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REVIEW

Molecular pathogenesis of malignant mesothelioma

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Malignant mesothelioma (MM) is an aggressive tumor arising primarily from the pleural or peritoneal cavities. It develops by asbestos exposure after a long latency, which is characterized by insidious growth and clinical presentation at an advanced stage of disease. MM is highly refractory to conventional therapies even with a combination of aggressive surgical intervention and multimodality strategies, with cure remaining elusive. Molecular genetic analysis has revealed several key genetic alterations, which are responsible for the development and progression of MM. The *cyclin-dependent kinase inhibitor 2A/alternative reading frame* (*CDKN2A/ARF*), *neurofibromatosis type 2* (*NF2*) and *BRCA1-associated protein-1* (*BAP1*) genes are the most frequently mutated tumor suppressor genes detected in MM cells; the alterations of the latter two are relatively characteristic of MM. Merlin, which is encoded by *NF2*, regulates multiple cell signaling cascades including the Hippo and mammalian target of rapamycin pathways, which regulate cell proliferation and growth. BAP1 is involved in histone modification and its inactivation induces the disturbance of global gene expression profiling. The discovery of a new familial cancer syndrome with germline mutation of *BAP1* also indicates the importance of genetic factors in MM susceptibility. Meanwhile, although frequent expression and functional activations of oncogene products such as receptor tyrosine kinases are observed in MM cells, activating mutations of these genes are rare. With further comprehensive genome analyses, new genetic and epigenetic alterations in MM cells are expected to be revealed more precisely, and the new knowledge based on them will be applied for developing new diagnostic tools and new target therapies against MMs.

Introduction

Malignant mesothelioma (MM) is an aggressive neoplasm that arises primarily from the surface serosal cells of the pleura and peritoneum (1,2). It can also develop from the serosal surfaces of the pericardium or the tunica vaginalis. Up to 80% of all cases are pleural in origin and are defined as malignant pleural mesothelioma (MPM). MM develops insidiously in patients and they are usually diagnosed at advanced stages because radiological diagnostic tools are not effective for its early detection, and serum biomarkers for early detection have not yet been established. The anatomical location and characteristics of the body cavities where MM initially develops also causes malignant cells to easily spread and invade the cavities. Pathologically, there are three major MM subtypes of epithelioid, sarcomatoid and biphasic type with both epithelioid and sarcomatoid components (3). Rare variants of histology are also included in this disease entity. As MM is largely unresponsive to conventional therapy, the prognosis is very poor. The median survival of patients with MPM is 9–12 months after diagnosis, regardless of the recent advancement in chemotherapeutical modalities combining cisplatin and pemetrexed, an antifolate drug (4). Although some new molecular target drugs show occasional stabilization of the disease, none of them seems to be currently recommended as standard treatment (5).

As MM is a relatively rare malignancy, the understanding of molecular pathogenesis of genetic/epigenetic alterations for MM development has lagged behind that of other common malignancies. However, recent development of global genetic and epigenetic analysis has served to reveal the fundamental molecular abnormalities of this rare, but highly aggressive tumor. Several recent reviews of MM describe comprehensive lists of genetic, epigenetic and signaling alterations (6), but this review focuses on asbestos-induced carcinogenic changes and three major tumor suppressor alterations in MM, which are currently considered to be fundamental abnormalities of MM development.

Genetic damages induced by asbestos

MM has been shown to be linked to asbestos exposure (7). Over 80% of MM patients have a history of asbestos exposure. Asbestos refers to a family of six mineral fibers and is classified into two subgroups: (i) the amphiboles, a group of rod-like fibers including amosite (brown asbestos), crocidolite (blue asbestos), anthophyllite, actinolite and tremolite; and (ii) the serpentine group, consisting of chrysotile (white asbestos). The association between amphibole asbestos exposure and MM development is well known. In particular, crocidolite is considered to be the most carcinogenic type of asbestos. Erionite, an asbestos-like mineral, also causes MM.

After long and thin asbestos fibers are inhaled deeply into the lung and penetrate the pleural space, interaction of asbestos fibers with mesothelial cells and inflammatory cells is thought to initiate prolonged cycles of tissue damage, repair and local inflammation, which finally lead to carcinogenesis of MM with unknown mechanisms. It also remains unclear why the initial affected site of MM development by asbestos exposure is the parietal, but not the visceral pleura. Compared with other cell types, human mesothelial cells are very susceptible to asbestos cytotoxicity, which raises a paradoxical issue of how asbestos causes MM if human mesothelial cells exposed to asbestos die (8).

There are several possible mechanisms involved in how asbestos fibers cause MM (9,10) (Figure 1). Four representative models by which asbestos fibers induce genetic/cellular damages of the cells and chronic inflammation, which is linked to carcinogenesis, are as follows. (i) Reactive oxygen species generated by asbestos fibers with their exposed surface lead to DNA damage and strand breaks of the cells. Macrophage, which phagocytoses asbestos fibers but is unable to digest them, also produces abundant reactive oxygen species. (ii) Asbestos fibers are also engulfed by mesothelial cells. Asbestos fibers taken up into the cells can physically interfere with the mitotic process of the cell cycle by disrupting mitotic spindles. Tangling of asbestos fibers with chromosomes or mitotic spindles may result in chromosomal structural abnormalities and aneuploidy of mesothelial cells. (iii) Asbestos fibers absorb a variety of proteins and chemicals to the broad surface of asbestos, which may result in the accumulation of hazardous molecules including carcinogens. Asbestos fibers also bind important cellular proteins and the deficiency of such proteins may also be harmful for normal mesothelial cells. (iv) Finally, asbestos-exposed mesothelial cells and macrophages release a variety of cytokines and growth factors, which induce inflammation and tumor promotion. Those include tumor necrosis factor-α, interleukin-1β, transforming growth factor-β and platelet-derived growth factor. Tumor necrosis factor-α has been shown to activate nuclear factor-κB, which leads to mesothelial cell survival and

Abbreviations: ARF, alternative reading frame; BAP1, BRCA1-associated protein-1; CDKN2A, cyclin-dependent kinase inhibitor 2A; FISH, fluorescence *in situ* hybridization; HPMCs, human peritoneal mesothelial cells; miR, micro RNA; MM, malignant mesothelioma; MPM, malignant pleural mesothelioma; mTOR, mammalian target of rapamycin; MWCNTs, multiwalled carbon nanotubes; NF2, neurofibromatosis type 2; PI3K, phosphoinositide-3 kinase; RTK, receptor tyrosine kinase; TSG, tumor suppressor gene.

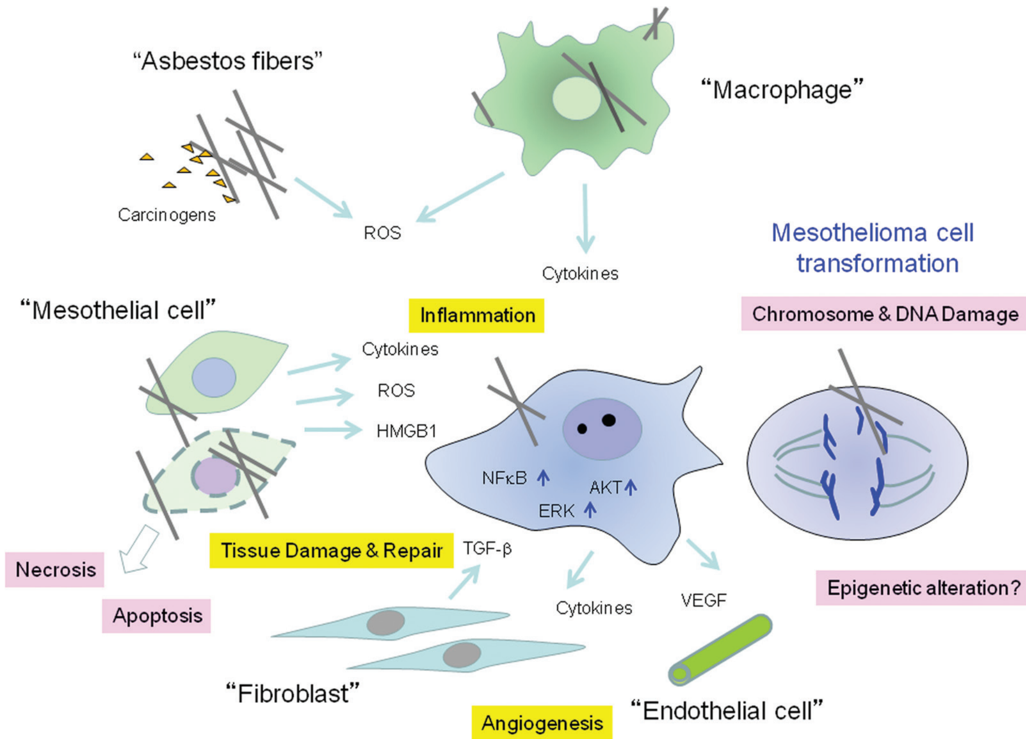

Fig. 1. Possible mechanisms of asbestos-induced carcinogenesis. HMGB1, high-mobility group box 1 protein; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

inhibits asbestos-induced cytotoxicity (11). High-mobility group box 1 protein has also been shown to be released from mesothelial cells, which are exposed by asbestos and then undergo necrotic cell death, promoting an inflammatory response (12). Thus, the aberrantly activated signaling network among mesothelial cells, inflammatory cells, fibroblasts and other stromal cells may create a pool of mesothelial cells, which harbor aneuploidy and DNA damage, potentially developing into cancer cells and together forming a tumor microenvironment that supports and nourishes them (Figure 1).

DNA damages in mesothelial cells induced by asbestos or other factors should be repaired in order to maintain DNA integrity. In mammalian cells, four major DNA damage repair systems are known to be responsible for repairing different DNA lesions. They include base excision repair, nucleotide excision repair, mismatch repair and recombinational system repair (homologous recombination and non-homologous end-joining) (13). Significant overexpression of genes involved in each DNA repair system in MMs, especially genes related to double-strand break repair, have been reported (14). Polymorphisms in genes encoding DNA repair proteins such as X-ray cross complementing group 1 have also been suggested to be associated with the risk of MM. It is also conceivable that the upregulation of DNA repair genes may account for both the chemo- and radio-resistance of MM cells (14).

Finally, multiwalled carbon nanotubes (MWCNTs) with a high aspect (length to width) ratio have been a concern in that they may also induce asbestos-like pathogenicity including MM because of their needle-like shape and high durability (15). When MWCNTs were inhaled into mice, they were shown to migrate to the subpleura (16). Thin MWCNTs (diameter ~50 nm) with high crystallinity have been demonstrated to show mesothelial cell membrane piercing and cytotoxicity *in vitro* and induction of inflammation and MM development *in vivo* (17). The MMs developed by MWCNTs showed frequent homozygous deletion of the *Cdkn2a/2b* genes.

Activation of oncogene cascades

Receptor tyrosine kinases (RTKs) are frequently activated in malignant cells. Activation of RTKs leads to constitutive upregulation of two major downstream cell signaling cascades including Raf-MEK-extracellular signal-regulated kinase and phosphoinositide-3 kinase (PI3K)-AKT pathways, which are critical for proliferation and/or survival of cells. However, activating mutations of oncogenes whose products are involved in these cascades such as epidermal growth factor receptor families, K-Ras and PIK3CA are rare in MMs. Nevertheless, constitutive and simultaneous activation of several RTKs such as epidermal growth factor receptor and MET has been reported in most MM cells (18). Other RTK receptors including AXL have also been suggested to be related with more malignant phenotypes to MM cells. Based on the observations of frequent activation of RTKs in MMs, small molecule inhibitors of specific RTK such as gefinitib and imatinib were applied to clinical studies, but no clear effectiveness was observed. Multiple RTK inhibitors such as sunitinib (19) and sorafenib (20) also showed only limited activity in advanced MM patients.

Activation of the mammalian target of rapamycin (mTOR) signaling contributes to the pathogenesis of many tumor types, which is also one of the PI3K/AKT downstream pathways. Regarding mesothelioma, rapamycin, an mTORC1 inhibitor, showed enhanced cell death with cisplatin on MM cell lines (21). When MM cells were grown as three-dimensional spheroids, which were highly resistant to a variety of apoptotic stimuli compared with monolayer culture, rapamycin was shown to block the acquired resistance of the spheroids (22). In patients with malignant peritoneal mesothelioma, the activation of both PI3K and mTOR signaling pathways was shown to be associated with a shortened survival (23).

Besides the PI3K-AKT and mitogen-activated protein kinase pathways, the signal transducer and activator of transcription 1 signaling axis has been shown to be aberrantly activated in MM cells using a phosphotyrosine proteomic screen (24). Although signal transducer and activator of transcription 1 is considered to be a tumor suppressor, it was also shown to promote radioresistance and tumorigenesis. Thus, signal transducer and activator of transcription 1 activation might be required for the development of MM, which may also be linked to inflammation. The SRC family kinases, including SRC and FYN, have also been reported to frequently activate in MM cells (24).

Angiogenesis plays a significant role in MM progression. MM expresses vascular endothelial growth factor and vascular endothelial growth factor receptors, which consist of an autocrine growth loop of

MM cells and stimulate angiogenesis. A phase II trial suggested that cediranib, an oral pan-vascular endothelial growth factor receptor, Kit and platelet-derived growth factor inhibitor, showed a high sensitivity to some patient tumors (25,26).

Cyclin-dependent kinase inhibitor 2A/alternative reading frame inactivation

The *cyclin-dependent kinase inhibitor 2A* (*CDKN2A*)*/alternative reading frame* (*ARF*) gene is the most frequently inactivated tumor suppressor gene (TSG) in human MM (27). *CDKN2A/ARF* is located at chromosome 9p21.3 and *CDKN2A* encodes $p16^{INK4a}$ with exon 1α, 2 and 3, whereas ARF encodes $p14^{ARF}$ with exon 1β, 2 and 3 with an alternative open reading frame. $p16^{INK4a}$ controls the cell cycle via the cyclin-dependent kinase 4/cyclin D-retinoblastoma protein pathway, whereas $p14^{ARF}$ regulates p53 through inactivation of the human ortholog of mouse double minute 2, which is an upstream regulator of p53. Thus, the homozygous deletion of *CDKN2A/ARF* indicates the inactivation of two major tumor suppressing pathways of retinoblastoma and p53 in the cell.

With the fluorescence *in situ* hybridization (FISH) analysis of primary MM tissue samples or MM cells from the pleural effusion, over 70% of cases showed homozygous deletions of the *CDKN2A/ARF* locus (28–34). According to the histological subclassification, MM cases of epithelioid type showed ~70% of homozygous deletion of *CDKN2A* and those of sarcomatoid type showed ~100% of homozygous deletion (Table I). Because the targeted deletion region of 9p21.3 is often large, other genes located in the same gene cluster such as *CDKN2B* (*$p15^{INK4b}$*) and methylthioadenosine phosphorylase are also co-deleted, which are thought to be responsible for granting more malignant phenotype to MM cells. Furthermore, microRNA (miR)-31, which is located ~0.5 Mb telomeric to *CDKN2A*, was found to be co-deleted with *CDKN2A*, and reintroduction of miR-31 in mesothelioma cells was demonstrated to show a suppressive effect on MM cells (35). One of the miR-31 target genes is the protein phosphatase (PPP6C), which was shown to be upregulated in MM specimens (35). Meanwhile, although *p53* is the most frequently inactivated TSG in human malignancies, only a limited number of MM cases show a *p53* mutation.

Although the pathological roles of $p16^{INK4a}$ have been well established in human cancers including MM, genetically engineered mouse studies showed that mice deficient for *Arf*, but not *$p16^{INK4a}$*, were also susceptible to accelerated asbestos-induced MM, indicating that Arf inactivation has a significant role in driving MM pathogenesis *in vivo* (41). The inactivation of both $p16^{INK4a}$ and Arf has been suggested to cooperate to accelerate asbestos-induced tumorigenesis *in vivo* (42). In addition, using rat peritoneal mesotheliomas, which were induced by iron overload of ferric saccharate, homozygous deletion of *CDKN2A* and *CDKN2B* was found to be the most frequent genomic abnormality, indicating that *CDKN2A/2B* deletion is the most fundamental genomic abnormality in the development of MM in mammalians (43).

A series of experiments of human peritoneal mesothelial cells (HPMCs) also suggest the importance of $p16^{INK4a}$ in mesothelial cells (44). When culturing, HPMCs become senescent relatively quickly within only a few rounds of replication. HPMCs undergo senescence without telomere shortening but show high $p16^{INK4a}$ expression, suggesting that HPMC senescence is telomere independent. Thus, the inactivation of $p16^{INK4a}$ might be needed to avoid cellular senescence, which is dependent on $p16^{INK4a}$.

Neurofibromatosis type 2 inactivation

The *neurofibromatosis type 2* (*NF2*) gene encodes a tumor suppressor protein, merlin (moesin-ezrin-radixin-like protein), a member of the Band 4.1 family of cytoskeletal linker proteins. NF2 cancer syndrome is characterized by the development of tumors of the nervous system such as bilateral vestibular schwannomas at the eighth cranial nerve, spinal schwannomas and meningiomas. Biallelic *NF2* mutations are also frequently detected in sporadic cases of these tumors.

Table I. Alteration frequencies of three major tumor suppressor genes in malignant mesothelioma

Gene	Type of mutation	Epithelioid	Sarcomatoid	Biphasic	Not specified	Reference	Method[a]
CDKN2A	HD[b]	67% (20/30)[c]	100% (3/3)	100% (6/6)	—	Bott *et al.* (36)	Seq
(*$p16^{INK4a}$/$p14^{ARF}$*)	HD	69% (49/71)	100% (5/5)	84% (16/19)	—	Illei *et al.* (29)	FISH
	HD	56% (10/18)	100% (22/22)	88% (7/8)	—	Wu *et al.* (30)	FISH
	HD	77% (23/30)	100% (5/5)	100% (7/7)	—	Takeda *et al.* (31)	FISH
	HD	—	—	—	67% (35/52)	Chiosea *et al.* (32)	FISH
	HD (or heterozygous D)	—	—	—	49% (42%) [16(14)/33]	Onofre *et al.* (33)	FISH
	HD (or heterozygous D)	—	—	—	80% (20%) [12(3)/15]	Matsumoto *et al.* (34)	FISH
	Mutation	42% (35/83)	81% (22/27)	44% (17/39)	57% (59/104)	COSMIC[d]	Seq
NF2	Truncation form	50% (13/26)	—	22% (4/18)	—	Thurneysen *et al.* (37)	Seq
	HD	33% (10/30)	40% (2/5)	43% (3/7)	—	Takeda *et al.* (31)	FISH
	Mutation including HD[e]	—	—	—	56% (14/25)	Cheng *et al.* (38)	Seq
	Mutation including HD[e]	—	—	—	50% (10/20)	Murakami *et al.* (39)	Seq
	Mutation (or heterozygous D)	—	—	—	21% (53%) [11(28)/53]	Bott *et al.* (36)	Seq
	Mutation	—	—	0% (0/1)	31% (8/26)	COSMIC	Seq
BAP1	Mutation	21% (8/38)	0% (0/5)	40% (4/10)	18% (12/68)	Bott *et al.* (36)	Seq
	Mutation[e]	—	—	—	24% (6/25)	Bott *et al.* (36)	Seq
	Mutation	81% (13/16)	0% (0/2)	20% (1/5)	—	Yoshikawa *et al.* (40)	Seq
	Mutation	38% (26/68)	0% (0/7)	29% (6/21)	20% (19/93)	COSMIC	Seq

[a]Methods in each study vary with different sensitivity/specificity rates, and definitions of mutations such as 'homozygous deletion' in FISH are different. Although each study used various genetic analytical techniques including PCR, reverse transcriptase–PCR, Sanger sequencing, single-strand conformation polymorphism analysis, comparative genomic hybridization analysis, and/or next-generation sequencing, and western blot analysis, they are described together as 'Seq'. Seq, sequencing.
[b]HD, homozygous deletion.
[c]Data are presented as % (number of positive/total cases).
[d]COSMIC MutantExport version 64 (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/).
[e]Cell line data.

The *NF2* gene was shown to be the target TSG of 22q12 loss in MM (45,46), with 40–50% of MM cases harboring an inactivating mutation (31,38,39) (Table I). It has also been suggested that merlin can be inactivated not only genetically but also with other mechanisms (47). Merlin can be inactivated by phosphorylation on Ser518 with increased expression of 17 kDa protein kinase C potentiated inhibitor (CPI-17), an oncogene product that inhibits the merlin phosphatase, myosin phosphatase targeting subunit 1-protein phosphatase 1δ (MYPT1-PP1 δ) (37). Because a splicing variant of *NF2* at the C-terminus does not show tumor suppressive activity, the expression of the *NF2* splicing variants may also account for the functional inactivation of merlin (37). The other study suggested that upregulation of miR such as hsa-miR-885-3p might target *NF2* (48). However, it still remains to be determined how much these inactivation mechanisms are actually involved in MM cases.

As *NF2* mutation is frequently detected in MMs, genetically engineered *Nf2*-knockout mouse models have been developed to confirm the significance of *NF2* inactivation on MM pathogenesis. Asbestos-exposed *Nf2* (+/−) knockout mice exhibited markedly accelerated MM tumor formation compared with asbestos-treated wild-type littermates (49). Loss of the wild-type *Nf2* allele, leading to biallelic inactivation, was observed in all asbestos-induced MMs from *Nf2* (+/−) mice and in 50% of MMs from asbestos-exposed wild-type mice. These developed murine MMs also had homozygous deletion of $p16^{Ink4a}$, $p19^{Arf}$ (murine ortholog of human $p14^{ARF}$) and/or $p15^{Ink4b}$. In another mouse MM model, in which direct injection of adenoviruses encoding the site-specific recombinase Cre (Adeno-Cre) in the pleural cavity of adult mice carrying conditional TSG knockout alleles including *Nf2*, *Ink4a/Arf* and *p53* caused mesothelium-specific recombination and loss, mesothelioma was shown to develop at a higher incidence (50).

Merlin is regulated by extracellular signaling such as from CD44, adherence junction and RTKs (Figure 2). The active form of merlin for tumor suppressor takes a 'closed form' with Ser518 dephosphorylation and the inactive form takes an 'open form' with Ser518 phosphorylation. While interacting with various proteins, merlin modulates multiple signal transduction cascades of the cells, including mTOR pathway, and Hippo signaling pathway.

In addition, the underphosphorylated form of merlin was also shown to translocate to the nucleus, bind to the E3 ubiquitin ligase $CRL4^{DCAF1}$ and inhibit the $CRL4^{DCAF1}$-ubiquitination activity of target proteins, indicating that merlin functions as a negative regulator of $CRL4^{DCAF1}$ (51) (Figure 2). Using a MM cell line and MeT-5A immortalized mesothelial cell line, the tumor suppressive activity of merlin was shown to be mediated by $CRL4^{DCAF1}$ (51).

Merlin and mTOR signaling pathway. The mTOR pathway is activated in a variety of human malignancies, which is induced by several distinct mechanisms including the activation of the upstream PI3K-AKT cascade (52). Rapamycin (also known as sirolimus) and its analogs (rapalogs) such as evelorimus and temsirolimus have been tested for *in vitro* and *in vivo* studies of many human malignancies including MM.

Merlin has been shown to be a negative regulator of mTORC1 (53,54) (Figure 2). Integrin-mediated adhesion to fibronectin was shown to promote mTORC1 signaling through the inactivation of merlin. Merlin-negative, but not merlin-positive, MM cells displayed unregulated mTORC1 signaling including phosphorylation of 4E-BP1 and S6 (53). As expected, merlin-negative MM cells showed a much enhanced growth-inhibitory effect of rapamycin compared with merlin-positive cells (53). Thus, mTORC1 inhibitors seemed to be more effective for MM cells with *NF2* mutation. In addition, loss of merlin was shown to activate mTORC1 signaling also in meningioma cells (54).

Merlin and Hippo signaling pathway. The Hippo signaling pathway is a regulator of organ size, development and differentiation, and tissue regeneration by restricting cell growth, regulating cell division and promoting apoptosis (55). The four core components in this pathway are MST1/2, SAV1 (also called WW45), MOB1 and LATS1/2, all of which have been shown to act as a tumor suppressor (Figure 2). After receiving upstream signaling, MST1/2 kinase, which makes a complex with a scaffold protein SAV1, phosphorylates and activates LATS1/2. The latter, which is activated by another scaffold protein MOB1, phosphorylates and inactivates yes-associated protein (YAP), a transcriptional coactivator. YAP activates transcription factors of TEA domain family member family members.

The Merlin-Hippo signaling pathway has been shown to be frequently inactivated in MM cells. Besides the mutation of *NF2*, alterations of *large tumor suppressor homolog 2* were identified in several MM cell lines and its tumor suppressive role was also shown *in vitro* (39). One MM cell line had a homozygous deletion at *SAV1* (39). The Merlin-Hippo signaling inactivation leads to constitutive YAP activation; YAP expression was observed in >70% of primary MM tissues, with most positive cases showing greater YAP staining in the nucleus than in the cytoplasm (39). It was reported that YAP activation in MMs was also induced by occasional gene amplification of chromosome 11q22, which is the locus of the *YAP* gene (56).

The YAP activation induces transcription of multiple cancer-promoting genes. The important genes induced by YAP in MM cells are cell cycle promoting genes including *cyclin D1*, *forkhead box M1* and *connective tissue growth factor* (57). Noticeably, *connective tissue growth factor* expression was enhanced significantly both with Hippo signaling inactivation and transforming growth factor-β stimulation (58). Connective tissue growth factor expression was shown to be associated with abundant extracellular matrix formation of MM tissues.

BRCA1-associated protein-1 inactivation

BRCA1-associated protein-1 (*BAP1*), which is localized to chromosome 3p21.1, has been shown to be an important TSG of MM, with 12 (23%) of 53 cases having a somatic mutation (36). A subsequent study using Japanese MM patients also indicated a frequent *BAP1* mutation (40). *BAP1* encodes a nuclear ubiquitin C-terminal hydrolase, one of the classes of deubiquitinating enzymes. BAP1 interacts with multiple proteins including (i) the host cell factor 1 transcriptional scaffolding subunit; (ii) an O-linked *N*-acetylglucosamine transferase subunit, which modifies host cell factor 1; (iii) human orthologs of additional sex combs (ASXL1/ASXL2) and (iv) forkhead transcription factors (FOXK1/FOXK2). BAP1 has been functionally implicated in various biologic processes including chromatin dynamics, DNA damage response and regulation of the cell cycle and growth (59). Recent studies indicate that deubiquitination of host cell factor 1 and histone protein may play important roles in subsequent chromatin modification and gene expression. The histone modification is carried out by interacting with ASXL1 to form a complex, named the Polycomb repressive deubiquitinase complex, which mediates deubiquitination of monoubiquitinated histone 2A at lysine 119 (H2AK119). Thus, BAP1 is suggested to have a role in the regulation of Polycomb target gene expression in MM cells.

Quite surprisingly, germline mutations of *BAP1* were detected in two families with a high incidence of mesothelioma and some *BAP1* mutation carriers in the families developed other types of tumors including uveal melanoma (60). Interestingly, *BAP1* was also shown to be frequently mutated in 26 (84%) of 31 metastasizing uveal melanomas of the eye (61), and germline mutation of *BAP1* was identified in two families with melanocytic tumors (62). Somatic BAP1 mutation was also found in 15% of clear cell renal cell carcinomas (63). These studies indicate that *BAP1* is an important tumor suppressor in multiple tissues and its germline mutation may have a causative role in a cancer-related syndrome, which develops uveal and cutaneous melanoma, mesothelioma, melanocytic BAP1-mutated atypical intradermal tumors, and possibly renal cell and other cancers as well.

Epigenetic alteration

Promoter methylation of known traditional TSGs has been identified in MMs, suggesting that epigenetic inactivation of several common TSGs is involved in MM tumor development and progression as well (64). Those include *E-cadherin*, *fragile histidine triad*, *retinoic acid receptor-β* and *wnt inhibitory factor-1*. Comprehensive epigenetic analysis of promoter regions revealed distinct methylation profile classes in MMs (65,66). The methylation profiles of MM were

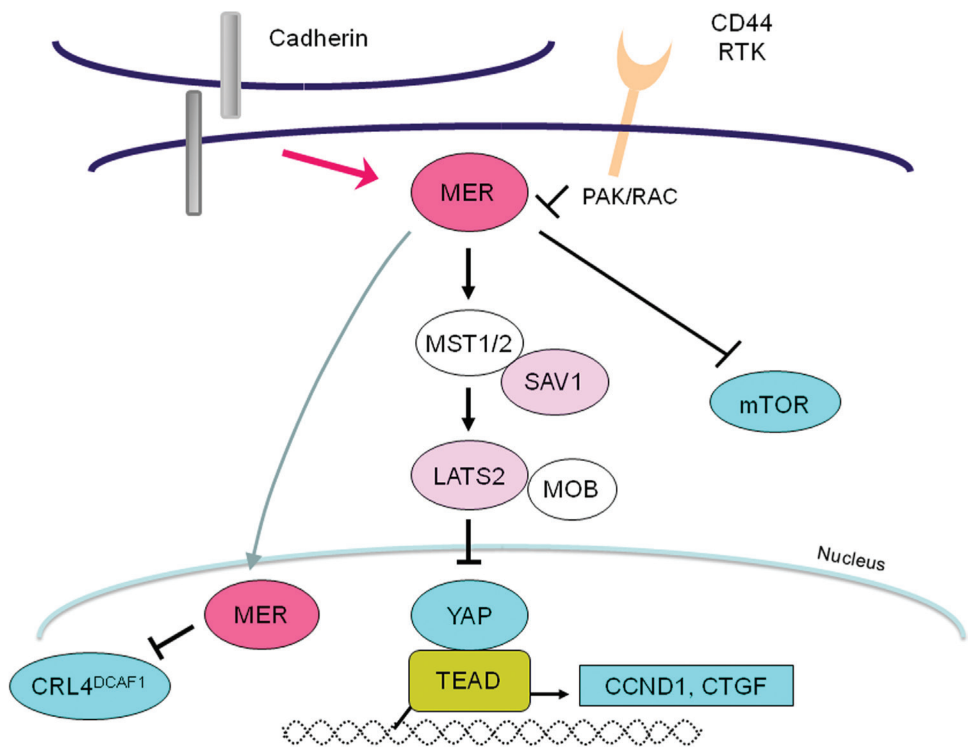

Fig. 2. Schematic representation of Merlin-Hippo signaling cascade. Signals from extracellular environment, transduced via cell–cell contact (cadherin), cell–matrix contact (CD44) or growth factors (RTKs) affect the tumor suppressive activity of merlin. Activated (underphosphorylated) merlin regulates the Hippo cascade, suppressing the activity of YAP transcriptional coactivator. Merlin also regulates mTOR signaling pathway in MM cells. CTGF, connective tissue growth factor; LATS2, large tumor suppressor homolog 2; Mer, merlin; MST, mammalian sterile 20-like kinase; SAV1, Salvador homolog 1; TEAD, TEA domain family member.

different from non-tumor pleura, and methylation class membership among tumors was associated with lung tissue asbestos body burden and patient survival (65). MMs exhibited distinct methylation patterns from lung adenocarcinoma, showing that MM had a relatively infrequent number of genes with hypermethylation compared with lung cancer (66). A possible relationship between gene copy number alterations and DNA methylation profiles was also investigated using 23 MM cases (67). No significant correlations between the copy number of single loci and methylation status of specific genes were found, suggesting two-hit gene inactivation is not commonly achieved by coordinate hypermethylation and allele loss in mesothelioma. However, an association of global genetic alteration and epigenetic dysregulation has been suggested, which was partially attributable to prevalent allele loss at the DNA methyltransferase gene *DNMT1* (67).

In addition, enhancer of zeste homolog 2 and embryonic ectoderm development, which encode components of polycomb repressor complex-2, were shown to be overexpressed in MMs (68). Knockdown of enhancer of zeste homolog 2 or embryonic ectoderm development, or treatment of 3-deazaneplanocin A inhibited MM cell proliferation and tumorigenicity, suggesting polycomb repressor complex-2 might be a novel target for mesothelioma therapy.

MiRs are short non-coding RNAs that regulate gene expression by inhibition of translation and play a major role in carcinogenesis. A distinct miR expression signature has also been found in MM, which is implicated in the potential diagnostic and prognostic utilities of MM (69). hsa-miR-29c*, a member of miR family 29, was expressed at higher levels in epithelial mesothelioma, and increased expression of hsa-miR-29c* was shown to link to a more favorable prognosis of MPM patients with this histology (70). Overexpression of hsa-miR-29c* induced significant decrease of proliferation and migration/invasion of MM cell lines. Noticeably, the possible targets of hsa-miR-29c* were suggested to be *DNMT* genes, which implied the importance of global epigenetic changes to acquire more malignant phenotypes of MM cells.

Taken together, it is yet to be clearly identified how the alteration of global gene expression profile is caused in MM cells; the above results and the discovery of *BAP1* mutation strongly indicate the significance of epigenetic alteration in the development, progression and possibly epithelial–mesenchymal transition of MM cells.

Other genetic clues of importance in MM development

Approximately, 80% of individuals with MM have a history of asbestos exposure, and other mineralogical and environmental factors also contribute to MM susceptibility (71). However, fewer than 5% of asbestos workers develop MM, which suggests that people have different genetic susceptibilities to MM development. For instance, genetic background has been indicated to have a role in determining susceptibility to mineral fiber carcinogenesis, specifically to erionite. A higher incidence of MM in certain families has been observed among residents exposed to erionite in several villages located in Cappadocia, Turkey (72). As mentioned previously, genetic variants of the DNA repair enzyme genes and epigenetics-related genes may account for the different susceptibilities, but a genome-wide association study may also be considered to clarify the genetic susceptibility of individuals in MM development in order to apply the information for the preventive tool.

Finally, newly developed DNA sequencing technologies have been applied for the characterization of genome-wide tumor-associated mutations in MM. A transcriptome sequencing study using complementary DNA from four MPMs detected 15 non-synonymous mutations including seven somatic mutations and three deletions, with each MM having a different mutation profile, suggesting that MM might have relatively limited numbers of genetic mutations (73). Among them, several genes were suggested to have a causative role in MM, including *X-ray cross complementing group 6* (encodes DNA repair Ku70), *PDZK1IP1*, *ACTR1A* (ARP1 actin-related protein 1 homolog) and *AVEN* (apoptosis, caspase activation inhibitor) (73). Using more MM samples with continuously evolving sequencing technology, the landscape of genetic alterations of MM may be more clearly mapped in the near future. In this regard, a collaborative effort of the International Cancer Genome Consortium and the Cancer Genome Atlas to perform exome sequencing of over 200 MM cases was launched in 2012.

Conclusions

The underlying molecular alterations in MM have not yet been clearly determined despite the massive efforts of independent laboratories or collaboration efforts. It was a truly surprising discovery that MM can develop as a familial cancer syndrome with the *BAP1* germline mutation. Although there are still many important unanswered questions, newly developed molecular analytical tools continue to unveil the key cellular events including genetic and epigenetic alterations, which can be applied for target therapy. Differences in individual susceptibility of MM among asbestos exposures of a similar level and duration need to be more precisely determined in order to establish a more effective preventive strategy. Thus, a more complete understanding of the molecular pathogenetical changes of MM is critically needed to develop more effective approaches for identifying and treating this devastating disease.

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