Mesothelin

NATHALIE SCHOLLER
Molecular Diagnostics Program, Translational and Outcomes Research Group, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Synonyms
CAK1 antigen; SMR (soluble mesothelin-related proteins); MPF

Definition
The name Mesothelin was given by K. Chang and I. Pastan to a 40 kDa, GPI-anchored ▶glycoprotein ▶(GPI-anchored protein) that is physiologically expressed at the cell surface of mesothelial cells lining the pleura, pericardium and peritoneum [1]. Mesothelin is an epithelial marker highly expressed by ▶cancer cells from diverse origins, including ▶ovarian adenocarcinomas, pancreatic adenocarcinomas, and ▶mesotheliomas. Soluble forms of mesothelin can be found in fluids from patients affected by these cancers.

Characteristics
Mesothelin results from the cleavage of a 69 kDa preproprotein encoded by the human MSLN gene (NC_000016) that spans over 16 exons and occupies about 8 kb of human chromosome 16 (Fig. 1) The alternative splicing of MSLN gene results in at least two mesothelin transcript variants, variant 1 encoded by MSLN1 (NM_005823) and variant 2 encoded by MSLN2 (NM_013404) (Fig. 2) The variant 1 is predominant and variant 2 differs from variant 1 by 24 bp inserted in exon 11. The cleavage of MSLN-encoded preproprotein at the cationic motif TILRP RF RREV in exon 9 releases the ▶megakaryocyte potentiating factor (MPF), a 31 kDa soluble protein, while mesothelin remains membrane-bound.

MSLN gene is composed of 16 exons spanned over 7733 bp. In human, MSLN gene is located in 16p13.3. MSLN1 and 2 gene variants encode a 69 kDa preproprotein that is cleaved in position 937 after the cationic motif RXRR into two mature proteins, MPF and mesothelin. MSLN1 encodes mesothelin variant 1 and MSLN2 encodes variant 2, a less abundant, alternate splice with a 24-bp insertion in position 1282. Soluble mesothelins arise through a cleavage of GPI-anchored variants 1 or 2, or less frequently, an 82-bp insertion in position 1828 of MSLN1 resulting in a 212-bp ▶frameshift mutation that transforms the GPI anchor motif into a hydrophilic domain (SMR or variant 3).

Soluble mesothelins arise through a cleavage of GPI-anchor (Fig. 3) at the C-terminal domain or less frequently, a frameshift mutation changing the GPI anchor motif into a hydrophilic region (SMR or variant 3). The frameshift mutation is due to the insertion of an 82-bp fragment resulting probably from a lack of splicing of the last intron (Figs. 1 and 2).

The core of a GPI anchor is composed of a hydrophobic phosphatidyl-inositol group and a carbohydrate-containing linker made of glucosamines and mannoses linked to a phosphoryl-ethanolamine residue. The GPI anchor is linked to the C-terminal amino acid of a mature protein via the phosphoryl-ethanolamine residue. R1 and R2 fatty acids anchor the protein to cell membranes.

Mesothelin homologues were described for chimpanzees (MSLN gene, Pan troglodytes, accession DQ052446), macaque (LOC698095 gene, Macaca mulatta, accession XM_001087333), bovine (LOC516237 gene, Bos Taurus, accession XM_594389), dog (LOC611363 gene, Canis familiaris, accession XM_849019), rat (Msln gene, Rattus norvegicus, accession NM_013658), mouse (Msln gene, Mus musculus, accession NM_018857) and chicken (LOC416534 gene, Gallus gallus, accession XM_414835). Mouse mesothelin is 55% homologous to its human counterpart and its protease target sequence TVHPRFRRDAE is conserved.

Tissue Expression
Serial analysis of gene expression (SAGE), oligonucleotide and cDNA arrays have been used by independent laboratories to identify large sets of genes expressed at higher levels in cancer tissues compared with normal tissues. MSLN transcripts were found consistently highly over-expressed in non-mucinous ovarian carcinomas and invasive ductal adenocarcinomas (▶Pancreatic ductal adenocarcinoma (DA)) and significantly over-expressed in mesotheliomas and pulmonary, gastric/esophageal and colorectal adenocarcinomas. By real time PCR-based
normal transcript levels of MSLN and CCL23, GAGED2, SPAG6, ST18,WT1 and PRAME genes were found to be associated with continuous complete remission of pediatric acute myeloid leukemia, while elevated levels of at least one of these genes were found prior to relapse in most patients. The up-regulation of MSLN transcripts correlates well with mesothelin cell-surface expression. Studies by immuno-histochemistry (IHC) confirm that mesothelin is significantly more expressed by pancreatic cancer cells as compared to very weak or no expression in chronic pancreatitis or normal pancreatic ducts; mesothelin is found with a higher frequency in invasive intraductal papillary mucinous neoplasms (IPMN) than in non-invasive. Comparative analyses with tissue microarrays showed that the mesothelin protein expression in ovarian cancer depends on the histological type (75% of expression in serous papillary tumors, 30% in endometrioid and less than 20% in mucinous). Diffuse mesothelin staining in primary high grade ovarian serous carcinomas may be correlated with prolonged survival. Finally, mesothelin was also found to be expressed at a low level by a variety of adenocarcinomas, including endometrium, stomach/esophagus, pulmonary, breast and colorectal. None or very rare mesothelin expressions (less than 5%) were reported for carcinomas of prostate, bladder/ureter, liver, kidney and thyroid.

Regulation
Mechanisms that regulate MSLN transcription levels and mesothelin cell-surface expression or release as a soluble form in patient fluids are not well understood. Several pathways have been explored. MSLN gene was found hypomethylated in pancreatic ductal adenocarcinoma, consistently with the inverse correlation between mRNA
expression and DNA methylation described in numerous cancers. Also, mesothelin up-regulation in carcinomas has been associated with a misregulation of Wnt signal transduction pathway. In mouse mammary epithelial cells, Wnt-5a down-regulates mesothelin expression, perhaps through antagonism of the Wnt/beta-catenin pathway, while in human colon cancer cells the forced expression of an N-terminal beta-catenin binding site-deficient high mobility group (HMG)-box T-cell factor 1 is associated with the up-regulation of several GPI-anchored adhesion molecules, including mesothelin. Furthermore, the overexpression of mesothelin in exon 9 GISTs suggests that mesothelin regulation could be linked to the intracellular signaling cascade triggered by ligand-independent activation of KIT receptor tyrosine kinase. Finally, the presence of soluble mesothelin derived from GPI-anchored forms in ovarian and mesothelioma patient fluids could also be related to the over-expression of GPI PLD observed in some cancer cells.

**Function**

Mesothelin knock-out mice have no obvious phenotype and produce normal offspring without anatomical or histological abnormalities, which suggests that mesothelin is a non-essential protein. However, mesothelin binds to CA125 in a specific and N-linked glycan-dependent manner, thus CA125-expressing tumor cells could bind specifically to mesothelin-expressing peritoneal lining. Consequently, CA125/mesothelin-dependent cell attachment may play an important role in the peritoneal implantation of ovarian tumor cells.

Some indirect experimental evidences suggest that mesothelin could also have a role in neoplastic progression. First mesothelin up-regulation in
pancreatic cancer corresponds to the transition from carcinoma in situ to DA. Second, mesothelin expression was found to be up-regulated after carcinogenic treatment in rats and its expression correlated with the risk of cancer development. Virgin and parous rats were compared for breast cancer incidence and mesothelin expression, before and after carcinogen exposure. Mesothelin was down-regulated before and after treatment in parous rats but only before treatment in virgin rats. It was increased after treatment in virgin rats, as were their cancer development risks.

Finally mesothelin is an immunogenic molecule. This observation is consistent with mesothelin GPI-anchor but puzzling considering its overexpression by cancer cells. In fact, most tumor antigens are weak immunogens and this has been a burden for the development of cancer vaccines. Nevertheless, anti-mesothelin autoantibodies have been found in about 40% of mesothelioma and epithelial ovarian cancer patients, and in some patients with pharynx/larynx squamous cell carcinoma. In addition, mesothelin-specific CD8-T cell responses are identifiable with a HLA-A2 mesothelin epitope in both normal and cancer patients, and have been reported to be increased after vaccination with pancreatic cancer lines in presence of GM-CSF.

**Diagnostic Marker**

The differential expression of mesothelin at the cell surface of some cancer cells and in patient fluids makes it suitable as a cancer marker. Mouse monoclonal antibodies (mAb) are commercially available for immunohistochemistry (IHC) staining, for example K1 mAb from Abcam and 5B2 mAb from BioGenex, and for ELISA assay (Mesomark™ kit from Fujirebio Diagnostics, Inc., corresponding to the assay described by Scholler et al. [2].

Anti-mesothelin mAbs stain mesotheliomas with a high sensitivity and a specificity of 75%. Several studies suggest than mesothelin can be useful for differential diagnostics, alone or combined with a biomarker panel. For example, mesothelin staining can help to identify the origin of mucinous tumors or metastatic adenocarcinomas. Mesothelin staining is much less frequent (less than 20%) in primary ovarian mucinous tumors than in metastatic pancreatic mucinous carcinomas (more than 70%). Used with PSCA, mesothelin appears highly specific for pancreatic adenocarcinoma in fine needle aspirate (FNA) specimens thus useful in categorizing suspicious lesions. In combination with p53, TAG-72, mCEA and loss of Dpc4, mesothelin staining distinguishes well differentiated liver metastasis of DA from bile duct adenomas (BDA) or hamartoma of the liver. Mesothelin, calretinin and cytokeratin 5/6 were reported to be the best positive mesothelioma markers for differentiating epithelioid mesotheliomas from renal cell carcinomas; mesothelin, WT1, p63 and MOC31 distinguish between epithelioid mesotheliomas and squamous carcinomas of the lung; mesothelin, calretinin, BG8 and MOC 31 distinguish between epithelioid mesothelioma and adenocarcinoma. Finally, a larger panel including mesothelin, CA125, CDX2, cytokeratins 7/20, estrogen receptor, gross cystic disease fluid protein 15, lysozyme, prostate specific antigen and thyroid transcription factor 1 was reported to correctly classify 88% of breast, colon, lung, ovary, pancreas, prostate and stomach adenocarcinomas.

Mesothelin measured in fluids is a promising marker for ovarian carcinomas and mesotheliomas. Mesothelin serum levels are elevated in most late stage ovarian cancer patients and in most patients with malignant mesotheliomas (MM) at diagnosis, and serum levels correlate with tumor size and increase during tumor progression. This suggests that mesothelin serum levels could be helpful to monitor disease progression and to screen asbestos-exposed individuals for early MM. In addition, the presence of mesothelin in MM pleural fluid can help to better discriminate mesothelioma from pleural metastasis. Recent results, taken together with mesothelin cell surface expression on pancreatic tumor, suggest that mesothelin could also be a serum marker for some pancreas cancers. A pancreatic tumor cell line was reported to release soluble mesothelin in culture supernatant using an acoustic wave device immuno-sensor, and mesothelin mRNA was isolated from pure pancreatic juice of pancreatic tumors and was found more abundantly in DA than in IPMN. Finally, various studies combined mesothelin with other biomarkers to form a composite marker (CM) and demonstrated that the use of CM can improve diagnostic test sensitivity. For ovarian carcinoma diagnostic, mesothelin titers have been evaluated in combination with CA125, HE4, M-CSF, kallikrein and/or soluble EGF receptor; for mesothelioma diagnostic, mesothelin has been combined with osteopontin.

Interestingly, mesothelin tumor cell expression and serum levels do not strictly correlate. Although mesothelin serum levels are more frequently increased in both MM and ovarian cancer patients whose tumor expressed mesothelin (≥30% expression by tumor cells), some patients without detectable mesothelin expression on tumor cells have elevated titers of serum mesothelin. The absence of detectable mesothelin by IHC could be due to technical artifact, or alternatively, in some cases soluble mesothelin might be mainly released from normal mesothelial cells that are in contact with the tumor microenvironment, such as pleural effusion or peritoneal fluid.
**Therapeutic Applications**

Because of its high expression in mesothelioma, ovarian and pancreatic carcinomas, its immunogenicity and its non-essential function, mesothelin is an antigen of choice for targeted therapies and cancer vaccines. Anti-mesothelin natural and recombinant antibodies have been generated and conjugated to Pseudomonas exotoxin A (SS1P), 125I or 111In, and used alone or in combination with Taxol *in vitro* and *in vivo* in mouse model systems. Recent results demonstrated that SS1P synergizes with Taxol *in vivo* but not *in vitro* which underlines the importance of the tumor microenvironment for therapeutic strategies. Phase I trials with recombinant anti-mesothelin immunotoxin were completed in patients with mesothelin-expressing tumors without evidence of non-manageable side effects; about a fourth of the treated patients developed anti-SS1P antibodies. In addition, a DNA vaccine with a single-chain trimer of HLA-A2 linked to human mesothelin peptides has been successfully used to prevent the growth *in vivo* of HLA-A2 positive human mesothelin-expressing tumor cell lines in HLA-A2 transgenic mice. These results suggest possible clinical translation of mesothelin-targeted therapy and DNA vaccines for immunotherapy of gynecologic cancers against mesothelin.

Finally, the specific binding of mesothelin to CA125 suggests that agents able to compete with or to block CA125/mesothelin-dependent cell attachment could prevent or delay the development of peritoneal metastasis.

**References**

2. Scholler N, Fu N, Yang Y et al. (1999) Soluble member(s) of the esothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma. Proc Natl Acad Sci USA 96:11531–11536
**Author Query Form**

Title: Encyclopedia of Cancer  
Alpha - M

<table>
<thead>
<tr>
<th>Query Refs.</th>
<th>Details Required</th>
<th>Author's response</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU1</td>
<td>Please check whether the affiliations is OK as typeset.</td>
<td></td>
</tr>
<tr>
<td>AU2</td>
<td>Please check whether the heading level for the heading &quot;Theapeutic Applications&quot; is OK as given.</td>
<td></td>
</tr>
</tbody>
</table>