Cancer Chemotherapy and Pharmacology (2019) 84:241–253
https://doi.org/10.1007/s00280-019-03878-3

REVIEW ARTICLE

The role of apoptosis defects in malignant mesothelioma pathogenesis with an impact on prognosis and treatment

Vasiliki Galani[1] · Anna Varouktsi[2] · Stamatis S. Papadatos[3] · Antigoni Mitselou[4] · Ioannis Sainis[5] · Stavros Constantopoulos[6] · Yotanna Dalavanga[1]

Received: 14 March 2019 / Accepted: 18 May 2019 / Published online: 22 May 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Malignant mesothelioma (MM) is a highly aggressive tumor that is strongly related to asbestos fiber exposure. The tumorigenesis procedure in MM is complex, and many pathogenetic mechanisms including chronic inflammation, deregulation of cell death, and the genomic copy-number losses and gains may contribute to carcinogenesis. MM cells are resistant to TRAIL-mediated apoptosis due to defects in extrinsic apoptotic pathway. CAPS, a regulator of cell cycle and death, may contribute to the MM development as well. *BAP1* is the most frequently inactivated gene in MPM; *BAP1* deficiency triggers malignant transformation via disruption of DNA repair, transcription regulation, cell metabolism, apoptosis, and ferroptosis. In addition, bcl-2 family proteins as well as abnormal activation of PI3 K/Akt/mTOR pathway and deregulation of the Wnt signaling pathway may result in MM tumorigenesis. Finally, the Hippo pathway plays a critical role in MPM development. Mutations of *NF2* and *LATS* lead to YAP activation in MPM. Thus, inhibition of YAP activity by YAP inhibitors could be a potentially promising treatment option for MM. In conclusion, extensive genetic alterations exist in mesotheliomas associated with the signaling of apoptotic HM cells death. The comprehension of these pathways may contribute to enhancing survival via developing new effective therapeutic strategies.

Keywords Malignant mesothelioma · Apoptosis defects · Genomic losses · Genomic gains · Prognosis and treatment

Abbreviations

MM	Malignant mesothelioma
HM	Human mesothelial
HMGB1	High-mobility group protein B1
MΦs	Macrophages
IL	Interleukin
BAL	Bronchoalveolar lavage
S100-A9	Calcium- and zinc-binding protein
HSP27	Heat shock protein 27
MTAP	Methylthioadenosine phosphorylase
MPM	Malignant pleural mesothelioma
CFAP45	Cilia- and flagella-associated protein 45
ULK2	Unc-51-like kinase 2
RyR2	Ryanodine receptor
FLIP	FLICE-like inhibitory protein
ERK	Extracellular-signal-regulated kinase
EGF	Epidermal growth factor
CASP1	Caspase 1
TRAF1	TNF receptor-associated factor 1
SEMA3E	Semaphorin 3E
RPS6KA2	Ribosomal protein S6 kinase alpha-2
ARF	ADP-ribosylation factor
mcl-1	Myeloid cell leukemia 1
JNKs	c-Jun N-terminal kinases
AVEN	Apoptosis and Caspase Activation Inhibitor
APAF1	Apoptotic protease activating factor 1
PI3-K	Phosphatidylinositol-3-kinases

✉ Vasiliki Galani
vgalani@uoi.gr

[1] Department of Anatomy, Histology and Embryology, Medical School, University of Ioannina, 45110 Ioannina, Greece

[2] Department of Internal Medicine, Ippokratio Hospital, Thessaloníki, Greece

[3] 3rd Department of Internal Medicine, Sotiria General Hospital, National and Kapodistrian University of Athens Medical School, Athens, Greece

[4] Department of Forensic Pathology, Medical School, University of Ioannina, Ioannina, Greece

[5] Department of Cancer Biobank Center, University of Ioannina, Ioannina, Greece

[6] Department of Pneumonology, University of Ioannina, Medical School, Ioannina, Greece

MTOR	Mammalian target of rapamycin
S6K1	Ribosomal protein S6 kinase beta-1
MapK	Mitogen-activated protein kinase
WNT1	Wnt family member 1
MYC	MYC Proto-Oncogene
SAPK	Stress-activated protein kinases
CCND1	Cyclin D1
SFRP2/4	Secreted Frizzled-Related Protein 2/4
DKK1	Dickkopf-related protein 1
MDM2	Mouse double minute 2 homolog
pRB	Retinoblastoma protein
SLC7A11	Solute Carrier Family 7 Member 11
IAP	Inhibitors of apoptosis proteins
TRAF	TNF receptor-associated factors
CARP-1	Cell division cycle and apoptosis regulator protein 1
XAF1	XIAP-associated factor 1
TROY	Tumor necrosis factor receptor superfamily, member 19
BIRC5	Baculoviral IAP Repeat Containing 5
YAP	Yes-associated protein
ROCK2	Rho-associated coiled-coil containing protein kinase 2
NF2	Neurofibromin 2
LATS2	Large tumor suppressor homolog 2
SAV1	Salvador homolog 1
RASSF	Ras-association domain family
TEAD1	Transcriptional enhancer factor TEF-1
ROCK	Rho-associated protein kinase
REN	Neural progenitor cell line
PPIX	Protoporphyrin IX
RAGE	Receptor for advanced glycation endproducts

Introduction

Malignant mesothelioma (MM) is an aggressive neoplasm that arises primarily from the surface serosal cells of the pleura and peritoneum, and has been classified histologically into three subtypes: epithelioid, sarcomatoid, and biphasic [1]. About (70% to 90%) of pleural mesotheliomas have been associated with exposure to carcinogenic mineral fibers, mainly asbestos; for peritoneal mesothelioma, the proportion is lower [2, 3]. Human mesothelial (HM) cells are very sensitive to asbestos cytotoxicity, and many pathogenetic mechanisms including chronic inflammation and genetic and epigenetic alterations may contribute to carcinogenesis. The primary pathogenic pathways that were thought to induce asbestos-related malignant transformation include inflammation, apoptosis, and oxidative stress. Asbestos causes apoptotic HM cells death, leading to translocation of high-mobility group protein B1 (HMGB1) from the nucleus to extracellular space. HMGB1 promotes macrophage accumulation and inflammatory cytokines secretion such as TNF-alpha, Interleukin-1beta (IL-1beta), and Interleukin-6 (IL-6) which correlates with neoplasia [4, 5]. TNF-alpha activates the NF-κB pathway, which increases survival of asbestos-damaged HM cells and promotes tumor formation and development. Basic genetic lesions accumulate within the HM cells which retain asbestos-induced DNA damage resulting in the onset of mesothelioma [6]. Moreover, different functional and phenotypical macrophages (MΦs) subpopulations are implicated in antitumoral or protumoral immunity [7]. The major subtype of activated MΦs has a proinflammatory antitumoral phenotype, while one second subtype of activated MΦs is associated with immunosuppression [8]. Tumor-associated macrophages (TAM) represent one of the major populations of immune cells infiltrating tumors, and generally obtain functional characteristics similar to MΦs that are associated with immunosuppression [9]. MM has a big number of TAM, suggesting that they play a pivotal role in this malignancy [10]. In addition, proteomic analysis (2D electrophoresis/mass spectrometry) of BAL fluid in Metsovites nonoccupationally exposed to asbestos revealed increased albumin fragments, α1-antitrypsin, S100-A9 (calcium- and zinc-binding protein), and HSP27, suggesting ongoing inflammation and induction of apoptosis [11].

However, the risk of developing MM in high-risk individuals professionally exposed to asbestos is ~ 5%, suggesting that other genetic factors are involved in MM pathogenesis [2]. *BAP1* mutations were the most frequent; the rest were met in genes associated with DNA damage sensing and repair (*BRCA2*), oxygen sensing (*VHL*), endosome trafficking (*ENTR1, TMEM127*), and cell growth (*CDKN2A, WT1*) [12, 13]. Indeed, recent studies have shown that genetic alterations in many mesotheliomas include frequent somatic mutations of *BAP1*, *NF2*, and to a lesser degree include *SETD2*, *TP53*, *DDX3X*, *ULK2*, *RYR2*, *CPAF45*, *SETDB1*, and *DDX51* [14]. Undeniably, asbestos has mutagenic and genotoxic effects and may induce the production of reactive oxygen and nitrogen species from mesothelial cells and macrophages, resulting in indirect genotoxicity such as base substitutions, deletions, rearrangements, insertions, sister chromatid exchanges, and chromosomal aberrations; all these lesions may lead to a wide range of mutations in mammalian cells [15]. In the same in vivo experiments (216 MPM tumors), Bueno et al. used RNA-seq data and they identified four distinct molecular subtypes: sarcomatoid, epithelioid, biphasic-epithelioid (biphasic-E), and biphasic-sarcomatoid (biphasic-S) [14]. Furthermore, in mesotheliomas, chromosomal losses occur on the 9p21 region containing *CDKN2A*, *CDKN2B,* and *MTAP*. Molecular differences are also revealed between pleural and peritoneal mesothelioma cells in genomic copy-number losses and gains, indicating that different genetic alterations may be related to the

different site [16, 17]. Further studies should be conducted, to elucidate specific apoptotic signalings that are associated with any combination of these mutations or deletions.

The aim of this review is to provide an overview of the advances in the underlying molecular mechanisms of mesothelioma cell death and their role in diagnosis, monitoring, and therapeutic interventions.

Apoptosis deregulation (Table 1)

Before the examination of the role of apoptosis in MM, a concise introduction of the basic pathways of apoptosis should be presented. Apoptosis is mediated by two signaling pathways, the extrinsic pathway and intrinsic pathway. The extrinsic pathway is initiated by death receptors belonging to the Tumor Necrosis Factor-Receptor (TNF-R) family, which includes TNF-R1, Fas/CD95, Death Receptor (DR) 3, DR4, DR5, and DR6. The specific ligands for the TNF-R family belong to the TNF family, which includes TNF-alpha, Fas-ligand, lymphotoxin (LT) a, apo-3-ligand, and TNF-related apoptosis-inducing ligand (TRAIL) or Apo2L. The intrinsic pathway (also known as mitochondrial) pathway is induced in response to stress stimuli, such as DNA damage caused by chemotherapeutic agents, UV- or γ-irradiation or withdrawal of survival signals, such as growth factors, cytokines, or hormones. The intrinsic pathway involves the release of cytochrome c from the mitochondrial intermembrane space. These two pathways are intimately connected and they finally converge into one common pathway resulting in activation of effector or executioner caspases 3, 6, and 7 [18]. In the intrinsic pathway, the mitochondrial membrane potential and permeability are regulated by the Bcl-2 family of proteins. Members of this family include both proapoptotic proteins such as Bax, Bak, Bad, Bid, or Bim, and antiapoptotic proteins, such as Bcl-2, Bcl-xL, and Mcl-1 [19].

Regulators in extrinsic apoptotic pathway

Tumor necrosis factor-related apoptosis-inducing ligand or Apo 2 ligand (TRAIL/Apo2L) selectively leads to apoptosis of a variety of tumor cells and transformed cells. This ligand initiates a death receptor pathway to apoptosis; four TRAIL receptors have been identified and two of them, namely TRAIL-R1/DR4 and TRAIL-R2/DR5, are capable of the transduction of the apoptotic signal, whereas the other two receptors (TRAIL-R3/DcR1, TRAIL-R4/DcR2) act as antagonists, since they lack death domains that are crucial for apoptosis induction [20].

Although a number of MPM cells express TRAIL-R1 and TRAIL-R2 with higher TRAIL-R2, most of them are resistant to TRAIL-mediated apoptosis [21]. In in vitro studies, using spheroid culture and resected tumor samples of three mesothelioma subtypes, the combination of FLIP-siRNA, chemotherapeutic drugs such as α-tocopheryl succinate or cycloheximide with TRAIL demonstrated an efficient apoptotic effect compared to treatment with TRAIL alone [22, 23]. In the same context, Belyanskaya et al. used MPM cell lines or primary cultures [(epithelioid: H2452, SCP111, ZL5, ZL55, H28), (biphasic: SDM4), (sarcomatoid: MSTO-211H) to TRAIL-sensitive] and treated the cells with agonistic monoclonal antibodies directed to TRAIL-R1 (Mapatumumab) and TRAIL-R2 (Lexatumumab). They found that combined cisplatin treatment with either Mapatumumab or Lexatumumab significantly increased apoptosis in TRAIL-sensitive MPM tumors that are resistant to cisplatin, Mapatumumab, or Lexatumumab monotherapy via caspase-dependent activation [21].

Moreover, Liu et al. demonstrated that TRAIL enhances the chemosensitivity of tumor cells to various therapeutic agents, such as doxorubicin, gemcitabine, cisplatinum, or etoposide in mesothelioma cell lines. However, treatment with TRAIL alone attenuated apoptosis on MM cell lines [(epithelioid:VAMT, M28, REN), and (MS-1; endothelial)] [24]. Thus, it is suggested that this resistance is associated with overexpression of the caspase-8 inhibitor (FLIP/CFLAR) and methylation of TRAIL receptors in MM cells [23]. Subsequently, the use of thermal pressure, subtoxic doses of succinate or anisomycin, showed that it can sensitize MM cells [(biphasic: MM-B1), (sarcomatoid: Meso-2), and (epithelioid: Ist-Mes)] to TRAIL and induce apoptosis in vitro through mitochondrial increase in Bid-dependent apoptotic signaling [25, 26]. Accordingly, Katz et al. studied the potential antiproliferative effects of multikinase inhibitor sorafenib with TRAIL on TRAIL-resistant cells [(sarcomatoid: MSTO-211H), (M30: Hybridoma), (epithelioid: H28, H2052 and H2452, REN)]. They confirmed the antiproliferative effects of TRAIL synergistically with sorafenib, by caspase-independent activation [27]. Therefore, although MPM cells express TRAIL-R1 and TRAIL-R2, most of them are resistant to TRAIL-mediated apoptosis due to defects in extrinsic apoptotic pathway.

In addition to the classical apoptotic pathway of death receptors, there is an alternative pathway, the NF-kB signaling. When asbestos fibers interact with mesothelial cells, asbestos induces multiple cell-signaling pathways that lead to the NF-kB pathway activation through ligand–receptor interaction, resulting in transcription of multiple prosurvival genes which inhibit apoptosis and induce tumor development [28]. Therefore, blocking TNF-alpha with etanercept, a recombinant decoy receptor for TNF-alpha and the disruption of the NF-κB pathway, for example with Onconase (Ranpirnase), a drug that inhibits the NF-κB pathway, had beneficial effects in some MM patients [29].

Moreover, Calcyphosine (CAPS), a Ca^{2+}-binding protein, is involved in several types of malignant tumors. CAPS is

Table 1 Potential therapeutic targets in the treatment of mesotheliomas and prognostic implications: apoptotic pathways and/or the signalings pathways associated with apoptosis

Apoptotic pathways expressed/reduced	Targets and potential therapeutic approaches	Refs.
TRAIL	TRAIL + chemotherapeutic agents TRAIL + histone deacetylase inhibitor α-Tocopheryl succinate alone or plus TRAIL Monoclonal antibodies against DR4 or DR5	[22, 26–28]
IAPs	XIAP antisense oligonucleotides + chemotherapeutics Small molecule chemical IAP inhibitors Proteasome inhibitor alone or plus chemotherapeutic drugs	[82, 83]
FLIP overexpression	Combination of $FLIP_L$ siRNA with chemotherapeutic drugs (α-tocopheryl succinate or cycloheximide) with TRAIL	[23, 24]
IAP1	Antisense therapy + cisplatin Proteasome inhibitors increase of IAP-1 protein level	[82, 84, 89]
Survivin	Antisense therapy Transfection with a dominant negative survivin mutant	[88, 89]
Bcl-2 family members Proapoptotic Bax and Bak Normally expression Antiapoptotic Bcl-xl, mcl-1 Normally expression Antiapoptotic AVEN Impairs activation of caspases	Antisense oligonucleotides against Bcl-xL, Bcl-2 alone or + chemotherapeutics Sodium phenylbutyrate and proapoptotic gene therapy Bcl-2 binding component 6 (bbc6) with potential therapeutic and prognostic implication	[38–40, 103]
BAP1 Low levels of BAP1 inhibit apoptosis	Loss of *BAP-1* may confer a better prognosis Immunotherapy treatment, according to the *BAP1* status PARP inhibitors	[64, 65, 70, 71]
NF-kB pathway	Proteasome inhibitors Regulation of IAPs Drugs Bortezomib, a selective inhibitor of the 20S proteasome and Onconase inhibit the NF-kB pathway Blocking TNF-α with etanercept or disruption of the NF-κB pathway with Onconase	[82, 84] [30]
p53 pathway: Expression of wild-type p53 gene	p53 is inactivated by binding to proteins mdm2 Ad-p53 combination with the first-line chemotherapeutics induces phosphorylation of p53 and death of cancer cells p53 inducible protein with potential therapeutic and prognostic implication	[37, 103]
Signalings pathways-associated with apoptosis		
PI3K/AKT/mTOR Abnormal activation-increase of AKT activity	Inhibition of mTOR PTEN gene therapy	[54, 55]
JNK Upregulation of Wnt and Wnt-related genes	Regulation of Wnt signaling pathway Anti Wnt2 monoclonal antibody alone or plus pemetrexed Transfection of DKK-1 cDna construct Transfection of the SFRP gene construct Mitogen-activated protein kinase kinase kinase 8 and 10 (MAPKKK 8, 10) with potential therapeutic and prognostic implications	[60, 61, 103]
ERK1/2 Increase of AP-1 activity	EGFR autophosphorylation EGFR inhibitors Monoclonal antibodies against EGFR Small molecule inhibitors Tissue EGFR a molecular prognostic factor: EGFR expression ↑ survival, not predictive	[35, 36, 102]
Hippo Deregulation is frequent with inactivation of *NF2*	YAP is a downstream negative effector of the Hippo signaling Down-regulation of YAP activity siRNA Small molecule verteporfin, PPIX	[89, 90, 95, 96, 98]

Table 1 (continued)

Apoptotic pathways expressed/reduced	Targets and potential therapeutic approaches	Refs.
HMGB1 Inhibited MM cell growth and motility in vitro Reduced tumor growth in vivo, and -prolonged survival of MM-bearing mice	Ethyl pyruvate (EP) Impairs HMGB1 secretion by MM cells leading to reduced RAGE expression and NF-κB activation EP impaired cell motility, cell proliferation, and anchorage-independent growth of MM cells Polypeptides 1. Recombinant HMG Box-A inhibits HMGB1 activity with more efficient HMGB1 targeting 2. AntiHMGB1 neutralizing monoclonal antibody inhibits HMGB1 and the MM malignant phenotype	[100] [5, 101]

associated with cell cycle regulation and death through Ca^{2+} signaling [11, 30]. CAPS is upregulated in response to thyrotropin and the cAMP cascade, and down-regulated via tissue plasminogen activator (TPA) and epidermal growth factor; CAPS can induce the tumorigenesis and tumor progression through crosstalk between cAMP and EGF signals [31]. The presence of CAPS in the BAL fluid of Metsovites nonoccupationally exposed to asbestos showed that this may be involved in the detoxification of mutagenic and toxic agents in patients without pleural calcifications; CAPS may also contribute to the MM development by disturbing intracellular Ca^{2+} homeostasis [11].

Asbestos fibers also stimulate the extracellular signal-regulated kinase (ERK1/2) signaling pathway via epidermal growth factor receptor (EGFR) autophosphorylation, which increases activator protein (AP)-1 activity and mesothelial cell mitosis [29, 32]. Increased ERK1/2 activation occurs in human malignant mesotheliomas. It has been shown that ERK1/2 activation may be associated with the development of asbestos-induced inflammation, neoplasias such as lung cancer and MM and fibrosis [33]. Its oncogenic potential is associated with increased cell survival, mainly by inducing the activity of antiapoptotic proteins and repressing proapoptotic proteins. Both ERK1 and ERK2 are involved in human MM cell progression in vitro, as well as tumor development of a sarcomatoid MM line, whereas ERK2 is essential for the growth of a human epithelioid MM in vivo [34]. Using a synthetic small molecule MEK1/2 inhibitor (U0126) and RNA silencing of ERK1/2, comparatively, Shukla et al. showed that ERK2 is significant concerning transformation and homeostasis of human epithelioid malignant mesotheliomas, asbestos-induced tumors with a poor prognosis. In in vivo and in vitro experiments, they transfected MM cell (biphasic: HMESO) lines with shERK1 or shERK2 and both exhibited significant decreases in cell propagation. Furthermore, the injection of shERK2 cells and not shERK1 cells, into immunocompromised SCID mice showed notable reduced tumor growth in contrast to shControl cells. In vivo results are similarly interesting; after injection of sarcomatoid human MM (PPMMill) cells into SCID mice, both shERK1 and shERK2 lines showed notable reduced tumor growth. They concluded that members of the ERK family may have distinct roles in the differentiation and carcinogenesis. In addition, microarray and qRT-PCR analyses disclosed significantly increased of gene expression such as *CASP1*, *TRAF1*, *FAS* and the decreased of gene expression such as *SEMA3E*, *RPS6KA2*, *EGF*, and *BCL2L1* in shERK2-transfected MM cells compared to scrambled control (shCon) transfected MM cells [34].

Given the fact that curcumin (CUR) can efficiently inhibit the ERK1/2 signaling pathway by altering the phosphorylation of ERK1/2, the use of Curcumin might induce cell death. Masuelli et al. studied the possible antiproliferative effects of CUR on human [(biphasic: MM-B1, H-Meso-1), (sarcomatoid: MM-F1)] and mouse (#40a) MM cells. They found that CUR-mediated apoptosis by the activation of caspase 9, cleavage of PARP-1, increase of the percentage of cells in the sub G1 phase which was attenuated (MM-F1 and #40a) or abrogated (MM-B1 and H-Meso-1) after MM cells co-treatment with the apoptosis inhibitor Z-VAD-FMK. They also found that the intraperitoneal administration of CUR increased the median survival of C57BL/6 mice intraperitoneally transplanted with #40a cells and decreased the risk of developing tumors [35]. Thus, ERK1/2 plays a central role in gene expression concerning the development of epithelial MM.

Finally, p53 can be deregulated leading to loss of control of cell cycle regulation and inactivation of the apoptotic response resulting in tumor formation. The majority of MM has the wild-type *p53*gene with a homologous deletion of the INK4A/ARF locus containing the *p14ARF* and the *CDKN2A* genes. Li et al. showed that Ad-p53 transduction, with adenoviruses bearing the *p53* gene, induces phosphorylation of p53, upregulation of Mdm2 and p21, and decreased phosphorylation of pRb in MM cell lines [(epithelioid: NCI-H2452, NCI-H2052, NCI-H226, and NCI-H28) and (sarcomatoid:MSTO-211H)]. This study also indicated that Ad-p53 transduction activates the extrinsic death receptor-mediated apoptosis through caspase-8 and that the intrinsic mitochondrial-mediated apoptosis is less likely to

be involved. In addition, it was found that intrapleural injection of Ad-p53 and systemic administration of CDDP (cisplatin) induced death of cancer cells in an orthotopic animal model. Therefore, Ad-p53 combination with the first-line chemotherapeutics may provide a potential agent for therapeutic approach in mesothelioma [36].

Regulators in intrinsic apoptotic pathway

There is accumulated evidence that the bcl-2 family of proteins, along with caspases-mediated signaling, plays a role in the regulation of apoptotic pathways and tumor conduct. Members of the bcl-2 family have been shown to be associated with resistance to chemotherapy and radiation. When referring to mesothelioma, a number of studies in tissue cultures as well as in histological sections from malignant mesotheliomas and metastatic adenocarcinomas had been performed. Soini et al. indicated that bcl-2 is inversely associated with the apoptotic index, but is relatively infrequently expressed, whereas bcl-X, mcl-1, and bax overexpression in malignant mesotheliomas and Mcl-1 has also been associated with the apoptotic resistance of mesothelioma cells [(biphasic: M10K, M24K, M25K, M33K, M38K), (epithelioid: M14K, M28K) [37]. Accordingly, in other studies, the effects of bcl-x antisense oligonucleotides, sodium phenylbutyrate, and the gene transfer of proapoptotic Bcl-2 family members such as Bak in mesothelioma cell lines cause bcl-x down-regulation, induce apoptosis, and decrease the cellular viability in p53-sensitive and p53-resistant mesothelioma cells. These studies confirmed that MPM lines and tumors rarely expressed the antiapoptotic Bcl-2 protein, but normally express the antiapoptotic protein Bcl-xl and the proapoptotic proteins Bax and Bak [37–39]. In addition, several in vitro studies using sarcomatoid:I-45 and epithelioid:REN cell lines and in vivo studies using severe combined immunodeficient (SCID) mouse model which accept xenogeneic cells, indicated that down-regulation of Bcl-xL enhances baseline tumor cell death and improves sensitivity to chemotherapeutic agents [40, 41].

Moreover, Varin et al. showed that the down-regulation of Bcl-xL, alone or in combination with cisplatin, and the concurrent inhibition of Mcl-1 induce the cell death in chemoresistant mesothelioma cell lines [(Sarcomatoid: MSTO-211H), (epithelioid:NCI-H28, NCI-H2052, IST-Mes2)] to cisplatin [42]. Thus, most members of the proapoptotic Bcl-2 family are expressed in mesothelioma with functional integrity, suggesting that the loss of their proapoptotic properties is due to confiscation by Bcl-xL or Mcl-1.

On the other hand, cytological analysis of pleural fluid often discloses the presence of free spheroid aggregates of malignant cells, due to the ability of non-adherent tumor cells to resist the loss of anchorage-induced apoptosis (so-called anoikis), resulting in new tumor foci in the pleural cavity. Daubriac et al. showed that the non-tumoral mesothelial cells MeT-5A (epithelial) enter anoikis (programmed cell death induced upon cell detachment from extracellular matrix) in an SAPK/JNK-, Bim-, and caspase-9-dependent pathway. The activation of SAPK/JNK with anisomycin can attenuate the survival of MPM through a Bim-dependent mitochondrial pathway. Therefore, the impairment of cell aggregation causes the activation of SAPK/JNK and Bim and induction of anoikis. In addition, the intercellular contacts are associated with the anoikis resistance of MPM cells [43]. Moreover, there are other (non-Bcl-2) antiapoptotic survival pathways such as AVEN that impairs Apaf-1-mediated activation of caspases, resulting in MPM tumorigenesis [44].

Besides the Bcl-2 family of proteins, there are survival signaling cascades such as the PI3K/Akt/mTOR pathway that are involved in the regulation of mitochondria-mediated apoptosis. Protein kinase B (Akt) is a key signaling part lying downstream of PI3-K (phosphatidylinositol 3-kinase). The PI3K–AKT pathway is involved in tumor development and carcinoma invasion. The PI3K–AKT pathway is activated by many growth factors and interacts with mTOR (Mammalian target of rapamycin) that can be activated in distinct ways to induce translation initiation [45–47]. In addition, the tumor suppressor PTEN encodes a lipid phosphatase that negatively regulates the PI3-K/AKT cell survival signaling [46, 48].

The abnormal activation of PI3K/AKT/mTOR and the increased AKT activity in mesothelioma have been well established [49–52]. Interestingly, treatment of mesothelioma cells (epithelioid:VAMT, M28, REN) in three-dimensional cultures and ex vivo, tumor fragment spheroids with inhibitors of the PI3K/AKT/mammalian mTOR pathway, particularly rapamycin, showed higher resistance to apoptosis than mesothelioma cell monolayers. This study provided evidence that mTOR/S6K pathway, a central growth signaling regulator, mediates the apoptotic resistance of mesothelioma cells. In addition, by isolating the apoptotic pathway through the Bid knockdown, they confirmed that mTOR controls a step distal to Bid, possibly at the mitochondrial level. Thus, the inhibition of mTOR could be a potentially promising therapy for MM [53, 54].

The activation of the JNK pathway is associated with the apoptosis and regulators of the JNK pathway can induce tumor cell apoptosis on radiation therapy or chemotherapy [55]. Expression of the glutathione-S-transferase (GSTP1) has been associated with the regulation of cell proliferation and apoptosis through a JNK-dependent mechanism or through a JNK-independent pathway mediated by ERK signaling pathways [56]. Archimandriti et al. found increased expression of acid ceramidase (AC) and glutathione-S-transferase in the BAL fluid of Metsovites nonoccupationally exposed to asbestos, using proteomics analysis consisting

of 2D electrophoresis, image analysis, and mass spectrometry. In addition, they found increased albumin fragments, α1-antitrypsin, S100-A9, a marker of inflammation [11]. This result further reinforces the previous finding that alveolar macrophages exposed to asbestos fibers release proteolytic enzymes and the presence of the small MW proteins in the BAL fluid of patients with asbestosis is a result of the activity of enzymes of activated macrophages [4]. Furthermore, Kastamoulas et al. provided evidence that cytokines such as IL-13, TNF-alpha, and IL-1 beta attenuate the procell death effects of Fas on human neoplastic alveolar epithelial cell line A549, at least partially, by pathways involving the ERK1/2, p38, JNK, and PI3-K/Akt [57].

In conclusion, the inhibition of tumor growth through Map kinase pathways is associated with the tumor profile in a special way. Yet, in vivo data concerning the effectiveness of Map inhibitors in MM patients or even animal models are still relatively few.

Another regulator pathway involved in JNK-mediated intrinsic apoptosis pathway is the Wnt signaling pathway. Binding of Wnt proteins to specific Frizzled family of seven-pass transmembrane receptors activates a number of signaling pathways that regulates developmental processes and cell proliferation. Loss of control of Wnt signaling pathways has been indicated in cancer and plays a critical role in tumor development [58]. Data from custom arrays studies indicate deregulation of gene expression involved in the Wnt signaling pathway and downstream of Wnt signaling in MM cell lines [(epithelioid: H28, LRK1A, NCI-H2052, NCI-H290, REN), (adenosquamous lung carcinoma: H513), (endothelial: MS-1)], and normal mesothelial cell line, LP9), primary MPM tumors, and normal pleural tissue [59]. In the same context, Mazieres et al. also studied 16 matched samples (malignant tissue and normal adjacent pleura) and found that numerous *Wnt* genes *Wnt1*, *Wnt2*, *Wnt5,* and *Wnt*-related genes *MYC*, *CCND1, JUN*, were upregulated, and *Wnt2* was more often upregulated. In contrast, *Wnt8A* and some Wnt antagonists such as *DKK1* (Dickkopf), *SFRP2*, and *SFRP4* were down-regulated. In addition, they used antiWnt2 antibody and Wnt2 small interfering RNA, and indicated the role of Wnt2 in cell survival through inhibition of the downstream effectors of the Wnt pathway [59]. In vivo and in vitro results are similarly interesting; Kashiwakura et al. demonstrated that one member of *DKK* gene family, the *REIC/Dickkopf-3* was lower expressed in 4 human MM cell lines [(sarcomatoid:211H), (epithelioid: H28, H2052, H2452) than in normal tissue and overexpression by transduction in 1 cell line (211H), induced apoptosis via a JNK-dependent pathway [60]. This study was followed by another in vivo experiment using orthotopic inoculation of *REIC*/*Dickkopf*-3–deficient, luciferase-labeled MM cells, followed by intrapleural injection of recombinant REIC/Dickkopf-3-adenovirus, indicating increased expression of phosphorylated JNK and inductance of tumor cell apoptosis [60]. Therefore, the deregulation of the Wnt signaling pathway can be associated with mesothelial carcinogenesis and gene therapy with *REIC/DKK-3* could be considered as an effective therapeutic target in MM.

The ubiquitin carboxyl terminal BAP1 is a member of deubiquitinating enzymes superfamily associated with Ca^{2+}-signaling-mediated cell death. BAP1 deubiquitylates the IP3R3 channel receptor and induces the release of Ca^{2+} from the endoplasmic reticulum into the cytoplasm and mitochondria. High levels of mitochondrial Ca^{2+} induce cytochrome c release from the mitochondria into the cytoplasm, resulting in apoptosis. BAP1 also regulates apoptosis and ferroptosis with its involvement in tumor suppressor function, in vivo [61]. Recently, Zhang et al. demonstrated that BAP1 promotes ferroptosis by suppressing the expression of *SLC7A11* gene, resulting in low levels of reduced glutathione and decreased antioxidant ability of the cells, promoting the lipid-ROS accumulation [62].

It has been shown that low levels of BAP1 inhibit both apoptosis and ferroptosis, facilitating the survival of DNA damage cells [61]. Bott et al. reported somatic BAP1 mutations in malignant pleural mesothelioma [63] and Testa et al. also found MMe patients with germline *BAP1* mutations [64]. *BAP1* mutations are associated with better prognosis in MMe patients [65] and somatic *BAP1* point mutations were found in up to 60% of sporadic MMe [63, 66–68].

On the other hand, the increased resistance of mutated *BAP1* appears to be inconsistent with the clinical evidence that MMe patients with *BAP1* germline mutations survive longer [12]. This obvious discrepancy could be attributed to the fact the *BAP1* WT induces cancer stem cell generation, resulting in increased survival despite the decrease in chemosensitivity, as the total observed survival benefit is a result of the lack of functional *BAP1* leading to the cancer stem cell generation [12]. The different sensitivity to DNA damage between *BAP1* mutant and WT has been proposed to be the basis of selection of patients for treatment with poly-ADP ribose polymerase (PARP) inhibitors, given that patients with *BAP1* mutated or *BAP1* WT are likely to respond differently to this type of inhibitors. In addition, it has already been suggested the defective DNA repair leading to chromosome instability and increased mutations to provide a basis for the categorization of patients for immunotherapy treatment, according to the *BAP1* status [69, 70]. Therefore, further studies should be conducted, both in vivo and in vitro, to confirm the role of *BAP1* status on chemosensitivity of MMe to other drugs such as pemetrexed and platinum-based treatments, as well as potential effects of gemcitabine on *BAP1* signal transduction [71].

There is increasing evidence that asbestos-induced MM pathogenesis is a complex model in which the chronic inflammation can have antitumoral or protumoral roles,

depending on the cellular and soluble mediators involved. Given the fact that there is intra- and inter-familial mutability of cancer types in germline BAP1 mutated carriers, Napolitano et al. used *BAP1*(±) mouse model and they found that *BAP1*(/−) mice had a notably higher incidence of mesothelioma after exposure to very low doses of asbestos, which infrequently induce mesothelioma in wild-type mice. They concluded that minimal exposure to carcinogenic fibers may significantly increase the risk of malignant mesothelioma in individuals carrying germline *BAP1* mutations, possibly due to changes in the inflammatory response [72]. Xu et al. came to similar conclusions when they generated *BAP1*(±) mice that developed mesothelioma after intraperitoneal injection of crocidolite asbestos, and additionally suggested that *BAP1*(±) mice might develop MM at an earlier age compared to wild-type neonates [73]. However, a study suggests that mesothelioma development occurs without asbestos exposure in *BAP1* knockout mice [74]. Ohar et al. reported germline *BAP1* mutations in 9 of 150 patients with mesothelioma and a family history of cancer (6%) compared to none of the individuals exposed to asbestos without a family history of cancer. They concluded that low-level asbestos exposure plays a role in the mesothelioma development [75]. However, these findings are unclear as the authors did not provide details about exposure to asbestos such as dose and latency, as well as tumor site [3].

Apart from experimental studies, there is clinical evidence that germline *BAP1* mutations may be associated with MM prognosis. Baumann et al. studied 23 patients (13 of the 23 subjects had a second malignancy) with germline *BAP1* mutations' male-to-female ratio was 9:14, and half of the patients were younger than 55 years; 10 of the tumors were peritoneal, 10 pleural, and 3 recorded as originating from both sites. The overall survival was 2 years for the patients with pleural tumors and 10 years for the patients with peritoneal tumors. They concluded that germline *BAP1*-mutated mesotheliomas are associated with longer survival than the usual sporadic mesothelioma [65]. Leblay et al. have also found that inactivation of *BAP1* is as common in peritoneal mesotheliomas as in pleural mesotheliomas and BAP1 protein nuclear expression is a good prognostic factor and a more reliable marker for the loss of *BAP1* activity than mutation [76]. Thus, loss of *BAP1* may confer a better prognosis.

Moreover, immunohistochemical (IHC) stains with a monoclonal antibody for BAP1 were used to differentiate MM from lung carcinomas. Carbone et al. stained 45 non-small cell lung cancer samples (32 adenocarcinomas and 13 squamous cell carcinomas) and 35 MM biopsies with a monoclonal antibody BAP1. They found that the absence of BAP1 nuclear staining was associated with MM and the lack of nuclear BAP1 stain helps to differentiate MM from lung carcinomas [77].

In summary, one of the suggested mechanisms of MM cell chemo-resistance is related to defects in apoptotic pathways and especially in the intrinsic pathway, as many antitumor agents exert their cytotoxic effects through its activation.

Regulators in both the extrinsic and intrinsic apoptotic pathways

Among the many factors involved in cancer cell immortality, the transcriptional factor NF-kB seems to play a significant role in MPM. Activation of the NF-kB pathway can stimulate proliferation and reduce the effectiveness of chemotherapy and ionizing radiation [78]. The NF-kB pathway can be activated either by the AKT pathway by the IkB kinase beta (IKKβ, canonical NF-κB pathway) or by binding of TRAF with the activated NF-kB-inducing kinase (NIK), which successively leads to activation of NF-kB by the IkB kinase alpha (IKKα) [47, 79].

If activated, the p65 subunit of NF-kB translocates to the nucleus to activate genes that protect the cell from apoptosis (IAPs, inhibitors of apoptosis). IAPs proteins are crucial regulators of both NF-κB pathways. Overexpression of XIAP efficiently inhibits caspase activation and apoptosis stimulated by the intrinsic apoptosis pathways and extrinsic apoptosis pathways. Conversely, cells that lack XIAP are sensitized to apoptosis [80]. There is evidence that the drugs Bortezomib (also known as Velcade), a selective inhibitor of the 20S proteasome, and Onconase inhibit the NF-kB pathway. The effects of Bortezomib are pleiotropic and include inhibition of NF-kB activation by preventing IkB degradation [81, 82]. IAPs are also positively regulated by NF-kB a transcription factor whose inhibitor (I-kB) is degraded by the proteasome during the activation of NF-kB in MPM. The same way, proteasome inhibitors induce anticancer activity in MPM cells [(epithelioid: STAV-AB), (biphasic: M14 K, M28 K, ZL34), (sarcomatoid: STAV-FCS)] and A549 cells through induction of G2/M and G1/S cell cycle arrest and through stabilization or increase of p21 and IAP-1 protein levels and, to a lesser degree, p27, IAP-2, and XIAP [81, 82].

Other in vitro results are interesting, as well; Gordon et al. suggest that IAP-1 facilitates at least some resistance to bortezomib in MPM cells. They examined the effect of bortezomib on apoptosis-related proteins IAP-1, IAP-2, survivin, and XIAP and found that viability of mesothelioma cell lines [(biphasic: JMN, MS589), (normal primary: HM3), (sarcomatoid: H2052), (epithelioid: H28), (lung adenocarcinoma: H23)] was repressed in a dose- and time-dependent manner. Bortezomib also stabilized or increased protein levels of p21/*WAF1* and IAP-1 and to a lesser degree p27, IAP-2, XIAP, and survivin. In addition, they showed that bortezomib in combination with cisplatin was generally

synergistic at high concentrations and antagonistic at low concentrations. It is worth noting that pretreatment with bortezomib resulted in increased cytotoxicity of cisplatin and pemetrexed in a concentration-dependent manner [83].

Wang et al. confirmed that treatment of MPM cells [(epithelioid: H2595, H2452, H2461, H2714), (sarcomatoid: H2373)] with Velcade causes; G2M phase arrest, increased expression of cyclin-dependent kinase inhibitor p21 and proapoptotic protein Bax; and pretreatment with Velcade induced the apoptosis by the following treatment with cisplatin, through activation of apoptosis signal transducing genes such as *CARP-1*, *XAF1,* and Troy proteins [84]. Thus, the effects of bortezomib in combination with various chemotherapeutics could positively affect the stability of IAP proteins and/or negatively regulate the production of de novo IAP proteins via NF-kB signaling pathway. Furthermore, it was also showed that the IL-13 and TNF-alpha cell survival effects on human neoplastic alveolar epithelial cell line A549 may, at least partially, be due to increase of expression of the antiapoptotic proteins such as FLIP [57]. Consequently, PI-3 K/Akt signaling pathway regulates c-FLIP expression [57, 85] and induction of the expression of antiapoptotic genes can protect the tumor cells such as MM cells from cell death.

On the other hand, survivin, a member of the *IAP2* gene family encoded by the *BIRC5* gene, has been indicated to be overexpressed in 34 paraffin-embedded tissue specimens of MM tumors and in mesothelioma cell lines, and was linked to an apoptotic defect, possibly by inhibiting the intrinsic, caspase-9-dependent apoptotic pathway [86]. Given the fact that survivin has been implicated in the control of apoptotic cell death and the overexpression of survivin in tumors correlates with resistance to chemotherapy, Xia et al. and Zaffaroni et al. used antisurvivin oligonucleotides in transfected MPM cells (epithelioid, biphasic, and sarcomatoid) with the survivin siRNA. They found a remarkable inhibition of survivin protein expression, a time-dependent decrease in cell growth, suggesting that down-regulation of survivin by a targeted antisense oligonucleotide is a potential agent for the treatment of mesothelioma with gene therapy [87, 88]. As mentioned before, XIAP is also often expressed in MM and XIAP inhibition is associated with increased sensitivity of mesothelioma cells to TRAIL-induced apoptosis. These results suggest that combined treatments, triggering the extrinsic and intrinsic pathways or the caspase cascade signaling, are potentially promising therapy for malignant mesothelioma.

Finally, the Hippo (also known as the Salvador–Warts–Hippo) tumor suppressor pathway is an essential regulator of cell proliferation and deregulation of this pathway leads to tumourigenesis [89]. The transcriptional coactivator YAP is a crucial downstream negative effector of the Hippo pathway and is negatively regulated by upstream tumour suppressors such as *NF2, LATS1/2, MST1/2,* and *SAV1* [90, 91]. The *NF2* gene, which encodes the Merlin suppressor protein, shows the highest frequency of inactivating mutation in the Hippo pathway of mesothelioma cells [92, 93]. In addition, the genetic inactivation of *LATS2, SAV1,* and *RASSF* has been identified in in vitro studies [94, 95]. Genetic inactivation of these molecules leads to the activation of YAP and binding of activated YAP to the TEAD transcription factors family (TEAD1–4) resulting in oncogenic transformation [89]. In an experimental MM study, Murakami et al. found that three cell lines [(biphasic: Y-MESO-14), (epithelioid: Y-MESO-21, Y-MESO-27)] have overlapping homozygous deletion at chromosome 13q12, in which the *LATS2* gene is located. They also found in six other MM cell lines (epithelioid, sarcomatoid, and lymphohistiocytoid) and 25 MM tumors, 10 inactivating homozygous deletions of *LATS2* among 45 MMs coming to similar conclusions that Hippo pathway deregulation is frequent in MM cells with inactivation of *LATS2* and additionally suggesting that the inactivation of *LATS2* is one of the key mechanisms for activation of YAP, which induces deregulation of MM cell proliferation [94]. In vitro results are similarly interesting; three mesothelioma cell lines [(sarcomatoid: 211H), (epithelioid: H2052, H290)] had decreased phospho-YAP-to-YAP protein ratio compared to LP9 (normal mesothelial cell line). Moreover, the same three cell lines were more sensitive than LP9 to the YAP/TEAD inhibitor verteporfin which aggravated invasion and sphere formation capability of H2052 and H290 epithelial cell lines. siYAP notably decreased YAP transcriptional activity and sphere formation capability of transfected H2052 and H290 cell lines [89]. NF2 activity is also associated with regulation of motility and invasiveness in MM cells, by down-regulation of focal adhesion kinase (FAK). Shapiro I et al. indicated that low Merlin expression predicts for increased sensitivity of MPM cells to an FAK inhibitor, VS-4718, in vitro and in tumor xenograft models. They concluded that interruption of MPM cell–cell or cell-extracellular matrix (ECM) contacts with blocking antibodies indicates that feeble cell–cell adhesions in Merlin-negative MPM cells underlie their greater reliance on cell-ECM-mediated FAK signaling [96].

Finally, the activation of RhoA/ROCK signaling induces YAP activation and inhibitors of RhoA/ROCK such as GSK269962A could be used to suppress YAP activity [89]. Therefore, down-regulated of YAP activity by siRNA and by small molecule (such as verteporfin, PPIX) have inhibitory effects on human mesothelioma cells and inhibition of YAP activation may be a potential therapeutic target in mesothelioma [97]. On the other hand, verteporfin also induces apoptosis in MPM by YAP-independent mechanisms [98]. In the same context, Tranchant R et al. used MPM primary cultures that were screened for mutations in LATS2, and

they found that NF2 and LATS2-mutated MPM are more sensitive to mTOR/PI3 K/AKT pathway-mediated apoptosis [99]. Therefore, it is important to determine the combining molecular classification and genetic changes of MPM to generate a new potential therapeutic strategy.

Conclusions

The tumorigenesis procedure in MM is complex and mainly results from deregulation in apoptosis. A large number of molecules which regulate the intrinsic apoptotic pathway (bcl-2 family, PI3K/Akt/mTOR, Wnt, BAP1), the extrinsic apoptotic pathway (TRAIL, NF-kB, CAPS, ERK1/2, p53) as well as both pathways (IAP, Hippo, YAP) seem to play an important role in MM tumorigenesis and have possible therapeutic implications and, therefore, clinical interest. As a result, further studies should be carried out to encourage the development of new therapeutic approaches.

Funding This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

1. Husain AN, Colby TV, Ordonez NG, Krausz T, Borczuk A, Cagle PT et al (2009) Guidelines for pathologic diagnosis of malignant mesothelioma: a consensus statement from the International Mesothelioma Interest Group. Arch Pathol Lab Med 133(8):1317–1331
2. Carbone M, Ly BH, Dodson RF, Pagano I, Morris PT, Dogan UA et al (2012) Malignant mesothelioma: facts, myths, and hypotheses. J Cell Physiol 227(1):44–58
3. Attanoos RL, Churg A, Galateau-Salle F, Gibbs AR, Roggli VL (2018) Malignant mesothelioma and its non-asbestos causes. Arch Pathol Lab Med 142(6):753–760
4. Galani V, Constantopoulos S, Manda-Stachouli C, Frangou-Lazaridis M, Mavridis A, Vassiliou M et al (2002) Additional proteins in BAL fluid of Metsovites environmentally exposed to asbestos: more evidence of "protection" against neoplasia? Chest 121(1):273–278
5. Yang H, Rivera Z, Jube S, Nasu M, Bertino P, Goparaju C et al (2010) Programmed necrosis induced by asbestos in human mesothelial cells causes high-mobility group box 1 protein release and resultant inflammation. Proc Natl Acad Sci USA 107(28):12611–12616
6. Carbone M, Yang H (2012) Molecular pathways: targeting mechanisms of asbestos and erionite carcinogenesis in mesothelioma. Clin Cancer Res 18:598–604
7. Whiteside TL (2008) The tumor microenvironment and its role in promoting tumor growth. Oncogene 27:5904–5912
8. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M (2013) Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol 229:176–185
9. Noy R, Pollard JW (2014) Tumor-associated macrophages: from mechanisms to therapy. Immunity 41:49–61
10. Burt BM, Rodig SJ, Tilleman TR, Elbardissi AW, Bueno R, Sugarbaker DJ (2011) Circulating and tumor-infiltrating myeloid cells predict survival in human pleural mesothelioma. Cancer 117:5234–5244
11. Archimandriti DT, Dalavanga YA, Cianti R, Bianchi L, Manda-Stachouli C, Armini A et al (2009) Proteome analysis of bronchoalveolar lavage in individuals from Metsovo, nonoccupationally exposed to asbestos. J Proteome Res 8(2):860–869
12. Pastorino S, Yoshikawa Y, Pass HI, Emi M, Nasu M, Pagano I et al (2018) A subset of mesotheliomas with improved survival occurring in carriers of BAP1 and other germline mutations. J Clin Oncol 36:3485–3494
13. Panou V, Gadiraju M, Wolin A, Weipert CM, Skarda E, Husain AN et al (2018) Frequency of germline mutations in cancer susceptibility genes in malignant mesothelioma. J Clin Oncol 36(28):2863–2871
14. Bueno R, Stawiski EW, Goldstein LD et al (2016) Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. Nat Genet 48(4):407–416
15. Huang SXL, Jaurand MC, Kamp DW, Whysner J, Hei TK (2011) Role of mutagenicity in asbestos fiber-induced carcinogenicity and other diseases. J Toxicol Environ Health B Crit Rev 14(1–4):179–245
16. Takeda M, Kasai T, Enomoto Y, Takano M, Morita K, Nakai T et al (2014) Comparison of genomic abnormality in malignant mesothelioma by the site of origin. J Clin Pathol 67(12):1038–1043
17. Borczuk AC, Pei J, Taub RN, Levy B, Nahum O, Chen J et al (2016) Genome-wide analysis of abdominal and pleural malignant mesothelioma with DNA arrays reveals both common and distinct regions of copy number alteration. Cancer Biol Ther 17(3):328–335
18. Galani V, Tatsaki E, Bai M, Kitsoulis P, Lekka M, Nakos G et al (2010) The role of apoptosis in the pathophysiology of Acute Respiratory Distress Syndrome (ARDS): an up-to-date cell-specific review. Pathol Res Pract 206:145–150
19. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P et al (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ 25(3):486–541
20. Igney FH, Krammer PH (2002) Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2(4):277–288
21. Belyanskaya LL, Marti TM, Hopkins-Donaldson S, Kurtz S, Felley-Bosco E, Stahel RA (2007) Human agonistic TRAIL receptor antibodies Mapatumumab and Lexatumumab induce apoptosis in malignant mesothelioma and act synergistically with cisplatin. Mol Cancer 6:66
22. Kim KU, Wilson SM, Abayasiriwardana KS, Collins R, Fjellbirkeland L, Xu Z et al (2005) A novel in vitro model of human mesothelioma for studying tumor biology and apoptotic resistance. Am J Respir Cell Mol Biol 33(6):541–548
23. Rippo MR, Moretti S, Vescovi S, Tomasetti M, Orecchia S, Amici G et al (2004) FLIP overexpression inhibits death receptor-induced apoptosis in malignant mesothelial cells. Oncogene 23(47):7753–7760
24. Liu W, Bodle E, Chen JY, Gao M, Rosen GD, Broaddus VC (2001) Tumor necrosis factor-related apoptosis-inducing ligand and chemotherapy cooperate to induce apoptosis in mesothelioma cell lines. Am J Respir Cell Mol Biol 25(1):111–118

25. Tomasetti M, Rippo MR, Alleva R, Moretti S, Andera L, Neuzil J et al (2004) Alpha-tocopheryl succinate and TRAIL selectively synergise in induction of apoptosis in human malignant mesothelioma cells. Br J Cancer 90(8):1644–1653
26. Abayasiriwardana KS, Barbone D, Kim KU, Vivo C, Lee KK, Dansen TB et al (2007) Malignant mesothelioma cells are rapidly sensitized to TRAIL-induced apoptosis by low-dose anisomycin via Bim. Mol Cancer Ther 6(10):2766–2776
27. Katz SI, Zhou L, Chao G, Smith CD, Ferrara T, Wang W et al (2009) Sorafenib inhibits ERK1/2 and MCL-1(L) phosphorylation levels resulting in caspase-independent cell death in malignant pleural mesothelioma. Cancer Biol Ther 8(24):2406–2416
28. Heintz NH, Janssen-Heininger YM, Mossman BT (2010) Asbestos, lung cancers, and mesotheliomas: from molecular approaches to targeting tumor survival pathways. Am J Respir Cell Mol Biol 42(2):133–139
29. Yang H, Bocchetta M, Kroczynska B, Elmishad AG, Chen Y, Liu Z et al (2006) TNF-alpha inhibits asbestos-induced cytotoxicity via a NF-kappaB-dependent pathway, a possible mechanism for asbestos-induced oncogenesis. Proc Natl Acad Sci USA 103(27):10397–10402
30. Raphaël M, Lehen'kyi V, Vandenberghe M, Beck B, Khalimonchyk S, Abeele F et al (2014) TRPV6 calcium channel translocates to the plasma membrane via Orai1-mediated mechanism and controls cancer cell survival. Proc Natl Acad Sci USA 111(37):E3870–E3879
31. Li F, Zhu D, Yang Y, Wu K, Zhao S (2017) Overexpression of calcyphosine is associated with poor prognosis in esophageal squamous cell carcinoma. Oncol Lett 14(5):6231–6237
32. Ramos-Nino ME, Timblin CR, Mossman BT (2002) Mesothelial cell transformation requires increased AP-1 binding activity and ERK-dependent Fra-1 expression. Cancer Res 62(21):6065–6069
33. Manning CB, Sabo-Attwood T, Robledo RF, Macpherson MB, Rincon M, Vacek P (2008) Targeting the MEK1 cascade in lung epithelium inhibits proliferation and fibrogenesis by asbestos. Am J Respir Cell Mol Biol 38:618–626
34. Shukla A, Hillegass JM, MacPherson MB, Beuschel SL, Vacek PM, Butnor KJ et al (2011) ERK2 is essential for the growth of human epithelioid malignant mesotheliomas. Int J Cancer 129(5):1075–1086
35. Masuelli L, Benvenuto M, Stefano ED, Mattera R, Fantini M, Feudis GD et al (2017) Curcumin blocks autophagy and activates apoptosis of malignant mesothelioma cell lines and increases the survival of mice intraperitoneally transplanted with a malignant mesothelioma cell line. Oncotarget 8(21):34405–34422
36. Li Q, Kawamura K, Yamanaka M, Okamoto S, Yang S, Yamauchi S et al (2012) Upregulated p53 expression activates apoptotic pathways in wild-type p53-bearing mesotheliomaand enhances cytotoxicity of cisplatin and pemetrexed. Cancer Gene Ther 19(3):218–228
37. Soini Y, Kinnula V, Kaarteenaho-Wiik R, Kurttila E, Linnainmaa K, Pääkkö P (1999) Apoptosis and expression of apoptosis regulating proteins bcl-2, mcl-1, bcl-X, and bax in malignant mesothelioma. Clin Cancer Res 5:3508–3515
38. Mohiuddin I, Cao X, Fang B, Nishizaki M, Smythe WR (2001) Significant augmentation of pro-apoptotic gene therapy by pharmacologic bcl-xl down-regulation in mesothelioma. Cancer Gene Ther 8:547–554
39. Smythe WR, Mohuiddin I, Ozveran M, Cao XX (2002) Antisense therapy for malignant mesothelioma with oligonucleotides targeting the bcl-xl gene product. J Thorac Cardiovasc Surg 123:1191–1198
40. Cao X, Rodarte C, Zhang L, Morgan CD, Littlejohn J, Smythe WR (2007) Bcl2/bcl-xL inhibitor engenders apoptosis and increases chemosensitivity in mesothelioma. Cancer Biol Ther 6(2):246–252
41. Littlejohn JE, Cao X, Miller SD, Ozvaran MK, Jupiter D, Zhang L et al (2008) Bcl-xL antisense oligonucleotide and cisplatin combination therapy extends survival in SCID mice with established mesothelioma xenografts. Int J Cancer 123(1):202–208
42. Varin E, Denoyelle C, Brotin E, Meryet-Figuière M, Giffard F, Abeilard E et al (2010) Downregulation of Bcl-xL and Mcl-1 is sufficient to induce cell death in mesothelioma cells highly refractory to conventional chemotherapy. Carcinogenesis 31(6):984–993
43. Daubriac J, Fleury-Feith J, Kheuang L, Galipon J, Saint-Albin A, Renier A et al (2009) Malignant pleural mesothelioma cells resist anoikis as quiescent pluricellular aggregates. Cell Death Differ 16(8):1146–1155
44. Sugarbaker DJ, Richards WG, Gordon GJ, Dong L, De Rienzo A, Maulik G et al (2008) Transcriptome sequencing of malignant pleural mesothelioma tumors. Proc Natl Acad Sci USA 105(9):3521–3526
45. Saxton RA, Sabatini DM (2017) mTOR signaling in growth, metabolism, and disease. Cell 168:960–976
46. Galani V, Kastamoulas M, Varouktsi A, Lampri E, Mitselou A, Arvanitis DL (2017) IFNs-signaling effects on lung cancer: an up-to-date pathways-specific review. Clin Exp Med 17(3):281–289
47. Galani V, Papadatos SS, Alexiou G, Galani A, Kyritsis AP (2017) In vitro and in vivo preclinical effects of type I IFNs on gliomas. J Interferon Cytokine Res 37(4):139–146
48. Hong TM, Yang PC, Peck K, Chen JJ, Yang SC, Chen YC et al (2000) Profiling the downstream genes of tumor suppressor PTEN in lung cancer cells by complementary DNA microarray. Am J Respir Cell Mol Biol 23(3):355–363
49. Zhou S, Liu L, Li H, Eilers G, Kuang Y, Shi S et al (2014) Multipoint targeting of the PI3K/mTOR pathway in mesothelioma. Br J Cancer 110(10):2479–2488
50. Altomare DA, You H, Xiao GH, Ramos-Nino ME, Skele KL, De Rienzo A et al (2005) Human and mouse mesotheliomas exhibit elevated AKT/PKB activity, which can be targeted pharmacologically to inhibit tumor cell growth. Oncogene 24(40):6080–6089
51. Suzuki Y, Murakami H, Kawaguchi K, Tanigushi T, Fujii M, Shinjo K et al (2009) Activation of the PI3K-AKT pathway in human malignant mesothelioma cells. Mol Med Rep 2(2):181–188
52. Pinton G, Manente AG, Angeli G, Mutti L, Moro L (2012) Perifosine as a potential novel anti-cancer agent inhibits EGFR/MET-AKT axis in malignant pleural mesothelioma. PLoS ONE 7(5):e36856
53. Barbone D, Yang TM, Morgan JR, Gaudino G, Broaddus VC (2008) Mammalian target of rapamycin contributes to the acquired apoptotic resistance of human mesothelioma multicellular spheroids. J Biol Chem 283(19):13021–13330
54. Wilson SM, Barbone D, Yang TM, Jablons DM, Bueno R, Sugarbaker DJ et al (2008) mTOR mediates survival signals in malignant mesothelioma grown as tumor fragment spheroids. Am J Respir Cell Mol Biol 39(5):576–583
55. Vivo C, Liu V, Broaddus VC (2003) c-Jun N-terminal kinase contributes to apoptotic synergy induced by tumor necrosis factor-related apoptosis-inducing ligand plus DNA damage in chemoresistant, p53 inactive mesothelioma cells. J Biol Chem 278(28):25461–25467
56. Holley SL, Fryer AA, Haycock JW, Grubb SE, Strange RC, Hoban PR (2007) Differential effects of glutathione S-transferase pi (GSTP1) haplotypes on cell proliferation and apoptosis. Carcinogenesis 28(11):2268–2273
57. Kastamoulas M, Chondrogiannis G, Kanavaros P, Vartholomatos G, Bai M, Briasoulis E et al (2013) Cytokine effects on cell survival and death of A549 lung carcinoma cells. Cytokine 61(3):816–825

58. Fox S, Dharmarajan A (2006) WNT signaling in malignant mesothelioma. Front Biosci 11:2106–2112
59. Mazieres J, You L, He B, Xu Z, Twogood S, Lee AY et al (2005) Wnt2 as a new therapeutic target in malignant pleural mesothelioma. Int J Cancer 117(2):326–332
60. Kashiwakura Y, Ochiai K, Watanabe M, Abarzua F, Sakaguchi M, Takaoka M et al (2008) Down-regulation of inhibition of differentiation-1 via activation of activating transcription factor 3 and Smad regulates REIC/Dickkopf-3-induced apoptosis. Cancer Res 68(20):8333–8341
61. Affar EB, Carbone M (2018) BAP1 regulates different mechanisms of cell death. Cell Death Dis 9:1151
62. Zhang Y, Shi J, Liu X, Feng L, Gong Z, Koppula P et al (2018) BAP1 links metabolic regulation of ferroptosis to tumour suppression. Nat Cell Biol 20:1181–1192
63. Bott M, Brevet M, Taylor BS, Shimizu S, Ito T, Wang L et al (2011) The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nat Genet 43:668–672
64. Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E et al (2011) Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet 43:1022–1025
65. Baumann F, Flores E, Napolitano A, Kanodia S, Taioli E, Pass H et al (2015) Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival. Carcinogenesis 36(1):76–81
66. Cheung M, Testa JR (2017) BAP1, a tumor suppressor gene driving malignant mesothelioma. Transl Lung Cancer Res 6:270–278
67. Nasu M, Emi M, Pastorino S, Tanji M, Powers A, Luk H et al (2015) High incidence of somatic BAP1 alterations in sporadic malignant mesothelioma. J Thorac Oncol 10:565–576
68. Yoshikawa Y, Emi M, Hashimoto-Tamaoki T, Ohmuraya M, Sato A, Tsujimura T et al (2016) High-density array-CGH with targeted NGS unmask multiple noncontiguous minute deletions on chromosome 3p21 in mesothelioma. Proc Natl Acad Sci USA 113:13432–13437
69. Bononi A, Giorgi C, Patergnani S, Larson D, Verbruggen K, Tanji M et al (2017) BAP1 regulates IP3R3-mediated Ca^{2+} flux to mitochondria suppressing cell transformation. Nature 546:549–553
70. Mouw KW, Goldberg MS, Konstantinopoulos PA, D'Andrea AD (2017) DNA damage and repair biomarkers of immunotherapy response. Cancer Discov 7:675–693
71. Guazzelli A, Meysami P, Bakker E, Demonacos C, Giordano A, Krstic-Demonacos M et al (2019) BAP1 Status Determines the sensitivity of malignant mesothelioma cells to gemcitabine treatment. Int J Mol Sci 20(2):E429
72. Napolitano A, Pellegrini L, Dey A, Larson D, Tanji M, Flores EG et al (2016) Minimal asbestos exposure in germline BAP1 heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma. Oncogene 35(15):1996–2002
73. Xu J, Kadariya Y, Cheung M, Pei J, Talarchek J, Sementino E et al (2014) Germline mutation of Bap1 accelerates development of asbestos-induced malignantmesothelioma. Cancer Res 74(16):4388–4397
74. Kadariya Y, Cheung M, Xu J, Pei J, Sementino E, Menges CW et al (2016) Bap1 is a bona fide tumor suppressor: genetic evidence from mouse models carrying heterozygous germline Bap1 mutations. Cancer Res 76(9):2836–2844
75. Ohar JA, Cheung M, Talarchek J, Howard SE, Howard TD, Hesdorffer M et al (2016) Germline BAP1 mutational landscape of asbestos-exposed malignant mesothelioma patients with family history of cancer. Cancer Res 76(2):206–215
76. Leblay N, Leprêtre F, Le Stang N, Gautier-Stein A, Villeneuve L, Isaac S et al (2017) BAP1 is altered by copy number loss, mutation, and/or loss of protein expression in more than 70% of malignant peritoneal mesotheliomas. J Thorac Oncol 12(4):724–733
77. Carbone M, Shimizu D, Napolitano A, Tanji M, Pass HI, Yang H et al (2016) Positive nuclear BAP1 immunostaining helps differentiate non-small cell lung carcinomas from malignant mesothelioma. Oncotarget 7(37):59314–59321
78. Zucali PA, Ceresoli GL, De Vincenzo F, Simonelli M, Lorenzi E, Gianoncelli L et al (2011) Advances in the biology of malignant pleural mesothelioma. Cancer Treat Rev 37(7):543–558
79. Hoesel B, Schmid JA (2013) The complexity of NF-kB signaling in inflammation and cancer. Mol Cancer 12(1):86
80. Gyrd-Hansen M, Meier P (2010) IAPs: from caspase inhibitors to modulators of NF-κB, inflammation and cancer. Nat Rev Cancer 10(8):561–574
81. Sun X, Gulyás M, Hjerpe A, Dobra K (2006) Proteasome inhibitor PSI induces apoptosis in human mesothelioma cells. Cancer Lett 232(2):161–169
82. Borczuk AC, Cappellini GC, Kim HK, Hesdorffer M, Taub RN, Powell CA (2007) Molecular profiling of malignant peritoneal mesothelioma identifies the ubiquitin-proteasome pathway as a therapeutic target in poor prognosis tumors. Oncogene 26(4):610–617
83. Gordon GJ, Mani M, Maulik G, Mukhopadhyay L, Yeap BY, Kindler HL et al (2008) Preclinical studies of the proteasome inhibitor bortezomib in malignant pleural mesothelioma. Cancer Chemother Pharmacol 61(4):549–558
84. Wang Y, Rishi AK, Puliyappadamba VT, Sharma S, Yang H, Tarca A et al (2010) Targeted proteasome inhibition by Velcade induces apoptosis in human mesothelioma and breast cancer cell lines. Cancer Chemother Pharmacol 66(3):455–466
85. Chondrogiannis G, Kastamoulas M, Kanavaros P, Vartholomatos G, Bai M, Baltogiannis D et al (2014) Cytokine effects on cell death and major signaling pathways in LNCaP prostate carcinoma cells. B Biomed Res Int 2014:536049
86. Jin L, Amatya VJ, Takeshima Y, Shrestha L, Kushitani K, Inai K (2010) Evaluation of apoptosis and immunohistochemical expression of the apoptosis-related proteins in mesothelioma. Hiroshima J Med Sci 59(2):27–33
87. Xia C, Xu Z, Yuan X, Uematsu K, You L, Li K et al (2002) Induction of apoptosis in mesothelioma cells by antisurvivin oligonucleotides. Mol Cancer Ther 1(9):687–694
88. Zaffaroni N, Costa A, Pennati M, De Marco C, Affini E, Madeo M et al (2007) Survivin is highly expressed and promotes cell survival in malignant peritoneal mesothelioma. Cell Oncol 29(6):453–466
89. Zhang WQ, Dai YY, Hsu PC, Wang H, Cheng L, Yang YL et al (2017) Targeting YAP in malignant pleural mesothelioma. J Cell Mol Med 21(11):2663–2676
90. Park HW, Guan KL (2013) Regulation of the Hippo pathway and implications for anticancer drug development. Trends Pharmacol Sci 34:581–589
91. Gomez M, Gomez V, Hergovich A (2014) The Hippo pathway in disease and therapy: cancer and beyond. Clin Transl Med 3:22–33
92. Bianchi AB, Mitsunaga SI, Cheng JQ, Klein WM, Jhanwar SC, Seizinger B et al (1995) High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. Proc Natl Acad Sci USA 92:10854–10858
93. Sekido Y, Pass HI, Bader S, Mew DJ, Christman MF, Gazdar AF et al (1995) Neurofibromatosis Type-2 (Nf2) gene is somatically mutated in mesothelioma but not in lung-cancer. Cancer Res 55:1227–1231
94. Murakami H, Mizuno T, Taniguchi T, Fujii M, Ishiguro F, Fukui T et al (2011) LATS2 is a tumor suppressor gene of malignant mesothelioma. Cancer Res 71:873–883

95. Miyanaga A, Masuda M, Tsuta K, Kawasaki K, Nakamura Y, Sakuma T et al (2015) Hippo pathway gene mutations in malignant mesothelioma revealed by RNA and targeted exon sequencing. J Thorac Oncol 10:844–851
96. Shapiro IM, Kolev VN, Vidal CM, Kadariya Y, Ring JE, Wright Q et al (2014) Merlin deficiency predicts FAK inhibitor sensitivity: a synthetic lethal relationship. Sci Transl Med 6(237):237ra68
97. Woodard GA, Yang YL, You L, Jablons DM (2017) Drug development against the hippo pathway in mesothelioma. Transl Lung Cancer Res 6(3):335–342
98. Tranchant R, Quetel L, Montagne F, De Wolf J, Meiller C, De Koning L et al (2018) Assessment of signaling pathway inhibitors and identification of predictive biomarkers in malignant pleural mesothelioma. Lung Cancer 126:15–24
99. Tranchant R, Quetel L, Tallet A, Meiller C, Renier A, de Koning L et al (2017) Co-occurring mutations of tumor suppressor genes, LATS2 and NF2, in malignant pleural mesothelioma. Clin Cancer Res 23(12):3191–3202
100. Pellegrini L, Xue J, Larson D, Pastorino S, Jube S, Forest KH et al (2017) HMGB1 targeting by ethyl pyruvate suppresses malignant phenotype of human mesothelioma. Oncotarget 8(14):22649–22661
101. Jube S, Rivera ZS, Bianchi ME, Powers A, Wang E, Pagano I et al (2012) Cancer cell secretion of the DAMP protein HMGB1 supports progression in malignant mesothelioma. Can Res 72(13):3290–3301
102. Edwards JC, Swinson DE, Jones JL, Waller DA, O'Byrne KJ (2006) EGFR expression: associations with outcome and clinicopathological variables in malignant pleural mesothelioma. Lung Cancer 54:399–407
103. Singhal S, Wiewrodt R, Malden LD, Amin KM, Matzie K, Friedberg J et al (2003) Gene expression profiling of malignant mesothelioma. Clin Cancer Res 9(8):3080–3097

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.