Biomolecular Pathways and Malignant Pleural Mesothelioma

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Abstract: Although asbestos is banned in several countries, Malignant Pleural Mesothelioma (MPM) incidence is increasing worldwide, and this cancer remains a disease of concern. MPM is a very severe cancer, with no curative treatment despite the development of different kinds of therapeutic approaches. To date the treatments are based on multimodal chemotherapy and surgery. The longest survival data in patients is obtained with the combination of chemotherapy, radical surgery and radiotherapy. The development of targeted therapies offers new strategies to kill cancer cells. To be efficient, they need a precise identification of the critical targets. This objective can be reached by a deep knowledge of the molecular and physiological changes associated with the neoplastic transformation of MPM cells. One approach consists in the identification of gene mutations, epigenetic alterations, study of gene expression profiles and identification of the deregulated signalling pathways in malignant cells, with the goal to select molecules or mechanisms that could kill cancer cells or abolish tumour growth. The present knowledge on the main alterations in genes and signalling pathways indicates that MPM have recurrent mutations in a limited number of tumour suppressor genes, and oncogenic mutation in the promoter of *TERT*. A number of studies have emphasized the role of receptor tyrosine kinase (RTK) driven signalling, although not related to mutations in RTK. Multiple signalling pathways are altered in MPM. Transcriptomic analyses permitted to classify mesotheliomas in subgroups, according to prognosis. They showed heterogeneity of MPM, not only defined by the histological subtype, but also by molecular features. So far, targeted therapy was unsuccessful, at least partly due to the heterogeneity of MPM. Moreover, the complexity stands in the interconnection between pathways, which is a challenge to choose the most critical target for an efficient therapy. This review summarizes the main alterations identified in genes and signalling pathways in MPM, the impact on therapeutics, and discusses the future of these approaches to improve MPM outcome, especially knowing molecular and physiological characteristics of MPM to define their diversity.

Keywords: Mesothelioma; signalling pathways; targeted therapy

BACKGROUND

Malignant pleural mesothelioma (MPM) is an uncommon and severe neoplasm of poor prognosis with a median survival of 8 to 14 months. Since the publication of the association between mesothelioma and asbestos exposure in South Africa, epidemiological and fundamental researches have been developed to define the populations at risk and evaluate the risk level, to investigate the mechanism of action of asbestos and of mesothelioma carcinogenesis, and to treat the disease [1]. However, pathobiological and clinical researches are difficult in the case of uncommon diseases, and MPM suffers from a deficit of medical and scientific knowledge. Nevertheless, the interest on MPM is now increasing; national tools and international studies are implemented to improve patients' care, and our knowledge on the biological features of MPM. Presently, researchers can take advantage of the new methodologies to describe the molecular characteristics, and work through networks to improve their efficiency. In the future, these advances will be beneficial to the patients and to overcome this cancer.

To date, there is no cure for this type of cancer, and the treatments do not sufficiently improve patients' survival. Chemotherapy improved the management of symptoms by a stabilisation of the disease, but increase survival of only few months. To date association of platinum-based chemotherapy combined with anti-folate pemetrexed is accepted as standard treatment. The longest survival data in patients is obtained with the multimodal treatment consisting of chemotherapy, radical surgery and radiotherapy [2, 3]. Surgical resection is offered to few selected cases, depending on patient's performance status, and its interest is debated [4, 5].

Mesothelioma incidence varies markedly from one country to another. Asbestos is the main etiological factor of MPM, which is diagnosed 30 to 40 years after exposure [6]. MPM is related to both occupational and environmental asbestos exposures. Although banned in several countries, asbestos still remains used in many other countries [7, 8]. From a recent review mesothelioma epidemic does not show signs of attenuation[9]. Epidemiological studies have demonstrated that exposure to other factors such as erionite fibres and non-asbestos amphiboles lead to MPM occurrence [10]. Co-exposure to asbestos and man made mineral fibres

enhances the risk of MPM[11]. Although debated, the virus SV40 has been associated to the risk of MPM as a cofactor in some studies [12, 13]. Moreover, MPM diagnosis is sometimes difficult, because of the lack of specific biomarkers and the similitudes with pleural metastatic carcinomas, frequently from lung and breast carcinomas [14]. Therefore, because of the natural history of MPM, and for pathological, clinical, environmental and societal reasons, mesothelioma is still a worldwide major health concern. A precise characterization of mesothelioma hallmarks is a great challenge to improve current therapies and to deliver specific and effective treatments.

Carcinogenesis is a long-term term process in which cells differentiate from normal cells by the acquirement of new phenotypes and genomic changes. Tumour cells express specific characteristics consisting in dedifferentiated morphological features, expression of specific biomarkers (miRNA, protein), chromosome alterations, allelic disequilibrium, gene mutations, and deregulation of signalling pathways. Several mechanisms can lead to tumour growth; they are evidenced by multiple mutations (mutator phenotype) or chromosomal misseggregation (aneuploidy) [15]. Intratumoral heterogeneity is observed in solid cancers; it may arise from a clonal evolution from differentiated cell transformation or follow the cancer stem cell model[16, 17]. Tumour growth is also dependent on the cell microenvironment that may produce many types of regulatory factors (cytokines, growth factors…), and interactions with stromal cells in the tumour (fibroblasts and endothelial cells, immune cells such as tumour associated macrophages and lymphocytes) [18]. Because of the variety of the different mechanisms by which cells can be modified and move forward in the neoplastic process, tumour cells exhibit an heterogeneity which is highlighted at the morphological, molecular and cell signalling levels. The identification of these characteristics is a basic issue to progress in the science of mesothelioma.

PATHOBIOLOGY OF MPM

Mesothelioma diagnosis

Presently, MPM is classified according to pathological features. Histologically, three major types of MPM are described: epithelioid, biphasic, or sarcomatoid, with more than ten uncommon subtypes for the epithelioid form and five for the

sarcomatoid type [19]. Biphasic MPM include both epithelial and sarcomatoid component, the latter being less than 10% of the tumour cell population [19]. The epithelial subtype is the most common form of MPM, accounting for approximately 50-70% of cases. Histologic MPM type is associated with different median survival time of the patients, the better prognosis being for the epithelioid type. As for many other types of cancers, the diversity of histological features of MPM suggests that individual tumours have different specificities. The more recent large scale molecular studies have confirmed the MPM heterogeneity [20]. So far, MPM diagnosis is based on pathological features and biomarkers, but the development of genetic, epigenetic and cell regulation studies in MPM will permit to better know the biology of the MPM subtypes, and will provide more precise diagnosis to define therapeutic decisions.

The main results from researches carried out during the last 10 years on gene expression profiles in MPM and primary tumours allowed advances in our knowledge of the molecular biology of malignant mesothelioma. They demonstrated that differential gene expression profiles exist between malignant mesotheliomas, most often linked to the histological subtypes [21, 22]. However, recent findings based on unsupervised hierarchical clustering on transcriptomic data in MPM primary cultures and primary tumours defined two MPM subgroups, C1 and C2, closely related to prognosis and partly to histological subtypes, demonstrating that MPM occurs with different molecular profiles, gene alterations, and survival outcomes [23].

Signalling pathways in MPM

Tumour growth is controlled by numerous pathways, which are regulated by the activity of both intrinsic effectors and extrinsic factors, produced either by the tumour cells or by nearby non-tumour cells, which activate or negatively regulate signalling pathways of importance to the maintenance of cell homeostasis. These pathways can also be deregulated by alterations in key members, due to mutations in genes encoding driving proteins, gene silencing by epigenetic mechanisms, changes in gene expression of initiating factors or unscheduled gene expression. In this work, we will summarize the data showing the alteration of the pathways of

importance to the evolution of the carcinogenic process in MPM, and discuss their interest to implement more precise therapies.

Gene mutations in MPM. Numerous studies focused on gene mutations in candidate genes, oncogenes, tumour suppressor genes and key regulatory genes, accounting for mesothelioma oncogenesis. Earlier sequencing studies identified the genes most recurrently mutated in MPM as *CDKN2A/2B* (cyclin-dependent kinase inhibitor 2A/2B), *NF2* (neurofibrin 2) and *BAP1* (*BRCA1* Associated Protein-1), and showed low frequency of mutation in the well-known tumour suppressor gene *TP53* [24, 25];. *CDKN2A/2B* are regulatory genes in cell cycle, *NF2* is involved in several signalling pathways including the Hippo pathway and plays a role in contact inhibition of cell proliferation, and *BAP1* belongs to the ubiquitin carboxy-terminal hydrolase subfamily of deubiquitinating enzymes that are involved in the removal of ubiquitin from nuclear proteins. Interestingly, all are tumour suppressor genes and no recurrent oncogenic mutation has been reported until recently. The first recurrent oncogenic mutation was identified in the *TERT* promoter [26]. Most recent studies using next-generation sequencing (NGS) assays confirmed mutation in these tumour suppressor genes and provided new information on the mutational status of a series of genes in MPM. A whole exome sequencing on DNA from 22 frozen MPM tumour samples and matched blood samples identified frequent somatic genetic alterations in *CUL1* (cullin1), which encodes a core component of SCF complex (Skp, Cullin, F-box containing complex), a E3 ubiquitin-protein ligase complex that mediates the ubiquitination of proteins involved in cell cycle progression, signal transduction and transcription [27]. Another series of 123 formalin-fixed, paraffin-embedded tissue samples was analysed for mutations in 50 cancer genes by NGS. Frequent variations were detected in seven new genes related to signalling pathways: *PDGFRA*, *KIT*, *KDR*, *HRAS*, *PIK3CA*, *STK11* and *SMARCB1*, including genes involved in the PI3K/AKT and MAPK pathways or playing a role in the P53/DNA repair pathway [28]. Patients with multiple genetic variations in *PIK3CA*, *KIT*, *STK11* and *TP53* correlated with shorter time to progressive disease and reduced overall survival [28]. A NGS sequencing in six MPM cell lines suggests mutation in genes involved in Hippo, MAPK, Wnt, nuclear factor kappa B, and angiogenesis pathways [29].

Receptor Tyrosine Kinases (RTKs) in MPM. RTKs are membrane receptors driving downstream cell signalling, involved in several pathways [30, 31]. From the studies carried out with other types of cancers, it is known that mutations in RTKs play a role in the neoplastic transformation of normal cells. Then, the status of RTKs has been investigated in MPM.

Epidermal growth factor receptor (EGFR)**.** From immunohistochemical studies, EGFR was expressed in about 50% of MPM cases, suggesting an overexpression. This was not found in benign mesothelial lesion, and EGFR expression seems to be a factor negatively affecting prognosis [32-35]. However, the relationship between EGFR expression and patient's survival is not clear. Either no relationship or a favourable prognosis was reported [20]. *EGFR* is generally not mutated in human MPM, although a few papers reported a low percentage of mutations. No *EGFR* gene mutation was detected from histological samples or frozen tissues of MPM [36-38], and no mutation or amplification in *EGFR* in and *ERBB2* [39]. *EGFR* mutations were found in 6% and 16% of MPM samples [33, 40]. A phosphorylation of EGFR was reported in 10 of 14 mesothelioma cell lines, not due to mutation in the kinase domain [41]. By DNA sequencing, *EGFR* mutations were not detected in 34 primary cultures from MPM, in contrast to lung cancer [42]. Interestingly, *EGFR* mutations in lung cancer were not linked with asbestos exposure, suggesting that *EGFR* mutations do not result from the effect of asbestos, consistent with the absence of mutations in MPM that is mainly due to asbestos exposure [42].

Vascular endothelial growth factor receptors (VEGFR). MPM cells express both vascular endothelial growth factors (VEGF), and the VEGFR receptors fms-related tyrosine kinases (FLT1 and FLT4) and fetal liver kinase (KDR/FLK1) [20]. Several immunohistochemical studies have demonstrated an enhanced expression of VEGF in comparison with non neoplastic specimens [43]. The results of *in vitro* studies using neutralizing antibodies against VEGF or VEGFR, or antisense oligonucleotides against VEGF have suggested an autocrine role of VEGF in the proliferation of MPM cells. Contradictory results were found regarding the correlation between VEGF expression and survival [20].

Platelet-derived growth factor receptor (PDGFR). Normal human mesothelial cells express platelet-derived growth factor (PDGF) PDGF-A mRNA and protein, but

these no detectable PDGF-B. In contrast, human MPM cells express high levels of both PDGF-A and PDGF-B, as well as PDGFR-B [44]. An autocrine proliferation involving receptor and ligand has been suggested in MPM. Regulation of proliferation by PDGF may occur, either directly or indirectly, via the hyaluronan, an important component of the extracellular matrix, and CD44 pathway. PDGF-B–stimulated normal human mesothelial cells express both hyaluronan synthase and hyaluronan [20]. Gain in copy number of PDGFR-B was reported in 40% of 88 MPM tumours, with a potential role as prognostic biomarker[45].

Mesenchymal epithelial transition factor, c-MET oncogene (MET). MET, also known as hepatocyte growth factor receptor (HGFR) transduces signals from binding its ligand, hepatocyte growth factor/scatter (HGF), leading to the activation of several signalling cascades including the MAPK and PI3K/AKT. MET is overexpressed and activated in a majority of cases of mesothelioma when compared with normal tissues, and MET siRNA and MET inhibitor (SU11274) inhibited cell growth and migration [13]. MET protein was overexpressed in 6 of 14 mesothelioma cell lines, and co-expression of phosphorylated EGFR and MET was observed in 8 of 14 (57%) cell lines [41]. MET expression was detected in 82% of MPM tissue samples, and in mesothelioma cell lines, but not in normal tissue, or at low level in non-malignant mesothelial cell lines [46]. No mutation was found in cell lines, but 2 mutations were found among 70 MPM tumour samples [41]. Mutations of *MET* were identified in 5 of 43 (11.6%) primary tumours and 2 of 7 (28.6%) cell lines [46].

Other RTKs: AXL, Insulin growth factor (IGFR); fibroblast growth factor (FGFR).

AXL belongs to the TAM family of RTKs. It is activated by binding growth factor GAS6 (growth arrest-specific 6), and regulates cell survival, cell proliferation, migration and differentiation, via the PI3K/AKT pathway. AXL is overexpressed and activated in many human cancers, including MPM with spindle cell morphology, but somatic AXL mutations were not found in MPM [47, 48].

Human MM cells express insulin growth factor (IGF) and IGFR, and IGF-1 appears to function as an autocrine growth stimulus to MPM cells [20]. Insulin-like growth factor–binding proteins (IGFBP) form a complex with IGFR subunit and IGF, and have been shown to either inhibit or stimulate the growth-promoting effect of IGF. IGFBP can be either expressed or unexpressed in MM, modulating

the aggressiveness of the MM phenotype [20]. IGF-1 stimulation resulted in activation of PI3K and ERK1/2 pathways leading to a stimulation of the eIF4F complex and cap-dependent translation [49]. Few data are available on the activity of FGFR in MPM. A study of mutations in *FGFR1* and *FGFR2* detected one mutation in *FGFR3* from 42 MPM patients [39]. No amplification of FGFR1, a common feature in other cancers, was found by FISH analysis in 19 MPM tissues [50]. Otherwise, FGFR1 and FGFR2 were coexpressed in three of seven MPM cell lines, and FGF2/FGFR1 may form an autocrine signal [51]. KIT is a receptor for stem cell factor or kit ligand. It activates several regulatory pathways, including PI3K and MAPK. KIT expression has been mostly studied by immunohistochemistry, showing a low percentage of positivity in MPM tumours [52]. No expression was detected by RT-PCR in a study of 37 MPMs [53]. KIT has not been shown to be characteristic of MPM at the present time. Ou et al [54] determined the relative levels of tyrosine phosphorylation of 42 distinct RTKs in mesothelioma cell lines established from surgical specimens. They found coordinated activation of several RTKs: EGFR, ERBB3, AXL, and MET [54]. Frequent coactivation of multiple RTKs in MPM cells including EGFR, ERBB3 and MET, but also ERBB2 was also observed in another study [55, 56].

Activity of signalling pathways in MPM

***MAPK pathway*.** MAPK pathway comprises several signalling cascades of protein phosphorylations, which activation depends on the nature of the external stimuli [31]. In MPM, the activation and role of several MAPK, extracellular-regulated kinases (ERK1/2), Jun amino-terminal kinases/stress-activated kinases (JNK/SAPK) and MAPKp38 have been studied. A phosphorylation of ERK, JNK and p38 MAPK was observed in several studies, by immunohistochemistry or western blots [20]. MAPK activation does not seem differentiate between benign and malignant mesothelial cells, but MAPK expression and phosphorylation were better predictive factors of outcome [57]. The ERK and SAPK/JNK signalling pathways are activated in adherent 2D cultures of MPM cells, but not when cells are cultured in 3D conditions [58]. In 3D cultures, MPM are resistant to anoïkis (the death process due to loss of anchorage). This resistance is likely a consequence of the aggregation state of the cells in 3D conditions; it can be reversed by activating SAPK/JNK with anisomycin, according to a Bim-dependent

mitochondrial pathway [58]. The toxicity of drugs able to interact with the MAPK pathways has been investigated in MPM cells. *In vitro* end points concern inhibition of proliferation and induction of apoptosis and in animals, tumour growth in immunodeficient mice (xenograft), or in syngenic mice. Activation of EGFR, MET and AXL in MPM is associated with activation of MAPK signalling cascade [59]. Activation of p21-activated kinases (PAKs), which regulate signalling pathways, was investigated in MPM by IHC [60]. All 15 MPM tumours were more positive in comparison with normal pleural tissue. In MPM cell lines, inhibition of PAKs reduced mesothelioma cell proliferation and survival, and RAF-MAPK signalling [60]. Silencing of the RTK, AXL, by shRNA suppressed mesothelioma migration, invasiveness and cellular proliferation [47]. Arsenic trioxide (As_2O_3) induced apoptosis and phosphorylation of ERK1/2 and JNK1/2, but not p38MAPK, after As_2O_3 treatment, indicating the involvement of the ERK-dependent and JNK-dependent, pathway in the cell response. In contrast, As_2O_3 treatment did not alter phosphorylation of either AKT or SRC [61]. The mechanism seems complex as combination of As_2O_3 treatment and EGFR inhibitor exerted a synergistic effect [62].

PI3K/AKT/mTOR pathway.

The phosphatidylinositol 3'-kinase(PI3K)-Akt (PI3K/AKT) signalling pathway regulates transcription, translation, proliferation, growth, and survival. It is negatively regulated by the phosphatase and tensin homolog (PTEN) and can activate many downstream targets such as Mechanistic Target Of Rapamycin (mTOR) (Figure 2). An alteration of the PI3K/AKT pathway has been reported in several studies [63]. Phosphorylation of AKT protein, the active form of the protein, was demonstrated in MPM cells by IHC and western blots analyses. AKT activation was observed in 13 (62%) of the 21 mesothelioma cell lines under serum-starved conditions [64]. The relationship between AKT and mTOR was observed in tumours demonstrating an association of elevated phospho-mTOR positivity with of AKT pathway activation [65]. However, while the PI3K-Akt signalling pathway was activated in adherent MPM cells, loss of anchorage resulted in inactivation of this pathway and failed to restore apoptosis [58]. AKT phosphorylation and expression of downstream signalling factors, 4E-BP1, p4E-BP1, eIF-4E, peIF-4E, pS6 and FOXO3a were studied by IHC in 30 mesothelioma

cases, demonstrating a positive immunophenotype of the PI3K signalling pathway [66]. Correlation of IHC expression levels and progression free survival (PFS) showed that patients with concomitant low expression of pS6 and p4E-BP1 and overexpression of FOXO3a had significantly better prognosis [66]. IGF stimulation of MPM cells resulted in an increased phosphorylation of AKT, S6 and 4EBP1. Moreover the fraction of side-population (SP, a potential stem cell fraction) was increased, showing that active PI3K signalling may play a role in regulating the SP phenotype in MPM cell lines [67].

Inactivation of *PTEN* could account for the activation of the AKT pathway. No clear-cut data are available on PTEN status in MPM. *PTEN* homozygous deletion has been reported in 2 of 9 of MPM cell lines, and low or no mRNA expression in 9/21 cell lines compared to expression levels in the non-tumour MeT-5A cell line [64, 68]. Loss of PTEN expression was found in 62% of 206 MPM, by tissue microarray, but lower percentage (26.7%) was found in 68 mesothelioma tissues, where no relationship was found with survival [69, 70]. More recently, enhanced expression pAKT in comparison with normal samples, and no difference in PTEN expression was reported in tissue microarray of 213 MPM samples [71]. *PTEN* expression correlated with survival was an independent prognostic biomarker in patients with mesothelioma [70].

The mTOR and PI3K/AKT pathways are strongly interconnected and may be considered as a single pathway [72-74]. The mTOR pathway is activated in mesotheliomas, and the mTOR inhibitor, rapamycin, reduces apoptosis resistance and cell spreading [75-77]. Targeting of PI3K/AKT pathway in MPM cell lines was carried out with inhibitors of either AKT, or dual AKT and mTOR inhibitors (GDC-0980; NVP-BEZ235). Cell viability was reduced with GDC-0980 or NVP-BEZ235 [71]. The combination of MET inhibition with ARQ 197 (Tivantinib) and PI3K/mTOR dual inhibitors enhanced reduction of cell viability [71]. ARQ 197 and GDC-0980 inhibited significantly the growth of MPM xenografts in mice, and the combination of the two drugs was synergistic [71]. The PI3K/mTOR inhibitor NVP-BEZ235 and PI3K inhibitor wortmannin reduced the phosphorylation of downstream target AKT, S6 and 4EBP1, decreased the SP fraction and sensitized SP cells to chemotherapy, via the regulation of ABCG2, a potential marker of stem cells [67]. Other inhibitors were tested in mesothelioma cell lines. Perifosine, an

analogue of lysophospholipids tested in clinical trials for other types of cancers, that targets PI3K/AKT signalling reduced AKT activation, and also affected EGFR and MET phosphorylation in mesothelioma cells [59, 78] studied phosphorylation of the PI3K/AKT pathway in five human mesothelioma cell lines in comparison non-neoplastic mesothelial cells. Curiously, activation of EGFR, MET and AXL activated AKT but not MAPK pathways. AKT inhibitors reduced proliferation and cell viability, but the apoptosis yield was low. Inhibition of both AKT and mTOR induced maximal inhibition, but did not abolish cell proliferation or induced strong apoptosis [59]. Moreover, AKT inhibition by shRNAs enhanced mTOR activation [59]. These results suggest that other control of cell survival operates in mesothelioma cells. Moreover, mTOR pathway also cross-talks with other regulatory pathways, MAPK and Hippo [79, 80] TSC2 can be phosphorylated by ERK or RSK [74]. Collectively, these results show that inhibitors can impact factors of different pathways or cross-talk between distinct pathways, rendering difficult to specifically target a given signalling pathway.

Hippo signalling pathway. The Hippo Pathway is a regulator of organ size, development and differentiation. It is involved in negative control of cell proliferation, and also tissue regeneration and mechanotransduction. It is initiated by extracellular signals that activate NF2, also called merlin by dephosphorylation In confluent cells, merlin inhibits the activity of the cofactor of transcription, YAP, then inhibiting cell growth (Figure 3) [80-83]. In MPM, an inactivation of the *NF2* gene has been evidenced by allelic or biallelic losses and point mutations, occurring in 40-60% of the cases [20, 80]. Large deletion is the main type of *NF2* inactivation. Genetic alterations were also described in other members of the Hippo pathway, SAV1, LATS1 and LATS2, with the higher frequency in LATS2 [24, 29, 84]. Conversely, YAP seems to be overexpressed in MPM and knockdown of YAP expression inhibits proliferation of MPM cells *in vitro* [85]. Other members of these pathways such as *AJUBA* are also deregulated at the expression level [86]. These findings converge to conclude to an inactivation of the Hippo pathway, in a situation where NF2 cannot regulate YAP, which in turn allows its transcriptional and oncogenic activity. Moreover, *NF2* is a target of miRNAs upregulated in MPM samples [87]. Despite a wild-type status for *NF2*,

merlin also appears to be present in an inactivated phosphorylated form in some MPM cells [88].

Hedgehog signalling pathway. The Hedgehog pathway works with a family of secreted signalling proteins; it plays a central role in the development and for tissue homeostasis; it is also involved in control of stem cell proliferation [89]. Its activation is initiated through binding of hedgehog (Hh) proteins (SHH: Sonic hedgehog; DHH: Desert hedgehog, and IHH: Indian hedgehog) to the receptor Patched 1 (PTCH1). PTCH1 is a negative regulator of the Hh signalling pathway via its association with Smoothened (SMO) and triggers a cascade of signalling events downstream from SMO. PTCH1 is considered to have a tumour suppressor function. Activation of the hedgehog pathway results in PTCH1 degradation and SMO release and the transduction of the hedgehog's proteins signal. This leads to the activation of the GLI family of transcription factors (GLI 1-3), through complex interactions of Costal2 (Cos2), Fused (Fu) and Suppressor of fu [Su(fu)] [89].

Mutations of Hh signalling pathway member are infrequent in MPM [90]. A role of the Hh signalling pathway in MPM has been suggested in several works and some members may be regarded as therapeutic targets. An overexpression of genes involved in Hh signalling, *SMO* and *GLI1*, was reported in primary cultures derived from MPM tumour samples [91-93]. Hh pathway may cross-talk with other signalling pathways. Interestingly, inhibition also resulted in a decrease in the expression of Yap [92]. So far, the activity of antagonists was investigated in a few studies, either alone or in association. The inhibition of *SMO* or *GLI1* by RNA interference or Hh drug antagonists (HhAntag) inhibited cell viability, cell growth and tumour growth in xenografts, accompanied by decreased Ki-67 [92]. Using siRNA strategy and small molecule inhibitors, vismodegib and cyclopamine, *in vitro*, inhibition of GLI was more efficient than that of SMO. Combination of GLI-I and pemetrexed, as well as GLI-I and vismodegib demonstrated synergistic effects in suppression of MPM proliferation [91]. In another study, SMO inhibitor GDC-0449 and the Gli inhibitor GANT61 reduced Hh signalling in MPM cells, as assessed by the level of *GLI1* expression [93].

Other pathways: WNT; ubiquitin/proteasome system; p53 network

The WNT signalling pathway normally acts in development; it increases cell proliferation and decrease apoptosis, and is misregulated in cancer [94]. WNT signals develop according to three different pathways: the canonical pathway, the planar cell polarity (PCP) pathway and the Wnt/Ca^{2+} pathway.

An activation of WNT signalling pathway has been reported in mesothelioma [63]. The protein expression of WNT1, WNT2B and WNT5A was studied in 107 MPM tumours by immunohistochemistry [95]. The percentage of WNT2B-positive tumours was significantly higher compared to that of the other WNTs, and was significantly correlated with the expression of survivin and c-Myc. A Cox multivariate analysis demonstrated the WNT2B status to be a significant prognostic factor in MPM patients [95]. Gene expression profiling of MM cell lines, primary MPM tumours, and normal pleural tissue has been studied to determine the expression of genes involved in the WNT signalling pathway, and downstream of WNT signalling [20, 96]. Numerous WNT genes (WNT1, WNT2, WNT5) and WNT-related genes (MYC, CCND1, JUN) were upregulated. WNT2 was most frequently upregulated and played a role for in cell survival [97]. In another study, WNT3 and WNT5A, as well as FZD2, 4 and 6 were expressed, and several members, WNT4, FZD3, sFRP4, APC and axin2 were downregulated in comparison to mesothelial cells. Moreover, LEF1 was overexpressed in mesothelioma cells [96]. WNT signalling inhibition is dependent on several factors including the Dickkopf (DKK) gene family. REIC/Dickkopf-3 expression was lower in mesothelioma than in normal tissue, and orthotopic inoculation of REIC/Dickkopf-3–deficient cells followed by intrapleural injection of recombinant REIC/Dickkopf-3-adenovirus resulted in a strong antitumor effect [98]. Conflicting data are reported on β-catenin localization in mesothelioma cells [99]. One study carried out to study miRNAs that were downregulated in MPM compared to lung adenocarcinoma identified potential regulators of WNT signalling [100]. Collectively, these results provide evidence for altered expression of members of WNT pathway and that identification of key targets could be of interest to therapeutic approaches.

The ubiquitin/proteasome system is the main system for controlled protein degradation, and a key regulator of fundamental cellular processes. It mediates proteolysis following two main steps, protein labelling for destruction, and protein

degradation by the 26S proteasome [101]. This pathway degrades the damaged or unwanted proteins and prevents constant activation of specific molecular signalling pathways. The process of ubiquitination can be reversed by the action of deubiquitinases (DUB) that counteract E3 ligases. There is some indication that ubiquitin and DUB proteins may play a role in mesothelioma. Identification of proteins interacting with NF2/merlin revealed that the unphosphorylated, growth-inhibiting form of NF2/merlin, accumulates in the nucleus and binds to $CRL4^{DCAF1}$ (Cul4-Roc1-DDB1-DCAF1) E3 ubiquitin ligase, suppressing NF2 activity and promoting YAP-dependent transcription activity [102, 103]. Further studies would be necessary to determine whether the tumour suppressive activity of NF2/merlin is due to its localisation and/or by inhibiting the E3 ubiquitin ligase $CRL4^{DCAF1}$ in the nucleus in mesothelioma [103]. Otherwise, as discussed above, mutations were detected in *CUL1*, which is an essential component of the SCF (SKP1–CUL1–F-box protein) E3 ubiquitin ligase complex, and in *BAP1* that encodes a DUB enzyme that mediates deubiquitination of histone H2A and HCFC1.

Another important issue accounting for lack of response of MPM cells to antitumoural agents stands in the cells' resistance to apoptosis. Although not mutated, P53 is expressed in MPM cells despite of the presence of negative regulators and does not seem critical in human MPM. However, P53 levels are also regulated by members in PI3K, mTOR and Hippo pathways [82, 83, 104]. Further understanding of the implication of P53 in drug-related apoptosis of MPM cells would be of interest.

TARGETED THERAPY

RTKs targeting

Preclinical studies suggested that EGFR targeting with RTKs inhibitors such as gefitinib or erlotinib resulted in reduction of cell proliferation and tumour growth in immunosuppressed mice by down regulation of the PI3K/AKT pathway inhibition. Several clinical trials have been performed, but the results were disappointing as no benefit was found in comparison with current therapies [105, 106]. Similar results were obtained with drugs targeting PDFGR such as imatinib, even when associated with chemotherapy [45, 107]. Several agents synthetized to

target VEGFR, such as bevacizumab, sorafenib, cediranib, demonstrated some effects in pre-clinical assays [105]. Association with cytotoxic molecule generally enhanced the cell response. Despite unsuccessful early trials of anti-VEGF therapy, new clinical trials have been carried out, in combination with chemotherapy, and the agents often targeted RTKs other than VEGFR. Only few responses were observed in these assays combining inhibitors and chemotherapy [106]. RTK analyses in MPM showed no or low rate of mutations, frequent autocrine processes and coactivation of several RTK. These findings may account for the lack of responsiveness to RTK inhibitors, and emphasize the need for a better knowledge of the mechanism of mesothelial carcinogenesis.

Targeting of pathways

So far, *in vivo* targeting of PI3K/AKT pathway in MPM patients did not entail encouraging results [106, 108]. Several trials showed low responsiveness to the mTOR inhibitor, everolimus, likely due to a compensatory upregulation of pathways. Recently, the results of a phase II trial using everolimus in 59 MPM patients who had received at least one but no more than two platinum-based chemotherapy demonstrated that everolimus has limited clinical activity in advanced MPM patients. Authors concluded that additional studies of single-agent everolimus in advanced MPM are not warranted [109].

Synthetic lethality approach could be pertinent for mesothelioma therapy as mutations in MPM mainly concern tumour suppressor genes and not oncogenes. The method consists in provoking cell death by the inactivation of 2 genes cells [110]. When mutated alone, cells are viable, while both inactivation are lethal. In MPM mutations mainly provoke inactivation of tumour suppressor genes and that confers viability to the cells. Targeting tumour suppressor genes by inhibitors is not pertinent, as their activity should be restored. Recently, this type of therapeutic strategy has been validated in preclinical models to NF2 mutated mesothelioma cells using inhibitor of FAK (Focal Adhesion kinase) pathway, based on the hypothesis that FAK pathway became essential to cell adhesion, and consequently survival, when NF2 is inactivated [111]. A clinical trial using FAK inhibitor is ongoing and the results should bring new information on the potential of this strategy.

Targeting the Hippo pathway is of particular interest as this pathway is altered in a large proportion of mesotheliomas [112]. It has be found that several members may be altered, and the transcriptional co-activator YAP plays a central role. Targeting of YAP protein would be interesting. However, YAP is not only regulated by the Hippo pathway, but also by crosstalks between Hippo and other signalling pathways including WNT, TGFβ□□ Hedgehog [106]. This renders difficult the targeting of pathways without a clear knowledge of the concurrent activated pathways and cross-talks. However, one could take advantage of the redundancy between pathways to target co-activated members. Regarding the multiple alterations in individual mesothelioma tumours, one may assume that a precise molecular and physiological knowledge of mesotheliomas will benefit to targeted therapies.

CONCLUSIONS

Presently, targeted therapies remain unsuccessful, except for subsets of patients. Apart from patient status, one of the reasons likely stands in the role and importance of the targeted factors in the tumour status of mesothelioma cells, and the complexity of the mechanisms regulating the functions mesothelioma cells. So far, patients are not selected according to the characteristics of the tumour cells, except for morphological features. Moreover, the question of the nature of the target cell is discussed with regard to the existence of cancer stem cells in tumours. It could be necessary to develop new antitumoural drugs [113]. It brings an additional level of complexity related to the intratumoural heterogeneity. Targeting of cytokines and growth factors receptors that activate signalling pathways with inhibitors has been tested in clinical trials. Some growth factors have been associated with poor prognosis, and may be more relevant than non-significant factors. EGFR does not seem to be related to prognosis; some relation might be suggested with expression of AXL or PDGFR-B as good prognosis [114]. VEGF serum level has been linked to poor prognosis, but not IHC expression of FGF or TGFβ [114]. Although some molecules in cell cycle and signalling pathways ($P16^{INK4A}$ or MDM2) have been associated to poor prognosis, others were linked to better prognosis ($P27^{KIP1}$, $P21^{CIP1}$, AKT, PTEN) [114]. These observations demonstrate the interest of knowing the molecular status of targets the tumours, to

avoid unfavourable targeting, and not suppress the activity of favourable factors. However, further analyses are needed to establish the exact role of these factors in the viability and survival of mesothelioma cells. The studies carried out in MPM showed that the PI3K/AKT pathway deregulation likely plays a role in the viability of MPM cells, that other pathways are relevant and that the signalling intercrossed. Antitumoural targeted strategy should take these findings into consideration. Otherwise, the heterogeneity between the mesothelioma tumours is evidenced by the data concerning morphology, mutational status of genes, activity of signalling pathways. Knowledge of the different pathways involved in mesothelioma tumour progression is necessary to identify the relevant pathway, in a given patient, and to focus on this pathway. For this purpose, preclinical models reflecting mesothelioma diversity need to be developed. New 2D and 3D models using human mesothelioma cells, and co-cultures would be of interest. As quoted by Ceresoli [115], only a tight integration of preclinical and clinical studies will allow achievement of a real progress in MPM patients with this therapeutic strategy [115]. It can be added that a better knowledge of the perturbations in the regulation of cell homeostasis in mesothelioma cells is needed. The knowledge of the genetic and epigenetic changes that disrupt cell division, growth, mobility and apoptosis in individual tumours is also necessary to progress. Network organizations gathering epidemiologists, clinicobiologists, basic researchers and therapists, founded on databases and tissue biobanks are progressing and would have strong the impact to treat this disease [116].

Legends to Figures

Figure 1.

Schematic representation of the MAPKs pathways. Signalling occurs through three different MAPK pathways, extracellular-signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNK), or p38 MAPK. (1) ERK1/2 pathway is activated by mitogens and growth factors such as PDGF and EGF. Following receptor activation, RAS (a small GDP binding protein) is activated by exchange GDP/GTP; recruitment of other membrane components allows the phosphorylation of downstream members RAF, MEK and ERK1/2. ERK1/2 pathway activates various transcription factors (TF) such as ELK1, allowing the expression of the TF c-FOS that may dimerizes with c-JUN to form the TF complex AP-1, c-MYC or STAT3. The Ras/Raf/MEK/MAPK cascade involves members of the RAS family, which are recognized as oncogenes, such as H-*RAS*, K-*RAS* and N-*RAS*. Mutations in members of MAPK pathway have a very low frequency in MPM. Low frequency of mutations were found in *KRAS* and *NRAS* [37, 39, 117]). This pathway plays a role in the control of cell proliferation. (2) p38 pathway is mostly stimulated by inflammatory cytokines and in response to various cellular stresses (oxidative stress, UV radiations, hypoxia, ischemia). Several TF can be activated (ATF, ELK1, ETS1, P53) inducing the production of factors involved in immune and inflammatory responses, cell proliferation, survival, migration and gene expression. The p38a isoform negatively regulates cell cycle progression. (3) The third pathway activates JNK; it is also activated by cellular stresses (heat shock, ionizing radiation, UV radiation, oxidative stress, DNA damage, decrease in growth factors and reduction of protein synthesis). The activation of TF: c-JUN, ATF-2, JUNB, ALK1, STAT3, c-MYC, P53 induces control of cell proliferation. JNK isoforms play a role in the apoptotic response to cellular stresses. For details on activation and function of MAPKs, see review [118].

Figure 2

The PI3K/AKTmTOR pathway is activated in response to growth factors, such as PDGF, insulin-like growth factor (IGF), interleukin 7 (IL-7), and fms-like tyrosine

kinase 3 ligand (FLT3-L) following interaction with their respective RTK receptors, and induce intracellular PI3K/mTOR pathway signalling. There are three PI3K, which are lipid kinases, which convers PIP2 into PIP3, then recruits PDPK1 allowing the phosphorylation of AKT. Activation of AKT activates mTORC1 via the inhibition of the tuberous sclerosis complexes 1 and 2 (TSC1/TSC2), and small GTPases, Rheb, then mediating the phosphorylation of proteins S6K and the eukaryotic translation initiation factor 4E-binding protein (4E-BP1), and the release of the eukaryotic translation initiation factor 4E (eIF4E), resulting in increased protein synthesis and cell growth. A second complex of mTOR (mTORC2) also acts as activator of AKT. Negative regulation operates via the tumour suppressor gene, phosphatase and tensin homolog (PTEN) pathway. Inactivation of the PTEN pathway can account for PI3K/AKT activation. The PI3K-AKT cascade regulates cell growth processes, cell migration, and survival. Mutations in *PI3KCA*, a gene encoding a catalytic subunit of PI3K, have been detected at very low frequency in MPM [37, 39]. For review see [72, 74].

Figure 3

Members of the Hippo pathway include NF2/merlin, MST (mammalian sterile 20-like kinases), LATS1/2 (large tumour suppressor kinases), SAV1 (salvador homologue 1), AJUBA and KIBRA. NF2/merlin is regulated by interactions with cell-surface receptors or adherens junctions. The active form of NF2/merlin is unphosphorylated; it is phosphorylated and inactivated by RAC and PAK (p21-activated kinase). Active NF2/merlin activates LATS1/2 by phosphorylation. The activated form of LATS inhibits the transcriptional co-activators YAP and TAZ (Yes-associated protein/transcriptional and co-activator with PDZ-binding motif) through their phosphorylation, preventing their accumulation in the nucleus. Phosphorylation of YAP and TAZ leads to their sequestration in the cytoplasm by interaction with 14-3-3 proteins and ubiquitination-dependent proteosomal degradation. The underphosphorylated YAP and TAZ translocate into the nucleus and induce the activation of transcription factors (TF), including TEAD family (transcription enhancers activation domain) members and also TF involved in other signalling pathways such as TGFβ, WNT signalling and apoptosis. The underphosphorylated form of NF2/merlin can translocate to the nucleus, bind to

the E3 ubiquitin ligase $CRL4^{DCAF1}$ and inhibit its ubiquitination activity (see text). For reviews, see [119, 120].

Figure 4

Signalling is initiated by Wnt proteins, which bind to the cell surface receptor Frizzled (FZ) and low density lipoprotein receptor-related protein (LRP) complex. The signal is transduced to several intracellular proteins (dishevelled, DVL; glycogen synthase kinase-3β, GSK-3; axin; adenomatous polyposis coli, APC). Activation of WNT signalling releases the transcription factor, β-catenin, that is normally maintained at low level through continuous proteasome-mediated degradation by the GSK-3/APC/Axin complex that allows phosphorylated β-catenin to be recognized by β-TrCP. Then, β-catenin accumulates and is translocated to the nucleus where it interacts with T-cell factor/lymphoid enhancer factor (LEF/TCF) to affect transcription. Additional non-canonical Wnt signalling pathways, have bee described (see text). For review, see [94].

REFERENCES

1. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med. 1960; 17: 260-71.
2. Haas AR, Sterman DH. Malignant pleural mesothelioma: update on treatment options with a focus on novel therapies. Clin Chest Med. 2013; 34: 99-111.
3. Opitz I. Management of malignant pleural mesothelioma-The European experience. Journal of thoracic disease. 2014; 6 Suppl 2: S238-52.
4. Opitz I, Bueno R, Lim E, et al. Biomolecular and clinical practice in malignant pleural mesothelioma and lung cancer: what thoracic surgeons should know. Eur J Cardiothorac Surg. 2014; 46: 602-6.
5. Treasure T, Lang-Lazdunski L, Waller D, et al. Extra-pleural pneumonectomy versus no extra-pleural pneumonectomy for patients with malignant pleural mesothelioma: clinical outcomes of the Mesothelioma and Radical Surgery (MARS) randomised feasibility study. Lancet Oncol. 2011; 12: 763-72.
6. Reid A, de Klerk NH, Magnani C, et al. Mesothelioma risk after 40 years since first exposure to asbestos: a pooled analysis. Thorax. 2014; 69: 843-50.
7. Frank AL, Joshi TK. The global spread of asbestos. Ann Glob Health. 2014; 80: 257-62.
8. Leong SL, Zainudin R, Kazan-Allen L, et al. Asbestos in Asia. Respirology. 2015.
9. Bianchi C, Bianchi T. Global mesothelioma epidemic: Trend and features. Indian journal of occupational and environmental medicine. 2014; 18: 82-8.

10. Jean D, Le Pimpec-Barthes F, Andujar P, et al. Thoracic Neoplasia–Mesothelioma. In: Mitchell LMMaRN, editor. Pathobiology of Human Disease: A Dynamic Encyclopedia of Disease Mechanisms: Elsevier; 2014. p. 2690-700.
11. Guida F, Billon-Galland MA, Stucker I, et al. Effets sur la santé des fibres minérales artificielles. ECM - Pathologie professionnelle et de l'environnement. 2014; 9: 1-8.
12. Carbone M, Ly BH, Dodson RF, et al. Malignant mesothelioma: facts, myths, and hypotheses. J Cell Physiol. 2012; 227: 44-58.
13. Rascoe PA, Jupiter D, Cao X, et al. Molecular pathogenesis of malignant mesothelioma. Expert Rev Mol Med. [Review]. 2012; 14: e12.
14. Betta PG, Magnani C, Bensi T, et al. Immunohistochemistry and molecular diagnostics of pleural malignant mesothelioma. Arch Pathol Lab Med. 2012; 136: 253-61.
15. Loeb LA. Human cancers express mutator phenotypes: origin, consequences and targeting. Nat Rev Cancer. [Research Support, N.I.H., Extramural]. 2011; 11: 450-7.
16. Cabrera MC, Hollingsworth RE, Hurt EM. Cancer stem cell plasticity and tumor hierarchy. World journal of stem cells. 2015; 7: 27-36.
17. Shackleton M, Quintana E, Fearon ER, et al. Heterogeneity in cancer: cancer stem cells versus clonal evolution. Cell. 2009; 138: 822-9.
18. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012; 21: 309-22.
19. Husain AN, Colby T, Ordonez N, et al. Guidelines for Pathologic Diagnosis of Malignant Mesothelioma: 2012 Update of the Consensus Statement from the International Mesothelioma Interest Group. Arch Pathol Lab Med. 2013.
20. Jean D, Daubriac J, Le Pimpec-Barthes F, et al. Molecular changes in mesothelioma with an impact on prognosis and treatment. Arch Pathol Lab Med. [Review]. 2012; 136: 277-93.
21. Gordon GJ, Rockwell GN, Jensen RV, et al. Identification of novel candidate oncogenes and tumor suppressors in malignant pleural mesothelioma using large-scale transcriptional profiling. Am J Pathol. 2005; 166: 1827-40.
22. Hoang CD, D'Cunha J, Kratzke MG, et al. Gene expression profiling identifies matriptase overexpression in malignant mesothelioma. Chest. 2004; 125: 1843-52.
23. de Reynies A, Jaurand MC, Renier A, et al. Molecular classification of malignant pleural mesothelioma: Identification of a poor prognosis subgroup linked to the epithelial-to-mesenchymal transition. Clin Cancer Res. 2014; 20: 1323-34.
24. Bott M, Brevet M, Taylor BS, et al. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nat Genet. [Research Support, Non-U.S. Gov't]. 2011; 43: 668-72.
25. Jean D, Thomas E, Manie E, et al. Syntenic relationships between genomic profiles of fiber-induced murine and human malignant mesothelioma. Am J Pathol. 2011; 178: 881-94.
26. Tallet A, Nault JC, Renier A, et al. Overexpression and promoter mutation of the TERT gene in malignant pleural mesothelioma. Oncogene. 2013.
27. Guo G, Chmielecki J, Goparaju C, et al. Whole exome sequencing reveals frequent genetic alterations in BAP1, NF2, CDKN2A and CUL1 in malignant pleural mesothelioma. Cancer Res. 2015.
28. Lo Iacono M, Monica V, Righi L, et al. Targeted next-generation sequencing of cancer genes in advanced stage malignant pleural mesothelioma: a retrospective study. J Thorac Oncol. 2015; 10: 492-9.
29. Miyanaga A, Masuda M, Tsuta K, et al. Hippo pathway gene mutations in malignant mesothelioma: revealed by RNA and targeted exon sequencing. J Thorac Oncol. 2015; 10: 844-51.

30. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2010; 141: 1117-34.
31. Son Y, Kim S, Chung HT, et al. Reactive oxygen species in the activation of MAP kinases. Methods in enzymology. 2013; 528: 27-48.
32. Edwards JG, Swinson DE, Jones JL, et al. EGFR expression: associations with outcome and clinicopathological variables in malignant pleural mesothelioma. Lung Cancer. 2006; 54: 399-407.
33. Enomoto Y, Kasai T, Takeda M, et al. Epidermal growth factor receptor mutations in malignant pleural and peritoneal mesothelioma. J Clin Pathol. 2012; 65: 522-7.
34. Gaafar R, Bahnassy A, Abdelsalam I, et al. Tissue and serum EGFR as prognostic factors in malignant pleural mesothelioma. Lung Cancer. 2010; 70: 43-50.
35. Rena O, Boldorini LR, Gaudino E, et al. Epidermal growth factor receptor overexpression in malignant pleural mesothelioma: Prognostic correlations. J Surg Oncol. 2011; 104: 701-5.
36. Cortese JF, Gowda AL, Wali A, et al. Common EGFR mutations conferring sensitivity to gefitinib in lung adenocarcinoma are not prevalent in human malignant mesothelioma. Int J Cancer. 2006; 118: 521-2.
37. Mezzapelle R, Miglio U, Rena O, et al. Mutation analysis of the EGFR gene and downstream signalling pathway in histologic samples of malignant pleural mesothelioma. Br J Cancer. 2013; 108: 1743-9.
38. Velcheti V, Kasai Y, Viswanathan AK, et al. Absence of mutations in the epidermal growth factor receptor (EGFR) kinase domain in patients with mesothelioma. J Thorac Oncol. 2009; 4: 559.
39. Shukuya T, Serizawa M, Watanabe M, et al. Identification of actionable mutations in malignant pleural mesothelioma. Lung Cancer. 2014; 86: 35-40.
40. Schildgen V, Pabst O, Tillmann RL, et al. Low Frequency of EGFR Mutations in Pleural Mesothelioma Patients, Cologne, Germany. Diagnostic molecular pathology : the American journal of surgical pathology, part B. 2014.
41. Brevet M, Shimizu S, Bott MJ, et al. Coactivation of receptor tyrosine kinases in malignant mesothelioma as a rationale for combination targeted therapy. J Thorac Oncol. 2011; 6: 864-74.
42. Andujar P, Pairon JC, Renier A, et al. Differential mutation profiles and similar intronic TP53 polymorphisms in asbestos-related lung cancer and pleural mesothelioma. Mutagenesis. [Research Support, Non-U.S. Gov't]. 2013; 28: 323-31.
43. Lee AY, Raz DJ, He B, et al. Update on the molecular biology of malignant mesothelioma. Cancer. 2007; 109: 1454-61.
44. Gerwin BI, Lechner JF, Reddel RR, et al. Comparison of production of transforming growth factor-beta and platelet-derived growth factor by normal human mesothelial cells and mesothelioma cell lines. Cancer Res. 1987; 47: 6180-4.
45. Tsao AS, Harun N, Lee JJ, et al. Phase I trial of cisplatin, pemetrexed, and imatinib mesylate in chemonaive patients with unresectable malignant pleural mesothelioma. Clin Lung Cancer. 2014; 15: 197-201.
46. Jagadeeswaran R, Ma PC, Seiwert TY, et al. Functional analysis of c-Met/hepatocyte growth factor pathway in malignant pleural mesothelioma. Cancer Res. 2006; 66: 352-61.
47. Ou WB, Corson JM, Flynn DL, et al. AXL regulates mesothelioma proliferation and invasiveness. Oncogene. 2011; 30: 1643-52.
48. Paccez JD, Vogelsang M, Parker MI, et al. The receptor tyrosine kinase Axl in cancer: biological functions and therapeutic implications. Int J Cancer. 2014; 134: 1024-33.

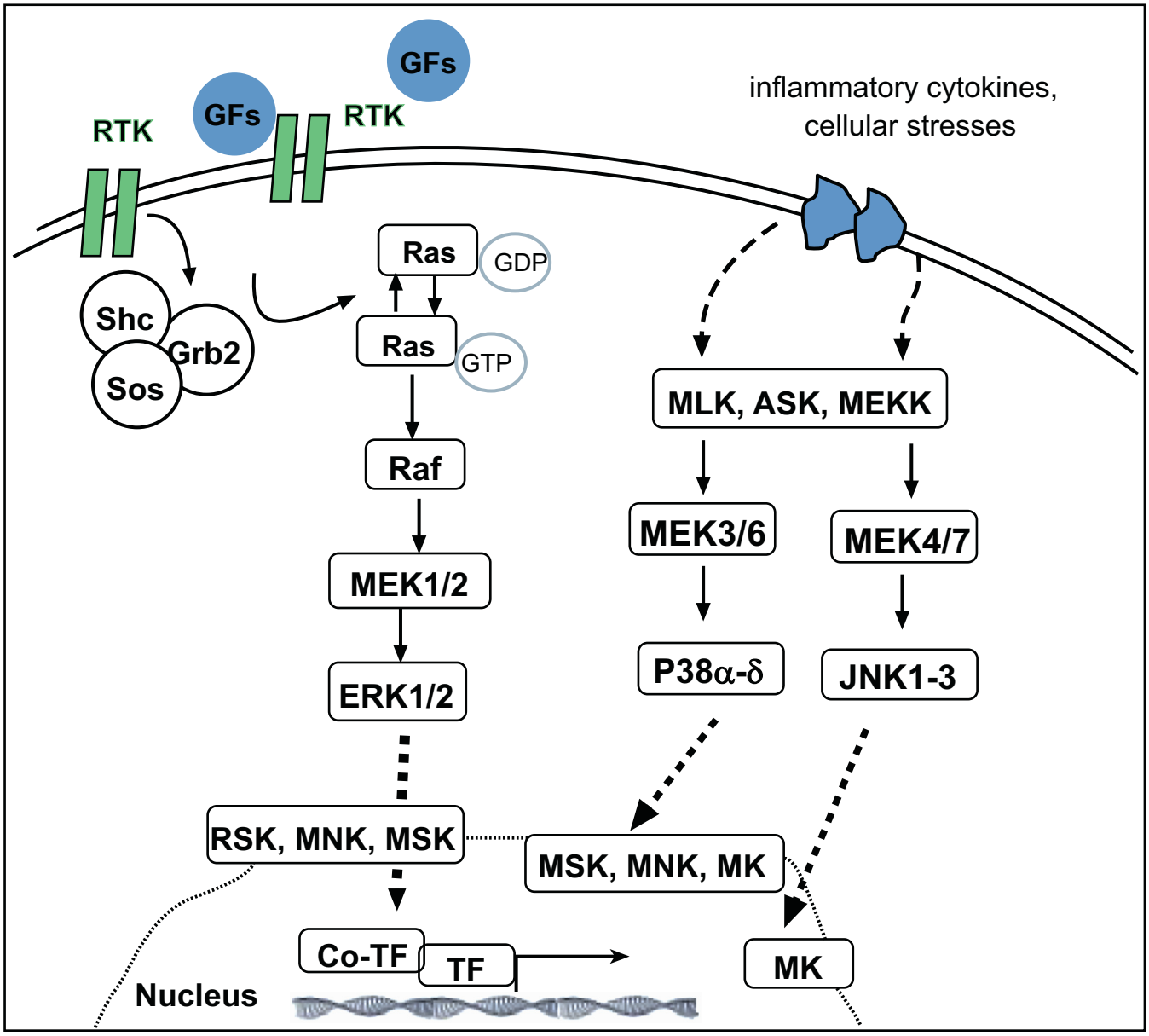
49. Jacobson BA, De A, Kratzke MG, et al. Activated 4E-BP1 represses tumourigenesis and IGF-I-mediated activation of the eIF4F complex in mesothelioma. Br J Cancer. 2009; 101: 424-31.
50. Plones T, Beckers F, Engel-Riedel W, et al. Absence of amplification of the FGFR1-gene in human malignant mesothelioma of the pleura: a pilot study. BMC research notes. 2014; 7: 549.
51. Marek LA, Hinz TK, von Massenhausen A, et al. Nonamplified FGFR1 is a growth driver in malignant pleural mesothelioma. Mol Cancer Res. 2014; 12: 1460-9.
52. Butnor KJ, Burchette JL, Sporn TA, et al. The spectrum of Kit (CD117) immunoreactivity in lung and pleural tumors: a study of 96 cases using a single-source antibody with a review of the literature. Arch Pathol Lab Med. 2004; 128: 538-43.
53. Horvai AE, Li L, Xu Z, et al. c-Kit is not expressed in malignant mesothelioma. Mod Pathol. 2003; 16: 818-22.
54. Ou WB, Hubert C, Corson JM, et al. Targeted inhibition of multiple receptor tyrosine kinases in mesothelioma. Neoplasia. 2011; 13: 12-22.
55. Kawaguchi K, Murakami H, Taniguchi T, et al. Combined inhibition of MET and EGFR suppresses proliferation of malignant mesothelioma cells. Carcinogenesis. 2009; 30: 1097-105.
56. Menges CW, Chen Y, Mossman BT, et al. A Phosphotyrosine Proteomic Screen Identifies Multiple Tyrosine Kinase Signaling Pathways Aberrantly Activated in Malignant Mesothelioma. Genes Cancer. 2010; 1: 493-505.
57. Vintman L, Nielsen S, Berner A, et al. Mitogen-activated protein kinase expression and activation does not differentiate benign from malignant mesothelial cells. Cancer. 2005; 103: 2427-33.
58. Daubriac J, Fleury-Feith J, Kheuang L, et al. Malignant pleural mesothelioma cells resist anoikis as quiescent pluricellular aggregates. Cell Death and Differentiation. 2009; 16 1146-55.
59. Zhou S, Liu L, Li H, et al. Multipoint targeting of the PI3K/mTOR pathway in mesothelioma. Br J Cancer. 2014; 110: 2479-88.
60. Menges CW, Sementino E, Talarchek J, et al. Group I p21-Activated Kinases (PAKs) Promote Tumor Cell Proliferation and Survival through the AKT1 and Raf-MAPK Pathways. Mol Cancer Res. 2012; 10: 1178-88.
61. Eguchi R, Fujimori Y, Takeda H, et al. Arsenic trioxide induces apoptosis through JNK and ERK in human mesothelioma cells. J Cell Physiol. 2011; 226: 762-8.
62. Kryeziu K, Jungwirth U, Hoda MA, et al. Synergistic anticancer activity of arsenic trioxide with erlotinib is based on inhibition of EGFR-mediated DNA double-strand break repair. Mol Cancer Ther. 2013; 12: 1073-84.
63. de Assis LV, Locatelli J, Isoldi MC. The role of key genes and pathways involved in the tumorigenesis of Malignant Mesothelioma. Biochim Biophys Acta. 2014; 1845: 232-47.
64. Suzuki Y, Murakami H, Kawaguchi K, et al. Activation of the PI3K-AKT pathway in human malignant mesothelioma cells. Molecular Medicine Reports. 2009; 2: 181-8.
65. Besson A, Robbins SM, Yong VW. PTEN/MMAC1/TEP1 in signal transduction and tumorigenesis. Eur J Biochem. 1999; 263: 605-11.
66. Cedres S, Montero MA, Martinez P, et al. Exploratory analysis of activation of PTEN-PI3K pathway and downstream proteins in malignant pleural mesothelioma (MPM). Lung Cancer. 2012; 77: 192-8.
67. Fischer B, Frei C, Moura U, et al. Inhibition of phosphoinositide-3 kinase pathway down regulates ABCG2 function and sensitizes malignant pleural mesothelioma to chemotherapy. Lung Cancer. 2012; 78: 23-9.

68. Altomare DA, You H, Xiao GH, et al. Human and mouse mesotheliomas exhibit elevated AKT/PKB activity, which can be targeted pharmacologically to inhibit tumor cell growth. Oncogene. 2005; 24: 6080-9.
69. Agarwal V, Campbell A, Beaumont KL, et al. PTEN protein expression in malignant pleural mesothelioma. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2013; 34: 847-51.
70. Opitz I, Soltermann A, Abaecherli M, et al. PTEN expression is a strong predictor of survival in mesothelioma patients. Eur J Cardiothorac Surg. 2008; 33: 502-6.
71. Kanteti R, Dhanasingh I, Kawada I, et al. MET and PI3K/mTOR as a Potential Combinatorial Therapeutic Target in Malignant Pleural Mesothelioma. PLoS ONE. 2014; 9: e105919.
72. Gao B, Roux PP. Translational control by oncogenic signaling pathways. Biochim Biophys Acta. 2014.
73. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR Signaling in Cancer. Frontiers in oncology. 2014; 4: 64.
74. Xu K, Liu P, Wei W. mTOR signaling in tumorigenesis. Biochim Biophys Acta. 2014; 1846: 638-54.
75. Barbone D, Yang TM, Morgan JR, et al. Mammalian target of rapamycin contributes to the acquired apoptotic resistance of human mesothelioma multicellular spheroids. J Biol Chem. 2008; 283: 13021-1330.
76. Ranzato E, Grosso S, Patrone M, et al. Spreading of mesothelioma cells is rapamycin-sensitive and requires continuing translation. J Cell Biochem. 2009; 108: 867-76.
77. Wilson SM, Barbone D, Yang TM, et al. mTOR mediates survival signals in malignant mesothelioma grown as tumor fragment spheroids. Am J Respir Cell Mol Biol. 2008; 39: 576-83.
78. Pinton G, Manente AG, Angeli G, et al. Perifosine as a potential novel anti-cancer agent inhibits EGFR/MET-AKT axis in malignant pleural mesothelioma. PLoS ONE. 2012; 7: e36856.
79. Lopez-Lago MA, Okada T, Murillo MM, et al. Loss of the tumor suppressor gene NF2, encoding merlin, constitutively activates integrin-dependent mTORC1 signaling. Mol Cell Biol. 2009; 29: 4235-49.
80. Sekido Y. Molecular pathogenesis of malignant mesothelioma. Carcinogenesis. 2013; 34: 1413-9.
81. Hao J, Zhang Y, Wang Y, et al. Role of extracellular matrix and YAP/TAZ in cell fate determination. Cell Signal. 2014; 26: 186-91.
82. Piccolo S, Dupont S, Cordenonsi M. The biology of YAP/TAZ: hippo signaling and beyond. Physiol Rev. 2014; 94: 1287-312.
83. Varelas X. The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease. Development (Cambridge, England). 2014; 141: 1614-26.
84. Murakami H, Mizuno T, Taniguchi T, et al. LATS2 Is a Tumor Suppressor Gene of Malignant Mesothelioma. Cancer Res. 2011; 71: 873-83.
85. Mizuno T, Murakami H, Fujii M, et al. YAP induces malignant mesothelioma cell proliferation by upregulating transcription of cell cycle-promoting genes. Oncogene. 2012; 31: 5177-22.
86. Tanaka I, Osada H, Fujii M, et al. LIM-domain protein AJUBA suppresses malignant mesothelioma cell proliferation via Hippo signaling cascade. Oncogene. 2015; 34: 73-83.
87. Guled M, Lahti L, Lindholm PM, et al. CDKN2A, NF2, and JUN are dysregulated among other genes by miRNAs in malignant mesothelioma -A miRNA microarray analysis. Genes Chromosomes Cancer. 2009; 48: 615-23.

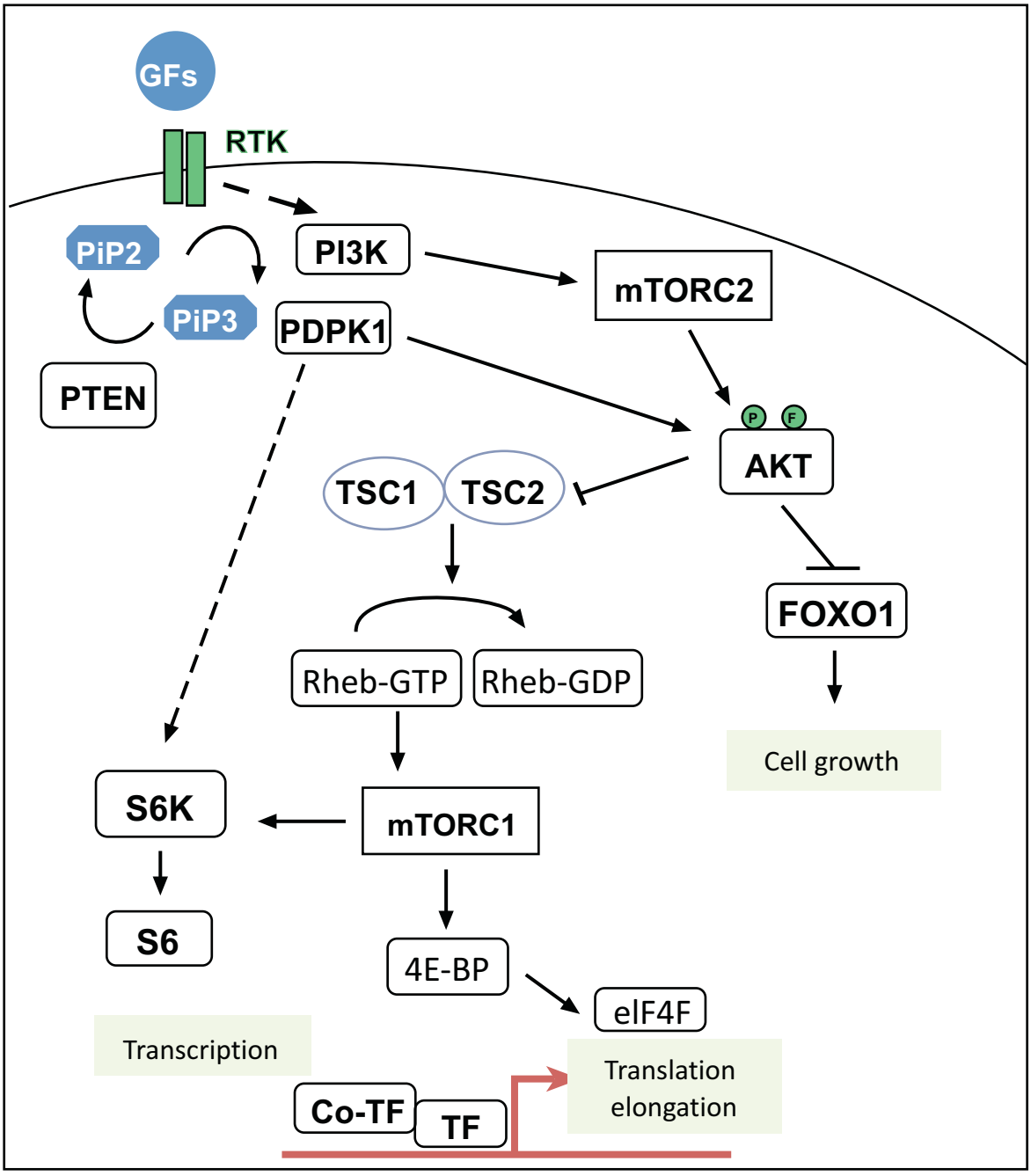
88. Thurneysen C, Opitz I, Kurtz S, et al. Functional inactivation of NF2/merlin in human mesothelioma. Lung Cancer. 2009; 64: 140-7.
89. Yao E, Chuang PT. Hedgehog signaling: From basic research to clinical applications. Journal of the Formosan Medical Association = Taiwan yi zhi. 2015.
90. Lim CB, Prele CM, Cheah HM, et al. Mutational analysis of hedgehog signaling pathway genes in human malignant mesothelioma. PLoS ONE. 2013; 8: e66685.
91. Li H, Lui N, Cheng T, et al. Gli as a novel therapeutic target in malignant pleural mesothelioma. PLoS ONE. 2013; 8: e57346.
92. Shi Y, Moura U, Opitz I, et al. Role of hedgehog signaling in malignant pleural mesothelioma. Clin Cancer Res. 2012; 18: 4646-56.
93. You M, Varona-Santos J, Singh S, et al. Targeting of the Hedgehog signal transduction pathway suppresses survival of malignant pleural mesothelioma cells in vitro. J Thorac Cardiovasc Surg. 2014; 147: 508-16.
94. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annual review of cell and developmental biology. 2004; 20: 781-810.
95. Kobayashi M, Huang CL, Sonobe M, et al. Intratumoral Wnt2B expression affects tumor proliferation and survival in malignant pleural mesothelioma patients. Experimental and therapeutic medicine. 2012; 3: 952-8.
96. Fox SA, Richards AK, Kusumah I, et al. Expression profile and function of Wnt signaling mechanisms in malignant mesothelioma cells. Biochem Biophys Res Commun. 2013; 440: 82-7.
97. Mazieres J, You L, He B, et al. Wnt2 as a new therapeutic target in malignant pleural mesothelioma. Int J Cancer. 2005; 117: 326-32.
98. Kashiwakura Y, Ochiai K, Watanabe M, et al. Down-regulation of inhibition of differentiation-1 via activation of activating transcription factor 3 and Smad regulates REIC/Dickkopf-3-induced apoptosis. Cancer Res. 2008; 68: 8333-41.
99. Anani W, Bruggeman R, Zander DS. beta-catenin expression in benign and malignant pleural disorders. Int J Clin Exp Pathol. 2011; 4: 742-7.
100. Gee GV, Koestler DC, Christensen BC, et al. Downregulated microRNAs in the differential diagnosis of malignant pleural mesothelioma. Int J Cancer. 2010; 127: 2859-69.
101. Liu J, Shaik S, Dai X, et al. Targeting the ubiquitin pathway for cancer treatment. Biochim Biophys Acta. 2014; 1855: 50-60.
102. Cooper J, Li W, You L, et al. Merlin/NF2 functions upstream of the nuclear E3 ubiquitin ligase CRL4DCAF1 to suppress oncogenic gene expression. Science signaling. 2011; 4: pt6.
103. Li W, Cooper J, Zhou L, et al. Merlin/NF2 loss-driven tumorigenesis linked to CRL4(DCAF1)-mediated inhibition of the hippo pathway kinases Lats1 and 2 in the nucleus. Cancer Cell. 2014; 26: 48-60.
104. Laplante M, Sabatini DM. mTOR signaling at a glance. J Cell Sci. 2009; 122: 3589-94.
105. Favoni RE, Florio T. Combined chemotherapy with cytotoxic and targeted compounds for the management of human malignant pleural mesothelioma. Trends in pharmacological sciences. 2011; 32: 463-79.
106. Stahel RA, Weder W, Felley-Bosco E, et al. Searching for targets for the systemic therapy of mesothelioma. Ann Oncol. 2015.
107. Mathy A, Baas P, Dalesio O, et al. Limited efficacy of imatinib mesylate in malignant mesothelioma: a phase II trial. Lung Cancer. 2005; 50: 83-6.
108. Remon J, Reguart N, Corral J, et al. Malignant pleural mesothelioma: new hope in the horizon with novel therapeutic strategies. Cancer Treat Rev. 2015; 41: 27-34.

109. Ou SH, Moon J, Garland LL, et al. SWOG S0722: phase II study of mTOR inhibitor everolimus (RAD001) in advanced malignant pleural mesothelioma (MPM). J Thorac Oncol. 2015; 10: 387-91.
110. Kaelin WG, Jr. The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer. 2005; 5: 689-98.
111. Shapiro IM, Kolev VN, Vidal CM, et al. Merlin deficiency predicts FAK inhibitor sensitivity: a synthetic lethal relationship. Science translational medicine. 2014; 6: 237ra68.
112. Felley-Bosco E, Stahel R. Hippo/YAP pathway for targeted therapy. Translational lung cancer research. 2014; 3: 75-83.
113. Favoni RE, Daga A, Malatesta P, et al. Preclinical studies identify novel targeted pharmacological strategies for treatment of human malignant pleural mesothelioma. British journal of pharmacology. 2012; 166: 532-53.
114. Davidson B. Prognostic factors in malignant pleural mesothelioma. Hum Pathol. 2015.
115. Ceresoli GL, Zucali PA. Anti-angiogenic therapies for malignant pleural mesothelioma. Expert Opin Investig Drugs. 2012; 21: 833-44.
116. Galateau-Salle F, Gilg Soit Ilg A, Le Stang N, et al. [The French mesothelioma network from 1998 to 2013]. Annales de pathologie. 2014; 34: 51-63.
117. Thomas RK, Baker AC, Debiasi RM, et al. High-throughput oncogene mutation profiling in human cancer. Nat Genet. 2007; 39: 347-51.
118. Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev. 2011; 75: 50-83.
119. Pan D. The hippo signaling pathway in development and cancer. Dev Cell. 2010; 19: 491-505.
120. Sekido Y. Inactivation of Merlin in malignant mesothelioma cells and the Hippo signaling cascade dysregulation. Pathol Int. 2011; 61: 331-44.

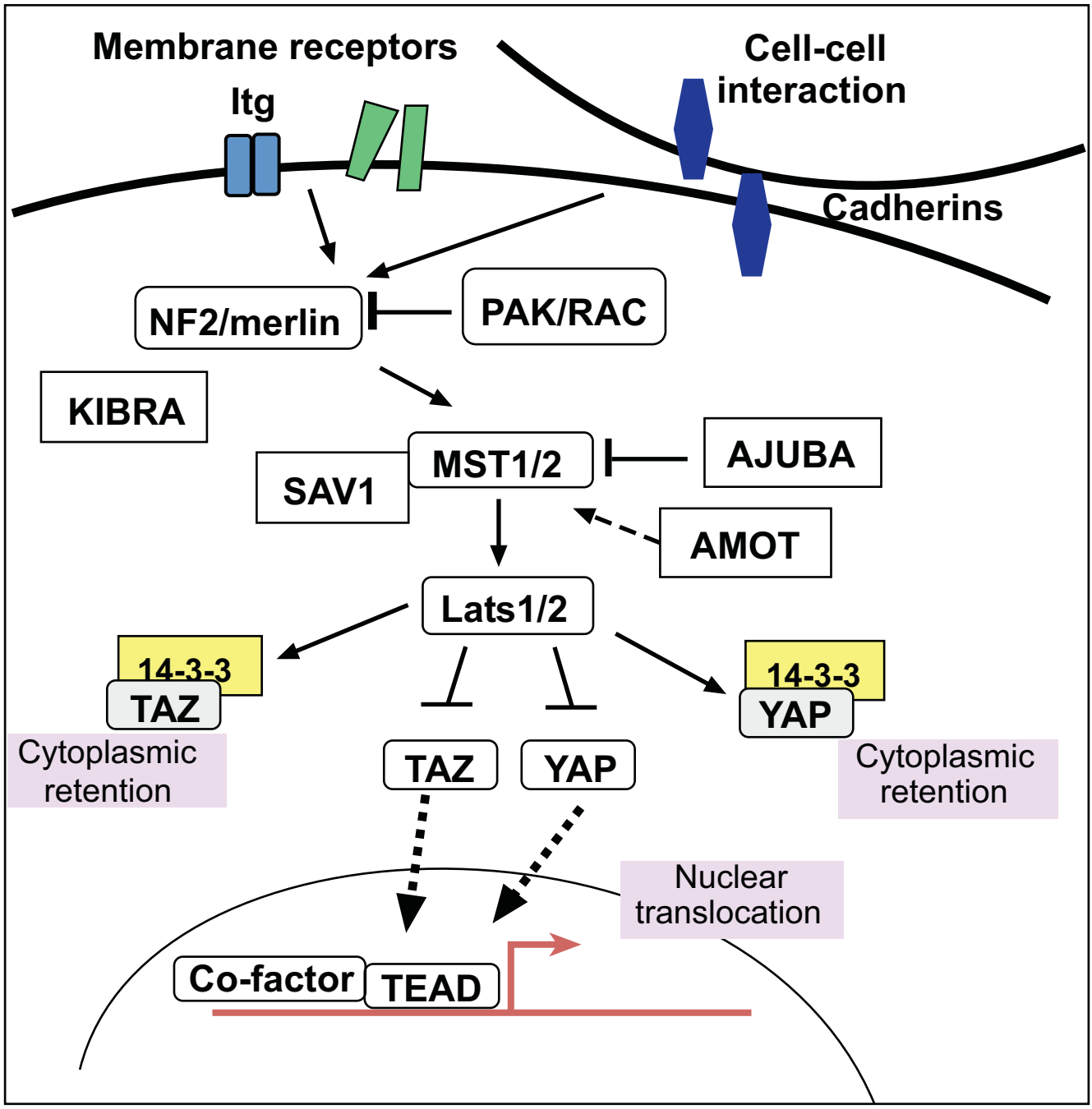
Improved Fig-1



Improved Fig-2



Improved Fig-3



Improved Fig-4