHC to select screen mesothelioma patients for germline \textit{BAP1} testing}. 

\textit{BAP1} IHC in mesothelioma
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Phosphorylation of BAP1 after DNA damage

- BAP1 is involved in the non-homologous end joining (NHEJ) pathway of double strand DNA repair through interaction with DNA-PK

- Proposed model: phosphorylation of BAP1 by DNA-PKcs upon DNA damage increases BAP1-mediated de-ubiquitination of DNA-PKcs, stabilizing it at foci of NHEJ DNA repair.

- These data suggest that synthetic lethal targets may exist in DNA repair pathways in BAP1-deficient cells.

Tatsuo Ito
Does BAP1 inactivation in MPM lead to new drug sensitivities based on its role in DNA repair?
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“Oncoprint” map of mesothelioma

N=53 tumors

Hippo signaling is an evolutionarily conserved pathway that regulates cell proliferation, apoptosis, and stem cell self renewal. The Hippo pathway includes a kinase cascade, wherein Mst1/2 kinases and Sav1 form a complex to phosphorylate and activate LATS1/2. LATS1/2 kinases in turn phosphorylate and inhibit the transcription co-activators YAP and TAZ, two major downstream effectors of the Hippo pathway. When dephosphorylated, YAP/TAZ translocate into the nucleus and interact with TEAD1-4 and other transcription factors to induce expression of genes that promote cell proliferation and inhibit apoptosis.

Adapted from Cell Signaling Technologies, Inc
LATS2 Is a Tumor Suppressor Gene of Malignant Mesothelioma

Hideki Murakami^1, Tetsuya Mizuno^1,4, Tetsuo Taniguchi^1,4, Makiko Fujii^1, Futoshi Ishiguro^1,4, Takayuki Fukui^2, Shinya Akatsuka^6, Yoshitsugu Horio^3, Toyoaki Hida^3, Yutaka Kondo^1, Shinya Toyokuni^6, Hirotaka Osada^1,5, and Yoshitaka Sekido^1,5

<table>
<thead>
<tr>
<th>Cell line</th>
<th>NF2^a</th>
<th>LATS2</th>
</tr>
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<tbody>
<tr>
<td>NCI-H290</td>
<td>HD</td>
<td>+</td>
</tr>
<tr>
<td>NCI-H2373</td>
<td>HD</td>
<td>+</td>
</tr>
<tr>
<td>ACC-MESO-1</td>
<td>Q389X</td>
<td></td>
</tr>
<tr>
<td>Y-MESO-9</td>
<td>NM_000268:c.527_528del2</td>
<td></td>
</tr>
<tr>
<td>Y-MESO-12</td>
<td>HD</td>
<td></td>
</tr>
<tr>
<td>Y-MESO-22</td>
<td>HD</td>
<td></td>
</tr>
<tr>
<td>Y-MESO-25</td>
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<td>Y-MESO-14</td>
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<td>Y-MESO-26B</td>
<td>HD</td>
<td></td>
</tr>
<tr>
<td>NCI-H2052</td>
<td>R341X</td>
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<tr>
<td>Y-MESO-21</td>
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<td>Y-MESO-27</td>
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<td>Y-MESO-30</td>
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<tr>
<td>MSTO-211H</td>
<td>+</td>
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<td>Y-MESO-28</td>
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<td>Y-MESO-8D</td>
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<tr>
<td>NCI-H2452</td>
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<td></td>
</tr>
<tr>
<td>ACC-MESO-4</td>
<td>+</td>
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LATS2 and YAP expression in mesothelioma cell lines.
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    • Higher in cell lines (up to 35%)
  – **TP53**: relatively rare but may define a distinct MPM subset
    • Fletcher 2014

• **Somatic mutations in oncogenes: rare**
Screening mesothelioma samples for oncogene point mutations common in other cancers

- Mass spectrometry-based mutation analysis (Sequenom) of known oncogenic point mutations in 87 tumors:
  
  \[ \text{KRAS, NRAS, HRAS, EGFR, BRAF, PIK3CA, ERBB2, MEK1, AKT} \]

  – Only 3 mutations identified in 87 MPM samples – confirmed by sequencing

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Mutation</th>
<th>Previously described in MM</th>
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<tbody>
<tr>
<td>18</td>
<td>BRAF V600E</td>
<td>No</td>
</tr>
<tr>
<td>38</td>
<td>KRAS G12D</td>
<td>No</td>
</tr>
<tr>
<td>97</td>
<td>NRAS Q61L</td>
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THANK YOU