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Didier Jean, Julien Daubriac, Françoise Le Pimpec-Barthes, Françoise Galateau-Salle, Marie-Claude Jaurand

▶ To cite this version:

Didier Jean, Julien Daubriac, Françoise Le Pimpec-Barthes, Françoise Galateau-Salle, Marie-Claude Jaurand. Molecular changes in mesothelioma with an impact on prognosis and treatment. Archives of Pathology and Laboratory Medicine, College of American Pathologists, 2012, 136 (3), pp.277-293. 10.5858/arpa.2011-0215-RA . inserm-02478588

HAL Id: inserm-02478588
https://www.hal.inserm.fr/inserm-02478588

Submitted on 14 Feb 2020

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1

2 **MOLECULAR CHANGES IN MESOTHELIOMA WITH AN IMPACT ON PROGNOSIS**

3 **AND TREATMENT**

4

5 **Abstract (250 words):**

6

7 Over recent decades, genetic and epigenetic abnormalities have been investigated in malignant pleural
8 mesothelioma (MPM) by studying gene mutations, DNA methylation, gene and miRNA expression
9 profiling. These researches have improved patients' outcomes by increasing our level of confidence in
10 MPM diagnosis and prognosis.
11 Molecular changes in MPM consist in altered expression, activation or inactivation of critical genes in
12 oncogenesis, especially tumor suppressor genes at the *INK4* and *NF2* loci. Deregulation of signaling
13 pathways related to differentiation, survival, proliferation, apoptosis, cell cycle control, metabolism,
14 migration and invasion have been demonstrated in complementary studies. Activation of membrane
15 receptor tyrosine kinase has been frequently observed. More recent data have indicated the presence of
16 alterations that could be targeted at a global level (methylation). Molecular analyses of series of MPM
17 cases showed that defined alterations are generally present in MPM subsets, consistent with inter-
18 individual variations of molecular alterations. This suggests that identification of patient subgroups
19 will be essential in order to develop more specific therapies.
20 Some of the findings have already been used as the basis for several studies testing various targeted
21 clinical approaches mainly on specific receptor tyrosine kinases, but mostly with limited success.
22 Various experimental researches have been also developed, especially to abolish proliferation and
23 trigger apoptosis in MPM cells. Further basic research studies are needed to predict a positive
24 response in MPM, in order to avoid a rapidly unfavorable course and to prevent wasting resources
25 with inappropriate treatments. Demonstration of multiple alterations present in MPM should
26 encourage research into combined or more global therapies.

27

28

29 **1. Introduction**

30 Over recent decades, various studies have been conducted to define the molecular characteristics of
31 malignant mesothelioma (MM) cells. Genome-wide array-based approaches have allowed progress in
32 MM research by identifying changes at the genetic and epigenetic levels. Genetic and epigenetic
33 abnormalities have been investigated by identification of gene mutations, copy number changes, DNA
34 methylation, and gene and miRNA expression profiling. The development of biological resources,
35 frozen tissue and serum banks, tissue arrays and virtual banks, has also provided efficient tools to
36 characterize MM cells and identify various types of tissue and serum markers. Reviews on genomic
37 abnormalities and signal transduction dysregulation have been previously published [1-3]. The goal of
38 this paper is to summarize the molecular changes in MM, focusing on more recent advances in
39 malignant pleural mesothelioma (MPM), and discussing the level of confidence and limitations of
40 these results, their impact on prognosis and treatment and the future research that is required to fill the
41 gaps and enhance the benefit of basic research to improve the patient's outcome.

42

43 **2. Genomic and epigenetic changes in mesothelioma**

44 Genomic and epigenetic changes of potential interest for MPM histology, diagnosis and prognosis are
45 described in **Table 1**.

46 **2.1. Chromosomal alterations**

47 Genomic alterations in human MPM have been previously reported in numerous studies based on
48 various methods: cytogenetic analysis of standard karyotype, classical comparative genomic
49 hybridization (CGH), CGH array, single nucleotide polymorphism (SNP) array and representational
50 oligonucleotide microarray analysis (ROMA). Cytogenetic studies first demonstrated that numerous
51 chromosomal abnormalities are associated with MPM, including both various structural and numerical
52 changes, and recurrent alterations [4-5]. These earlier studies have already been reviewed in detail [1-2].
53 **Table 2** shows the recurrent regions of chromosomal alterations reported in recent studies using high-
54 throughput analyses. MPM cell cultures and primary tumors both share similar patterns of
55 chromosomal alterations. However, the frequency of alterations in some particular chromosomal

56 regions is generally higher in cultured cells, likely due to the presence of normal cells in tumor
57 samples as mentioned by several authors. Losses of chromosomal regions are always more common
58 than gains. Frequent losses are localized on chromosomes 1p, 3p, 4q, 6q, 9p, 13q, 14q, and 22q and
59 gains involve chromosomes 1q, 5p, 7p, 8q and 17q [6-12]. A recent large-scale analysis of gene mutations
60 based on second-generation sequencing in one tumor specimen confirmed the presence of numerous
61 DNA rearrangements in MPM [13].

62 ***Chromosomal alterations and clinicopathological features.*** Differences in genomic alterations have
63 been described in MPM according to the histological subtype or the patient's asbestos exposure status.
64 Although recurrent regions of chromosomal alterations are roughly similar between epithelioid and
65 sarcomatoid MPM, significant differences in the frequency of genomic alterations have been observed,
66 such as losses in chromosomal regions 3p14-p21, 8p12-pter, and 17p12–pter or gain in 7q [6].
67 Experimental studies have shown that asbestos fibers induce chromosomal abnormalities in normal
68 human mesothelial cells [14-15]. Significant correlations have been described between high contents of
69 asbestos fibers in lung tissue and partial or total losses of chromosomes 1, 4 and 9, and chromosomal
70 rearrangements involving a breakpoint at 1p11-p22 [16-17]. More recently, comparison between recurrent
71 altered regions in asbestos-exposed and non-exposed patients showed a significant difference in the
72 14q11.2-q21 region, which was also lost in fiber-induced murine mesothelioma [12].

73 ***Chromosomal alterations and diagnosis.*** None of the individual genomic aberrations observed are
74 specific for MPM, as they are also found in other types of tumors. However, some of these genomic
75 aberrations could be used to distinguish benign mesothelial proliferations from MPM. This is the case
76 for the deletion involving the 9p21.3 locus, the site of the cyclin-dependent kinase inhibitor 2A gene
77 (*CDKN2A*) which is one of the most frequent alterations in MPM and is often homozygous. Detection
78 of *CDKN2A* deletion by fluorescence in-situ hybridization (FISH) has therefore been evaluated for the
79 diagnosis of MPM [18-20]. CGH analysis has also been used in an attempt to distinguish MPM from
80 adenocarcinoma and large-cell anaplastic carcinoma of the lung. The frequency of several genomic
81 alterations can be used to differentiate mesothelioma from lung carcinoma with a sensitivity and
82 specificity of 89% and 63%, respectively [21]. It has also been suggested that CGH analysis could be
83 useful to distinguish sarcomatoid MPM from other types of spindle cell tumors of the pleura [22].

84 ***Chromosomal alterations and patient outcome.*** Correlations between patient survival and
85 chromosomal imbalance have also been studied. Chromosome copy number and alterations of the
86 short arm of chromosome 7 have been reported to be inversely correlated with survival [16, 23].
87 Univariate and multivariate analyses in a larger number of MPM showed that homozygous *CDKN2A*
88 deletion, detected by FISH analysis, is a significant independent adverse prognostic factor [24-25].
89 Classification of MPM patients into two groups defined by short-term (less than 12 months) and long-
90 term recurrence after surgery also suggested an association between 9p21.3 deletion encompassing the
91 *CDKN2A* locus and the short-term group [9]. In the same ROMA analysis, chromosomal instability
92 corresponding to the number of genomic alterations was shown to be higher in MPM patients
93 characterized by a shorter time to relapse [9]. In deciduoid MPM, a variant of epithelioid MPM, survival
94 was also found to be longer in patients with a smaller number of losses [26]. Interestingly, a correlation
95 was demonstrated between chromosomal instability and tumorigenicity of human mesothelioma
96 xenografts in nude mice [12]. These data indicate a correlation between the number of genomic
97 alterations and the aggressive behavior of MPM and further studies are needed to determine whether
98 chromosomal instability can be used as a prognostic factor.
99 Data on chromosome imbalance could also be useful to design new treatment strategies: a relevant
100 example targets the methylthioadenosine phosphorylase (*MTAP*) gene. Homozygous co-deletion of the
101 *MTAP* gene and the *CDKN2A* gene has been observed in the majority of pleural mesotheliomas [24]. The
102 *MTAP* gene is a key enzyme in the salvage pathway of AMP synthesis complementary of the *de novo*
103 purine biosynthesis pathway. Inhibitors of *de novo* purine biosynthesis induced selective killing of
104 *MTAP*-negative cells in culture [27]. One clinical trial on MPM using L-alanosine showed that this
105 inhibitor was ineffective at the dose used [28]. Further studies are necessary to conclude on the value of
106 this treatment strategy.
107 Genomic alteration studies have already contributed to our knowledge on the mechanisms of
108 mesothelial carcinogenesis, especially by identifying or confirming the involvement of tumor
109 suppressor genes (TSG) such as *CDKN2A* in MPM. They have also identified potential markers for
110 diagnosis, prognosis and treatment. New genes of interest could be identified by using technologies
111 providing more precise localization of altered chromosomal regions and, especially, by performing

112 integrated mining of genomic data linked with epigenetic, miRNA profiling, and transcriptomic data
113 in the same cultured cells or primary tumors.

114 **2.2. DNA methylation**

115 Numerous genes have been shown to be downregulated in mesothelioma cells by epigenetic regulation
116 such as DNA methylation of their transcriptional promoters. These changes dysregulate several
117 signaling pathways, including the Wnt pathway, in which several negative regulators are silenced by
118 hypermethylation [29-32]. The global epigenetic profile determined by high-throughput methylation
119 analysis differs between MPM and normal pleura indicating that MPM, like other cancers, have
120 aberrant CpG island methylation [33-34]. Gene profiles of hypermethylation also differ between MPM
121 and other tumors [33-37]. These data support the hypothesis that a specific DNA methylation program is
122 induced during mesothelial carcinogenesis.

123 ***DNA methylation and clinicopathological features.*** DNA methylation of gene loci in MPM is
124 dependent on age, ethnic origin, histological subtype and asbestos exposure and could explain
125 discrepancies between the frequencies of DNA methylation in published studies as well as the
126 experimental method used to detect it. Age-dependent changes in DNA methylation have been
127 reported in the literature [38]. An age-associated increase of DNA methylation has been reported in
128 MPM patients [39]. The methylation status of the insulin growth factor binding protein *IGFBP2* and
129 bone morphogenetic protein *GDF10* loci has also been shown to be significantly higher in MPM from
130 Japanese patients than in USA patients [40-41].

131 The frequencies of DNA methylation of TRAIL receptors (*TNFRSF10C* and *TNFRSF10D*) and tumor
132 suppressor *RASSF1* have been reported to be significantly higher in epithelioid MPM than in
133 sarcomatoid MPM histological subtypes [35, 42]. These data were not confirmed in another study for
134 *RASSF1*, but methylation of another gene, *MT2A*, encoding heavy metal binding protein was shown to
135 differ between these two histological subtypes [43]. High-throughput methylation analysis showed that
136 epithelioid and sarcomatoid mesotheliomas had differential methylation at 87 CpG loci [44].

137 A significant association between asbestos exposure and DNA methylation at the *MT1A*, and *MT2A*
138 gene loci has also been described in MPM [43]. Methylation of TSG loci, *APC*, *CCND2*, *CDKN2A*,
139 *CDKN2B*, *HPPBP1* and *RASSF1* was studied in comparison with asbestos exposure. Only DNA

140 methylation at the *RASSF1* locus was correlated with an increased number of asbestos bodies in the
141 patient's lung. A trend towards an increasing number of methylated cell cycle control genes and
142 increasing asbestos body counts was also observed [39]. Recently, high-throughput methylation analysis
143 confirmed distinct methylation profiles between MPM from asbestos-exposed and non-exposed
144 patients and a significant positive association between asbestos fiber burden and methylation status of
145 *CDKN2A*, *CDKN2B*, *RASSF1* and *MT1A* in about one hundred other loci [33].
146 ***DNA methylation and diagnosis.*** DNA methylation could be useful for the diagnosis of MPM.
147 Differences in the frequency of DNA methylation have been described for several genes between
148 MPM and lung adenocarcinoma or non-malignant pulmonary tissues [35-36, 43]. High-throughput
149 methylation analysis covering several thousand CpG islands confirmed the potential value of DNA
150 methylation profile to distinguish MPM from these two other tissues. Accurate diagnosis could be
151 based on the global methylation profile, but further studies on larger populations are needed before
152 using a limited number of hypermethylated loci [33-34, 44]. It was recently suggested that DNA
153 methylation at the three loci *TMEM30B*, *KAZALD1* and *MAPK13,* could be useful in the differential
154 diagnosis of MPM [34].
155 ***DNA methylation and patient outcome.*** DNA methylation status of individual genes such as the
156 transcriptional repressor *HIC1*, the pro-apoptotic protein *PYCARD*, the tumor suppressor *LZTS1* and
157 the transporter *SLC6A20* has been associated with either a good or poor prognosis [43, 45]. High-
158 throughput methylation analysis showed that patients with MPM with a low frequency of DNA
159 methylation had a significantly longer survival [34]. Furthermore, classification based on the methylation
160 profile of patients undergoing surgical resection before any other treatment identified subgroups
161 characterized by different clinical outcomes [33]. These data highlight the potential prognostic value of
162 DNA methylation analysis.
163 In view of the aberrant epigenetic events observed in MPM, the clinical value of histone deacetylase
164 inhibitors (HDACi) has been studied in preclinical models using MPM cell lines and mouse xenograft
165 models. Phase I and II clinical trials in patients with MPM have been conducted using several different
166 HDACi, either alone or in combination with conventional chemotherapy. The encouraging results of

167 these early-phase trials led to a phase III, multicenter, randomized, placebo-controlled study of one of
168 these HDACi in patients with advanced MPM [46].
169 Like chromosome imbalance studies, epigenetic analyses identified genes or pathways potentially
170 involved in mesothelial carcinogenesis such as the Wnt pathway. At the present time, only the global
171 methylation profile appears to be relevant for diagnosis or to evaluate the patient's survival, thereby
172 limiting its clinical applications. Furthermore, epigenetic regulation mechanisms in MPM have been
173 mainly studied in terms of DNA methylation, but insufficient data are available on regulation of
174 histone modifications, despite their crucial role to maintain chromatin stability. Such data are
175 necessary to support clinical trials based on HDACi.

176 **2.3. miRNA expression**

177 Micro-RNAs (miRNAs) are emerging as key players in the control of a multitude of biological
178 processes and are aberrantly expressed in several tumors including MPM. MiRNA expression has
179 been shown to differ between MPM tumors and normal pleura [47] and between MPM cell lines and
180 immortalized mesothelial cells [48]. MPM histological subtypes also demonstrate a specific miRNA
181 expression pattern [47-48]. Potential targets of these deregulated miRNAs include TSGs, oncogenes and
182 genes involved in specific signaling pathways [47, 49]. However, a link between miRNA expression and
183 mesothelial carcinogenesis has been demonstrated by experimental analysis for only miR-31 and miR-
184 29c. MiR-31 is frequently lost in MPM due to its chromosomal location at 9p21.3, and miR-29c
185 expression is higher in epithelial MPM of patients with a good prognosis (time to progression greater
186 than one year). Overexpression induced by transfection of these two miRNAs decreased *in vitro*
187 proliferation, migration, invasion, and colony formation of the same two MPM cell lines [50-51].
188 ***MiRNA and diagnosis.*** MiRNAs have been proposed as diagnostic tools. Downregulation of seven
189 miRNAs (miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-205 and miR-429) was shown to
190 be characteristic of MPM regardless of their histological subtypes, and could be used to distinguish
191 MPM from adenocarcinoma [49]. Another study demonstrated that the selection of three miRNAs (miR-
192 193, miR-200c and miR-192) could distinguish MPM from various carcinomas invading the lung and
193 pleura [52].

194 ***MiRNA and patient outcome.*** Some recent data suggest that miRNA expression could also be used as
195 prognostic tools, as downregulation of both miR-17 and miR-30c in sarcomatoid MPM and
196 upregulation of miR-29c in epithelioid MPM were significantly associated with better patient survival
197 [48, 51].
198 MiRNA expression analysis is a promising tool to improve the accuracy of diagnosis and could be
199 complementary to immunohistochemical markers. This analysis also opens up new perspectives for
200 the prognostic assessment of MPM in the near future. However, a better knowledge of miRNA
201 signatures of MPM is still necessary, as certain discrepancies have been observed between miRNA
202 profiling studies. Functional studies in cultured cells and animal models are also needed to determine
203 the precise contribution of miRNAs to mesothelial carcinogenesis and whether or not they can be used
204 as potential targets for anticancer therapy.
205

206 **3. Molecular changes in malignant mesothelioma**

207 **3.1. Gene mutations**

208 Knowledge of gene mutations provides insight into specific mechanistic pathways that can be altered
209 in MPM cells, opening the way for future targeted therapies. A number of genes are known to be
210 recurrently mutated in MM.
211 ***TP53.*** The *TP53* gene, a TSG located at 17p13.1 that controls cell cycle and apoptosis, is mutated in
212 many types of human cancers. Its mutation frequency is about 20% in human MPM, a fairly low rate
213 in comparison with other human cancers [3]. Point mutations are the main types of alterations in MM.
214 Six point mutations are indicated in the IARC p53 database, five missense mutations and one stop
215 mutation (http://p53.free.fr/Database/p53_database.html). In a study conducted to determine the
216 frequency of simian virus 40 (SV40) in Egyptian MM patients, altered p53 and pRb expressions were
217 found in 57.5% and 52.5% of patients, respectively, with no p53 mutation [53]. These authors assessed
218 the prognostic impact of altered expression of *RB1* and *TP53* gene status. Univariate analysis showed
219 a significant correlation between overall survival and p53 overexpression (P = 0.05). Although
220 debated, SV40 has been associated with MM, and is assumed to act as a cofactor of asbestos in
221 carcinogenesis. In some MM, p53 protein function could be inactivated after binding to the Large T

222 (Tag) SV40 protein, but SV40Tag expression in MM remains controversial [54]. In a recent study, no
223 expression of SV40-specific miRNA was detected in human malignant pleural mesothelioma (MM)
224 samples [55].
225 No relationship has yet been established between *TP53* mutation and clinical impact. The uncertainties
226 concerning p53 status in MPM appear to make it difficult to establish relationships between p53 status
227 and prognosis and/or treatment.
228 **Neurofibromatosis 2 (*NF2*).** The *NF2* TSG, located on 22q12 was one of the first TSGs shown to be
229 inactivated in MPM [56-57]. Early conventional cytogenetic studies reported a loss of chromosome 22 in
230 human MM [58-59]. *NF2* inactivation is frequent, with rates ranging from 20 to 60% depending on the
231 material used, tissue or cells, and the method (classical CGH, DNA sequencing…). Various types of
232 lesions have been described, including small and large deletions, homozygous deletions, nonsense and
233 missense mutations. The role of *NF2* in mesothelial carcinogenesis will be described in the paragraph
234 on the hippo pathway.
235 ***INK4 locus.*** A second recurrent gene alteration occurring in human MM consists of inactivation of
236 genes located at the *CDKN2A* locus. The *CDKN2A* locus encodes both $p16^{INK4A}$ and $p14^{ARF}$ which
237 share common exons, but no common amino acid sequence. Alterations at this locus have been
238 demonstrated by DNA sequencing, FISH and methylation as reported above. The most frequent
239 alteration is homozygous deletion in about 70% of cases [3]. This alteration is related to asbestos
240 exposure in lung cancer and is also observed in mesotheliomas induced by mineral fibers in mice [60-61].
241 The *CDKN2B* gene adjacent to *CDKN2A* is also frequently codeleted in MPM, but at a lower
242 frequency [62].
243 FISH detection of *CDKN2A* deletion has been proposed to differentiate between reactive and
244 malignant mesothelial cells on paraffin-embedded sections and effusion cytology [19-20, 63]. Several
245 authors have reported that loss of the encoded protein $p16^{INK4A}$, as assessed by immunohistochemistry
246 and FISH analyses confirmed by gene profiling microarray studies, is associated with lower survival
247 [25, 64-67].
248 ***CTNNB1.*** The beta-catenin status in MPM cells was reported in one study of 2 primary tumors and 8
249 cell lines in which one homozygous deletion was found in one cell line [68]. A modification of the

250 subcellular localization of beta-catenin was reported in another study, consistent with activation of
251 beta-catenin as transcriptional cofactor [69].
252 There is now a general consensus that several TSGs are frequently altered in MPM: *NF2*, *CDKN2A*,
253 *CDKN2B*, and, less frequently, *TP53*. In contrast, no recurrent oncogene mutation has yet been
254 identified in MPM.

255 **3.2. Gene expression profiling**

256 Data from array-based studies indicate deregulation of gene expression in MPM. These studies were
257 conducted in order to improve histological classifications and prognosis (**Tables 3-5**). These data were
258 recently reviewed by Gray *et al.* [70].
259 ***Comparison with normal cells.*** Early studies were carried out with MPM cell lines compared to
260 normal pleural mesothelial cells (**Table 3**). Using a cDNA array including 588 genes : 26 genes that
261 play a role in signaling pathways (*MAP3K14*/*NIK*, a serine/threonine protein-kinase that stimulates
262 NF-kappaB activity; *JAG1*/*JAGGED1* a ligand of the notch1 receptor), cell cycle (cyclin D1, *CCND1;*
263 cyclin D3*, CCND3;* CDK phosphatase, *CDC25B)*, cell growth (fibroblast growth factor 3 and 12,
264 *FGF3* and *FGF12;* platelet-derived growth factor receptor B*, PDGFRB*) and DNA damage repair
265 (*XRCC5*/*Ku80*) were overexpressed, and 13 genes encoding growth factors such as *FGF1* and *FGF7*
266 (fibroblast growth factor 1 and 7), *CCND2* (a regulatory subunit of cyclin-dependent kinases, involved
267 in cell cycle G1/S transition), *KDR/VEGFR2* (vascular endothelial growth factor receptor 2), *PDGFRA*
268 (platelet-derived growth factor receptor), *RARß* (retinoic acid receptor ß2) and genes encoding
269 proteins involved in cell adhesion, motility and invasion were underexpressed [71].
270 Differentially expressed genes were also related to tumor invasiveness and resistance to anticancer
271 defenses [72]. In another study, in a series of 14 differentially expressed genes, 8 were upregulated: *CFB*
272 (complement factor B), *FTL* (ferritin light polypeptide), *IGFBP7* (insulin-like growth factor binding
273 protein 7), *RARRES1* (retinoic acid receptor responder 1), *RARRES2* (retinoic acid receptor responder
274 2), *RBP1* (retinol-binding protein 1), *SAT* (spermidine/spermine N1-acetyltransferase) and *TXN*
275 (thioredoxin), while 6 were downregulated: *ALOX5AP* (arachidonate 5-lipoxygenase-activating
276 protein), *CLNS1A* (chloride channel nucleotide-sensitive 1A), *EIF4A2* (eukaryotic translation

277 initiation factor 4A2), *ELK3* (ETS-domain protein, SRF accessory protein 2), *DF2/REQ* (apoptosis
278 response zinc finger gene), and *SYPL* (synaptophysin-like protein) [73].
279 The expression of 588 cancer-related genes was screened in 16 MPM tumors using normal mesothelial
280 cell lines and pleural mesothelium as references [74]. Eleven genes, *COL1A2*, *COL6A1* (collagen), *tPA*,
281 *MMP9* (protease), *CDH3*, *L1CAM*, *ITGB4, PLXNA3/PLXN3*, *KRT14/K14* (cell adhesion or cell
282 surface molecule), *SEMA3C* (semaphorin), and *CXCL10/INP10* (chemokine), were overexpressed in
283 MPM [74].
284 Microarray expression data of 40 MM tumor specimens, 4 normal lung specimens and 5 normal pleura
285 specimens were reported by Gordon *et al.* [75]. These authors identified genes that were significantly
286 differentially expressed in tumors compared to normal samples. There were 328 overexpressed genes
287 and 311 underexpressed genes in MM tumors. These authors proposed 3 novel candidate oncogenes
288 *NME2* (nucleoside diphosphate kinase), *EID1/CRI1* (regulator of EP300 and RB1) and the *PDGFC*
289 (platelet-derived growth factor), and one candidate tumor suppressor *GSN* (cytoskeleton regulator) in
290 MPM [75].
291 In another study, MM tissue specimens from 16 patients were compared to 4 control pleural tissue
292 samples using cDNA microarray filters with 4132 clones [76]. Interestingly, upregulation of many genes
293 involved in the glycolysis pathway and the Krebs cycle was observed, in agreement with the ability of
294 cancer cells to rely on aerobic glycolysis, the “Warburg effect”. Other upregulated genes were
295 involved in mRNA translation and cytoskeletal reorganization pathways. These authors also identified
296 gp96 (adenotin, GRP94, *HSP90B1*), LRP (lung-related resistance protein, *MVP*), galectin-3 binding
297 protein (*LGALS3BP*) and Mr 67,000 laminin receptor (*RPSA*), but this last gene was not expressed on
298 tumor cells, but on infiltrating vessels.
299 More recently, Crispi *et al*. [77] compared MPM tissues from 9 patients to normal pleural tissues from
300 patients undergoing resection for a non neoplastic disease. Components of the condensin complex
301 (e.g. *BRRN1*, *CNAP1*, *NCAPD3*) and members of the kinesin family (e.g. *KIF14*, *KIF23*, *KIFC1*) were
302 upregulated. Other upregulated genes were related to cell proliferation and its control such as cyclin-
303 dependent kinase *CDK1/CDC2*, cyclin genes *CCNA2, CCNB1, CCNB2* and *CCNL2*, the *DLG7*

304 component of the mitotic apparatus, the checkpoint kinase involved in response to DNA damage
305 *CHEK1/CHK1,* and *BUB1* and *MAD2L1,* components of the spindle checkpoint.
306 Romagnoli *et al.* [78] used a quantitative polymerase chain reaction (PCR)-based, low-density array
307 focusing on genes involved in cell cycle regulation. They studied 45 MPM tumor samples and normal
308 tissue samples obtained by pleural wiping of surgical samples with no evidence of pleural disease.
309 Several genes were differentially expressed: either downregulated in cancer cells (*UBE1L, CCND2*),
310 or upregulated (*CHEK1/CHK1, CCNH, CCNB1, p18-CDKN2C, CDC2, FOXM1, CDC6*).
311 Overexpression of the cell cycle regulator Chk1 was confirmed in an independent set of 87 MM by
312 immunohistochemistry using tissue microarrays. In another study, gene expression studies confirmed
313 by reverse transcriptase (RT)-PCR showed downregulation of the putative TSG *FUS1/TUSC2* and the
314 cytokine *OSM* (oncostatin M) compared to normal samples (matched normal peritoneum specimens) [9,
315 79]. Downregulation of *FUS1/TUSC2* and *PL6/TMEM115* was also observed in comparison with
316 matched normal pleura specimens [9].
317 ***Features of malignant mesothelioma cells related to MM histology.*** Several studies have provided
318 data on MM classification (**Table 4**). A microarray transcriptional profiling study of 10 MPM cell
319 lines and 4 MPM primary tumor specimens distinguished epithelial, sarcomatoid and biphasic MPM.
320 Upregulated genes included *ST14*, a gene encoding matriptase, and a membrane serine protease
321 degrading the ECM, overexpressed in epithelial MPM [80]. In the comparative study with normal cells
322 quoted above, *SEMA3C*, *ITGB4*, *CDH3* and *COL6A1* were highly expressed in the epithelioid MPM
323 subtype, *L1CAM*, *K14*, *INP10* were overexpressed in the mixed MPM subtype and *MMP9* and *PLXN3*
324 were overexpressed in the sarcomatoid MPM subtype [74]. Statistically significant distinct gene
325 expression patterns between epithelial and non-epithelial tumors were reported to be correlated with
326 distinctive subclasses from hierarchical clustering in a series of 40 MPM [75]. In a series of 99 tumors,
327 genes typical of epithelial differentiation, the cell-surface transmembrane proteins uroplakins 1B and
328 3B (*UPK1B* and *UPK3B*) and the protease kallikrein 11 (*KLK11*) were more highly expressed in
329 epithelioid MM [25]. Romagnoli *et al.* [78] compared epithelioid and nonepithelioid MPM using a
330 quantitative PCR-based low-density array. Two genes were overexpressed in epithelioid MPM, the
331 transcription factor *TFDP2* and the protooncogene *ABL1*, whereas the transcription factor *TWIST1*

332 was overexpressed in the nonepithelioid group. In an attempt to classify genes according to their
333 correlation with survival, more favorable genes were associated with epithelioid morphology and
334 unfavorable genes were associated with sarcomatoid type or epithelioid MM with poor outcome [25].
335 ***Features of malignant mesothelioma cells related to the outcome of MM patients.*** Other authors
336 have tried to improve prognostic assessment by using gene expression analyses (transcriptome and/or
337 quantitative RT-PCR) of MM primary tumors or cell lines (**Table 5**). In a study investigating
338 mesothelioma surgical specimens, calculation of three gene expression ratios, *KIAA0977*/*GDIA1*,
339 *L6*/*CTHBP*, and *L6*/*GDIA1* was found to be a good predictor of surgical treatment-related outcome.
340 Samples with geometric means greater than 1 and less than 1 were assigned to good-outcome and
341 poor-outcome groups, respectively [81]. In a cohort of 39 patients undergoing surgery, these authors
342 validated previous findings and identified new sets of gene expression ratios *CD9*/*KIAA1199*,
343 *CD9*/*THBD*, *DLG5*/*KIAA1199*, and *DLG5*/*THBD* allowing classification of tumors according to
344 patient outcome [82]. In a more recent study, Gordon *et al*. [83] investigated 120 consecutive patients with
345 malignant pleural mesothelioma treated by surgery. None of the patients had received preoperative
346 neoadjuvant chemotherapy or radiation therapy. By analyzing data for four genes, they defined three
347 ratios of gene expression (*TM4SF1/PKM2, TM4SF1/ARHDDIA, COBLL1/ARHDDIA*), which,
348 associated with other prognostic factors, were able to discriminate high-risk and low-risk patients.
349 A cohort of 1,153 samples from patients diagnosed with 11 distinct types of cancer including 17
350 patients with mesothelioma from the study by Gordon *et al.* [81] was investigated by microarray
351 analysis, looking for molecular signatures based on the polycomb group *BMI-1*-associated gene
352 expression pathway, a pathway essential for self-renewal of hematopoietic and neural stem cells [84].
353 Expression of the 11-gene signature was a powerful predictor of poor prognosis in cancer patients.
354 These 11 genes were *Gbx2*, *KI67*, *CCNB1*, *BUB1*, *KNTC2*, *USP22*, *HCFC1*, *RNF2*, *ANK3*, *FGFR2*,
355 and *CES1* [84].
356 Several studies have identified specific genes associated with patient outcome. In a study comparing
357 MM samples from patients with short-term recurrence after surgery (STR) and patients with longer
358 time to relapse (LTR), the cadherin *CDH2* was upregulated especially in the STR group. In contrast,

359 the chaperone protein *DNAJA1* showed reduced expression in the STR cohort. In addition, the authors
360 noted no discrimination between epithelial and biphasic histological types [9].
361 Aurora kinases A and B (*AURKA* and *AURKB*) are serine/threonine kinases that play an important role
362 in chromosome alignment, segregation and cytokinesis during mitosis. They were found to be
363 overexpressed in a study of 99 MPM [25]. The expression of aurora kinases and genes participating in
364 cell division and mitotic control was further investigated in 29 MPM [85]. Expressions of *AURKA* and
365 *AURKB* and related genes were correlated, and overexpression of *AURKB* determined by
366 immunohistochemistry was significantly correlated with poor outcome [85].
367 A correlation between metalloproteinase *MMP14* expression and overall survival was reported in one
368 study of 9 patients with MPM treated by standard thoracotomy for therapeutic purposes compared to 4
369 normal pleural samples [77]. High *MMP14* expression was associated with lower survival. This gene has
370 been proposed as a potential MPM biomarker. Upregulation of *MELK* (maternal embryonic leucine
371 zipper kinase) was associated with poor survival, confirming previous findings by Lopez-Rios *et al.* [25],
372 but *BTG2,* which plays a role in regulation of G1/S transition, was associated with different outcomes
373 in these 2 studies. Other genes, *BIRC5* an inhibitor of apoptosis, *KIF4A* an ATP-dependent
374 microtubule-based motor protein and *SEPT9*, a member of the septin family involved in cytokinesis
375 and cell cycle control, were upregulated and associated with poor prognosis [77]. In this study, a
376 favorable survival was associated with downregulation of transcription factor *WT1* in contrast with a
377 previous study, which associated long-term survival with upregulation of *WT1* [25].
378 Microarray analysis discriminated between normal and MM samples in a comparative study of 8
379 normal peritoneum and 7 stage I MM, subsequently validated on a large set of matched normal/MM
380 samples by RT-PCR. Intense overexpression of *HAPLN1* (hyaluronan and proteoglycan link protein
381 1), a protein of the extracellular matrix (ECM), was observed in MM samples. Immunostaining with
382 anti-HAPLN1 antibodies demonstrated that all MPM types (epithelial, mixed, and sarcomatoid) as
383 well as reactive mesothelium expressed this gene. Moreover, *HAPLN1* expression was negatively
384 correlated with time to progression and survival [86]. Functional studies using transfection assays
385 revealed that MM cells overexpressing full-length *HAPLN1* or its functional domains strongly
386 supported the protumorigenic role of *HAPLN1*.

387 A meta-analysis was carried out on published data on microarray analysis of gene expression profiles
388 in mesothelioma, glioma and prostate cancer [87]. Mesothelioma data were derived from the study by
389 Gordon *et al.* [81]. MM cases consisted of eight good responders who survived more than 17 months,
390 while 10 patients in the poor responder group survived less than 6 months. A list of genes generated
391 according to patient outcome showed similarities between the three types of cancers [87]. Thirteen highly
392 expressed genes and one gene expressed at low levels were identified as being equally related to poor
393 survival in the 3 types of cancers. These genes encode proteins of the ECM and regulators of ECM
394 assembly, and angiogenesis genes [87]. These results are consistent with a more aggressive state of
395 malignant cells, and a more deleterious tumor microenvironment. These results may be of interest for
396 combining tumor-specific and more global therapies.
397 An analysis of 6 MPM compared to normal visceral and parietal pleural tissues has focused on
398 differential gene expression and identification of pathways that could be related to the drug and
399 irradiation resistance of pleural MM [88]. Several genes encoding proteins known to control DNA
400 replication, cell cycle regulation and DNA repair were identified as over- or underexpressed in MPM
401 could account for MPM resistance mechanism to chemotherapies [88].
402 These studies show changes in the expression of genes involved in several regulatory pathways.
403 Discrimination between epithelioid and non-epithelioid MPM was reported in several studies without
404 apparent benefit for classification of MPM subtypes in comparison with classical histological analysis.
405 Other studies developed a gene ratio approach to predict outcome in patients having undergone
406 surgery. No extrapolation can be made to other therapeutic settings, such as chemotherapy, can be
407 made at the present time. Several specific genes were identified as potential predictors of patient
408 outcome. Although providing a number of candidate areas to kill cancer cells or abolish their growth,
409 these results need to be confirmed on a larger number of cases before proceeding to clinical
410 applications. An important issue is to determine the most pertinent individual approach in relation to
411 the various biological features of MM cells.

412 **3.3. Pathway regulation**

413 ***Receptor tyrosine kinases.*** Membrane receptor tyrosine kinases (RTKs) drive downstream cell
414 signaling to cell proliferation and cell cycle control, survival and differentiation [89]. Downstream

415 networks from RTKs can be activated by RTK mutation or sustained signaling by autocrine or
416 paracrine mechanisms, providing a useful context to therapeutically counter the effects of RTK
417 activation.

418 Epidermal growth factor receptor (*EGFR*) is generally not mutated in human MPM. However, in an
419 immunohistochemical study, EGFR was expressed in 44% of MPM cases [90]. EGFR protein status was
420 statistically significantly associated with a favorable prognosis, but was not an independent prognostic
421 factor, when compared to clinicopathological status [90]. A tissue array study was performed on
422 epithelioid tissue samples from 48 MPM cases for comparison between long-term survival and short-
423 term survival, associated with the expression of other proteins involved in the corresponding pathway
424 [91]. A relationship was found between EGFR expression and long-term survival, whereas PDGFR
425 signaling was more strongly associated with short-term survival [91]. In contrast, no relationship was
426 found between survival and EGFR protein or mRNA expression [92].

427 EGFR alteration cannot be considered to be critical in MPM at the present time, which could explain
428 why, although high EGFR expression is found in MPM, EGFR inhibitors, gefitinib and erlotinib, did
429 not induce any significant tumor response when applied in phase II studies in patients with MPM [93].
430 Response rates were situated between 0 and 4% and median overall survival was between 4.6 and
431 13.1 months in phase II trials including patients with either first-line chemotherapy failure or no
432 previous treatment [94-96].

433 *KIT/CD117* encodes a stem cell factor receptor. In MPM, *KIT* expression has mostly been studied by
434 immunohistochemistry, showing a low percentage of positive tumors [97]. No expression was detected
435 by RT-PCR in a study of 37 MPM [98]. *KIT* has not been shown to be characteristic of MPM at the
436 present time.

437 Vascular endothelial growth factor receptors (VEGFRs). Several immunohistochemical studies
438 demonstrated an enhanced expression of vascular endothelial growth factor (VEGF) in a large
439 proportion of MPM in comparison with non neoplastic specimens [99]. Contradictory results were found
440 regarding the correlation between VEGF expression and survival. VEGF was not identified as a
441 prognostic factor in studies of 52 and 37 MPM specimens, respectively [100-101]. In contrast, in a study of
442 40 MPM tissues, VEGF showed significant correlation with short survival, and was an independent

443 prognostic factor [102]. MPM cells express both VEGF and VEGFRs (fms-related tyrosine kinases, *FLT1*
444 and *FLT4*) and fetal liver kinase (*KDR/FLK1*) [103-106]. An autocrine role of VEGF has been suggested,
445 using neutralizing antibodies against VEGF or the VEGFR, or antisense oligonucleotides against
446 VEGF that significantly reduced MM cellular proliferation [105, 107]. VEGF expression can be regulated
447 by lipoxygenases. Human MPM cells, but not normal mesothelial cells, express a catalytically active
448 5-LO (arachidonate 5-lipoxygenase). A 5-LO antisense oligonucleotide potently and time-dependently
449 reduced VEGF mRNA and constitutive VEGF accumulation in the conditioned media of MPM cells
450 [108]. These results indicate that VEGF may have multiple effects, as a key regulator of MM growth via
451 activation of its tyrosine kinase receptors, and as promoter of tumor angiogenesis.
452 Despite unsuccessful early trials of anti-VEGF therapy, numerous clinical trials are testing the benefit
453 of VEGF inhibitors in combination with chemotherapy [95, 109].
454 <u>Platelet-derived growth factor receptors (PDGFRs)</u>. MM cell growth may be linked to autocrine or
455 paracrine stimulation by platelet-derived growth factor (PDGF), and the regulation by PDGF appears
456 to be complex in MM cells. Normal human mesothelial cells express low levels of PDGF-A mRNA
457 chain and the PDGF-B mRNA was not detectable [110]. These cells express PDGFR-A mRNA and
458 protein and had weak to undetectable levels of the PDGFR-B mRNA and protein [111]. In contrast,
459 human MM cells express high level of PDGF-A and PDGF-B, as well as PDGFR-B [110-111]. However,
460 expression of PDGFR-B is controversial and weak to undetectable levels were reported [110-113].
461 Nevertheless, an autocrine proliferation can be suggested in MM, as it may occur via binding of
462 homodimer of PDGF-B chains [114]. PDGF has been suggested as a regulatory factor for proliferation of
463 MM cells, either directly, or indirectly via the hyaluronan/CD44 pathway. Hyaluronan is an important
464 constituent of the extracellular matrix. PDGF-BB-stimulated normal human mesothelial cells express
465 both hyaluronan synthase and hyaluronan [115-116].
466 PDGF-A-stimulated autocrine loop does not seem to play a positive role in mesothelioma proliferation
467 *in vitro*, but nude mice injected with MM cells that over-express PDGF-A showed increased tumor
468 incidence and reduced latency period to tumor formation [117-118]. These data suggest that PDGF-A
469 could contribute to tumor formation via a paracrine mechanism to generate favorable environmental
470 conditions, e.g. by stimulating angiogenesis, for tumor proliferation [118].

471 Like EGFR targeted therapy, the PDGFRs inhibitor, imatinib mesylate, was ineffective in clinical
472 trials [119-120].
473 Insulin growth factor receptors (IGFRs). Human MM cells express IGF and IGFR [121]. IGF-I appears to
474 function as an autocrine growth stimulus in human mesothelial cells [122]. When activated, IGFR
475 phosphorylates multiple classes of signal transduction adaptators, including insulin receptor substrates
476 (IRS). IRS-1 was found to induce cell proliferation in response to IGF-1, whereas cell migration was
477 induced by IRS-2 [123]. In addition, various members of the insulin-like growth factor binding protein
478 (IGFBP) family have been investigated in MPM. IGFBPs form a complex with IGFR subunit and IGF,
479 and have been shown to either inhibit or stimulate the growth promoting effect of IGF. IGFBPs can be
480 either expressed or unexpressed in MM, modulating the aggressiveness of the MM phenotype [121, 124-
481 125].
482 Hepatocyte growth factor receptor (*MET*) is a proto-oncogene. Mutation in the *MET* gene appears to
483 be uncommon in MPM. No mutation was reported in a study of 20 cell lines [126], but 5 point mutations
484 and one deletion were identified in a series of 43 primary tumors and 7 cell lines [127]. The encoded
485 protein is involved in pathways regulating development, cell growth and survival, motility and
486 invasion. It is expressed in most MPM and in reactive mesothelium but not in normal mesothelial cells
487 [128-129]. Hepatocyte growth factor/Scattering factor (HGF/SF), the related Met ligand, is also expressed
488 in some but not all MPM cells. *In vitro* stimulation of MPM cells by HGF/SF increased spreading,
489 motility and/or invasiveness, but these effects were dependent on the cell line [127, 130-131]. Experimental
490 studies with cultured MPM cells demonstrated that inhibition of MET by RNA interference or protein
491 kinase inhibitor resulted in G1/S arrest and reduction of the activity of Akt and Erk1/2 signaling in
492 some cell lines [127, 131]. However, no correlation was found between levels of MET and ERK1/2
493 phosphorylation [126]. In the light of these results showing a tumor-dependent activation of HGF/MET
494 signaling, HGF/MET status could define various MPM subclasses.
495 The activation status of MET and other RTKs, EGFR family (Erb1, Erb2, Erb3), PDGF−A and
496 PDGFR−B was investigated in 20 MPM cell lines and 23 primary specimens of MPM, and the effect
497 of MET-specific inhibitors (MET-shRNA interference vector and RTK inhibitors) was investigated on

498 cell lines [126]. The results showed that inhibition of single RTK was not sufficient to obtain a tumor
499 suppressor effect but that inhibition of multiple RTK should be considered [126].
500
501 ***MAPK.*** As several RTK receptors are tyrosine-phosphorylated in some MM, downstream activation
502 of the MAPK (mitogen-activated protein kinase) proliferation-associated signaling pathway is likely.
503 Several studies have investigated phosphorylation of proteins of the MAPK cascade, extracellular-
504 regulated kinases (ERKs), Jun amino-terminal kinases/stress-activated kinases (JNKs/SAPKs), and
505 p38 MAPK. Other studies have tried to modulate MAPK pathways in order to inhibit cell survival and
506 induce apoptosis.
507 Phospho-ERK expression was studied by immunohistochemistry in 50 biopsy specimens including
508 non-small-cell lung cancer and normal lung, and pleural tissue comprising 10 MPM (6 epithelioid, 1
509 sarcomatoid and 3 biphasic) [132]. MPM showed significant ERK phosphorylation compared to lung
510 cancer and normal tissues [132]. Activation of ERK, JNK, and p38 MAPK was investigated in 28 MPM
511 and 8 peritoneal MM (32 effusions and 4 biopsies) and 14 samples of reactive mesothelium by
512 assessing the expression of phosphorylated proteins by immunohistochemistry and western blot.
513 MAPK activation did not differentiate between benign and malignant mesothelial cells [133]. The authors
514 argued against a major role for this pathway in the malignant transformation of mesothelial cells. They
515 also noted that MAPK expression and phosphorylation were better predictive factors of outcome, in
516 agreement with data obtained in ovarian cancer [133]. Arsenic trioxide (As_2O_3) is a chemical compound
517 that has been reported to inhibit cell proliferation and induce apoptosis in tumor cells via the MAPK
518 pathways. As_2O_3 inhibited proliferation and induced apoptosis in one mesothelioma cell line [134]. As_2O_3
519 did not alter phosphorylation of either Akt or Src, while ERK1/2 and JNK1/2, but not p38 MAPK,
520 were markedly phosphorylated after As_2O_3 treatment, indicating the involvement of the JNK-
521 dependent ERK-dependent pathway in the cell response [134]. However, p38 MAPK appears to be
522 involved in the response to TGF-beta. In 6 human MM cell lines, migration and invasion linked to the
523 production of metalloproteinases were stimulated by TGF-beta1 via phosphorylation of p38 MAP
524 kinase. The authors suggested that this pathway could be targeted to reduce mesothelioma progression
525 [113]. Ou *et al.* [135] determined the relative levels of tyrosine phosphorylation of 42 distinct RTKs in

526 mesothelioma cell lines established from surgical specimens and found coordinated activation of
527 RTKs EGFR, ERBB3, AXL and MET. As MAPK can be activated by heat shock proteins (HSP),
528 these authors studied the effect of HSP90 inhibition on ERK1/2 activation. HSP90 inhibition reduced
529 TKs phosphorylation and induced apoptosis [135]. The effect of other HSPs was also investigated in the
530 context of the possible use of hyperthermic chemotherapy [136]. HSP40 was upregulated in response to
531 heat stress, associated with activation of the ERK1/2 and p38 pathways in a study of 3 MPM cell lines,
532 suggesting that treatment could be more effective by blocking these pathways [136]. HSP90
533 overexpression has been reported in MPM [88], and *DNAJA1,* a member of the HSP40 family, showed
534 decreased expression in MPM with short-term recurrence of the disease [9].
535 These results show that regulation of mesothelioma cells via MAPK pathways is complex. Targeting
536 these pathways to abolish cell proliferation could be proposed, but the treatment strategy would be
537 difficult to define at the present time. MAPK activation is important for cell survival and can also be
538 linked to apoptosis events. More specific investigations taking into account specific tumor
539 characteristics and microenvironment must be conducted in order to trigger cell growth inhibition and
540 apoptosis.
541 ***PI3K/AKT.*** Constitutive activation of RTKs in MM results in downstream signaling cascades
542 including phosphatidylinositol-3-kinase (PI3K-AKT), a cascade regulating cell growth processes, cell
543 migration and apoptosis. Phosphorylation of AKT protein, the active form of the protein, has been
544 demonstrated in MM cells. Immunohistochemical analysis revealed elevated levels of phospho-AKT
545 in nearly two-thirds of human primary MPM. A strong association with elevated phospho-mTOR
546 positivity in the same tumors confirmed activation of the Akt pathway [137]. Activation of AKT triggers
547 anti-apoptotic mechanisms. However, while the PI3K-Akt signaling pathway was activated in
548 adherent MPM cells, loss of anchorage resulted in inactivation of this pathway and failed to restore
549 apoptosis [138]. Inactivation of PTEN (phosphatase and tensin homolog deleted from chromosome 10), a
550 TSG and negative regulator of the PI3K-AKT pathway, could account for PI3K-AKT activation.
551 PTEN homozygous deletion has been reported in a small subset of MPM cell lines [139-140]. A tissue
552 microarray-based study carried out on 206 tumor tissues demonstrated that loss of PTEN expression
553 was observed in 62% of cases [141]. In this study, PTEN expression was correlated with better survival

554 from data available in 129 patients. PTEN was an independent prognostic biomarker in mesothelioma
555 patients [141].
556 ***Wnt pathway.*** The Wnt signaling pathway regulates developmental processes, cell proliferation and
557 cell polarity. It is driven by membrane protein activation involving low-density lipoprotein receptor-
558 related protein (LRP) and Frizzled, and G-protein-coupled receptors. Activation of the Wnt signaling
559 pathway prevents beta-catenin phosphorylation and its subsequent ubiquitination and degradation.
560 Beta-catenin plays a central role in the Wnt pathway activity, as beta-catenin can act as a coactivator
561 of transcription, allowing the expression of a variety of genes exerting pleiotropic effects [142]. While no
562 recurrent mutation of beta-catenin has been described in MPM, the Wnt pathway could be altered as a
563 result of promoter hypermethylation of regulatory genes [29, 31-32]. Apart from this canonical Wnt/beta-
564 catenin pathway, a non-canonical beta-catenin-independent Wnt pathway can also transduce signals in
565 MPM cells. This was demonstrated in beta-catenin-deficient MPM cells, in which inhibition of Wnt
566 signaling produced growth reduction and apoptosis [30, 143].
567 Gene expression profiling of MM cell lines, primary MPM tumors and normal pleural tissue has been
568 studied by using a custom array designed to profile the expression of genes involved in the Wnt
569 signaling pathway and downstream to Wnt signaling [144]. In the sixteen matched samples (malignant
570 tissue and normal adjacent pleura) investigated, numerous Wnt genes (*WNT1*, *WNT2*, *WNT5*) and
571 Wnt-related genes (*MYC*, *CCND1*, *JUN*) were upregulated. *WNT2* was most frequently upregulated.
572 In contrast, *WNT8A* and some WNT antagonists (*DKK1*, *SFRP2* and *SFRP4*) were downregulated. A
573 role of *WNT2* in cell survival was demonstrated using anti-Wnt2 antibody and Wnt2 siRNA,
574 associated with inhibition of the downstream effectors of the Wnt pathway [144]. Wnt signaling
575 inhibition is dependent on several factors including the Dickkopf (*DKK*) gene family. One member,
576 *REIC/Dickkopf-3,* is downregulated in numerous human cancers [145]. In four human MM cell lines,
577 *REIC/Dickkopf-3* expression was lower than in normal tissue, and overexpression by transduction in
578 one cell line induced apoptosis via a JNK-dependent pathway [145]. Moreover, a preclinical study
579 consisting of orthotopic inoculation of *REIC/Dickkopf-3*-deficient luciferase-labeled MM cells
580 followed by intrapleural injection of recombinant *REIC/Dickkopf-3*-adenovirus resulted in a strong
581 antitumor effect [145]. These results suggest that deregulation of the Wnt signaling pathway can be

582 involved in mesothelial carcinogenesis, and that identification of key targets could be of interest to
583 suppress tumor development.
584 ***Hippo pathway.*** Merlin, the protein encoded by NF2, regulates cell growth by signaling via the Hippo
585 pathway to inhibit the function of the transcriptional coactivator and candidate oncogene *YAP1* via its
586 phosphorylation. Overexpression of *YAP1* was found in one MM cell line [146]. Moreover, Yap1 protein
587 physically and functionally interacted with merlin and, in *NF2*-transfected cells, merlin expression
588 reduced the nuclear localization of Yap1, suggesting that merlin can inhibit Yap1 function by
589 sequestration [146]. Inactivating homozygous deletions or mutations of *LATS2* were recently
590 demonstrated by CGH and DNA sequencing analyses in about 22% of MPM including 20 cell lines
591 and 25 primary tumors [147]. Disruption of *NF2* signaling plays a major role in the development of MPM
592 in view of the high rate of mutations in this tumor. Despite a wild-type status of *NF2,* the merlin also
593 appears to be present in an inactivated phosphorylated form in MPM cells [148]. Recent data suggest that
594 the Hippo pathway involving the merlin could be targeted for treatment strategies. There is now a
595 general consensus concerning inactivation of the Hippo pathway in MPM. To the best of our
596 knowledge, *NF2* expression has not been associated with any specific MPM subtype or specific
597 characteristics and has not been linked to prognosis. Investigation of merlin function in MPM could be
598 useful to develop new therapies. Some examples have been published in the literature. Using *NF2*-
599 negative MM cell lines transduced with a recombinant *NF2* Adenovirus (Ad*NF2*), cDNA microarray
600 analyses revealed differences in gene expression profiles characterized by a decrease in cyclin D1
601 (*CCND1*) expression, a gene upregulated in MPM, in cells transduced with Ad*NF2* compared to those
602 transduced with the control adenovirus. In parallel, CDK4, the catalytic partner of cyclin D1, was
603 inactivated and pRb was dephosphorylated, in agreement with efficient control of the G1/S transition
604 in NF2-expressing cells. G1 cell cycle arrest was confirmed by cell cycle analysis [149]. In this study, the
605 authors found that the effect of *NF2* was related to repression of cyclin-D1 promoter activity via
606 PAK1 inhibition [149]. *NF2* function could also be related to regulation of motility and invasiveness in
607 MM cells, as demonstrated by downregulation of focal adhesion kinase (FAK), and inhibition of
608 motility and invasiveness following *NF2*-transfection and overexpression of FAK in 2 *NF2*-deficient
609 mesothelioma cell lines [150]. A relationship between NF2 expression and apoptosis in MM cells has

610 been reported in other studies. In a study on the role of integrin-specific signaling in the control of
611 apoptosis factors, NF2 was demonstrated to have an inactivating role on integrin-dependent mTORC1
612 signaling [151]. In this study, eleven MM cell lines were analyzed, four not expressing merlin and 7
613 expressing merlin, for their activity in mTORC1, ERK, and AKT. While activation of ERK or AKT
614 was not correlated with the loss of merlin or activation of mTORC1, inactivation of merlin promoted
615 mTORC1 signaling independently of AKT or ERK [151].
616 ***Ubiquitin-proteasome.*** Differences of the expression of genes involved in the ubiquitin/proteasome
617 pathway have been observed between MM and normal tissue or according to histological subtype.
618 Several genes encoding proteasome complex subunits were upregulated in MPM tumors compared to
619 normal parietal pleura [88]. Others proteins involved in the ubiquitin/proteasome pathway, such as the
620 FAS-associated factor *FAF1* which inhibits protein degradation of ubiquitinylated proteins, were
621 recurrently altered at the genomic level in MM of $p19^{ARF}$ (+/-) mice and were downregulated in human
622 MM [152-153]. In peritoneal MM, several genes involved in the ubiquitin-proteasome pathway were
623 upregulated in biphasic tumors compared to epithelioid tumors [154]. In pleural MM, subunits of the
624 proteasome complex (*PSME3*, *PSMA3* and *PSMA4*) and ubiquitin-conjugating enzyme (*UBE2S*) were
625 upregulated in the epithelioid phenotype variant compared to the sarcomatoid phenotype variant of the
626 same MPM cell lines [155].
627 Several studies have analyzed the impact of proteasome inhibitors on MPM malignancy in preclinical
628 models. Bortezomib (PS-341 or Velcade®), a specific inhibitor of 20S proteasome activity, induces *in*
629 *vitro* apoptosis and *in vivo* tumor growth inhibition in mice of one MPM cell line [156]. Other
630 proteasome inhibitors, PSI or MG-132, were also shown to induce apoptosis in some MPM cell lines
631 [157-158]. Using MPM cell lines in monolayer culture, bortezomib was shown to increase the cytotoxicity
632 of chemotherapeutic agents [159]. However, MM cell lines, when grown as multicellular spheroids,
633 acquired resistance to apoptosis induced by a combination of the proteasome inhibitor MG-132 and
634 other apoptotic stimuli [160]. Results of ongoing phase II clinical trials using bortezomib combined with
635 cisplatin will indicate the efficacy of proteasome inhibitors in the management of MM
636 (ClinicalTrials.gov Identifier: NCT00458913).
637 ***Cell cycle regulation.***

638 Alteration of genes located at the *INK4* locus, encompassing *CDKN2A* and *CDKN2B,* is a feature of
639 human MM. Inactivation of these genes allows uncontrolled cell proliferation. While some MM do not
640 show mutation or methylation of these genes, another level of regulation could occur via deregulation
641 of miRNA expression (see above, chapter 2.3). Several authors have developed experimental studies
642 trying to restore cell cycle control in MM by adenovirus-mediated expression of $p16^{INK4A}$ and $p14^{ARF}$
643 in human MM cells, and found effects on both cell cycle progression and reduction of tumor growth in
644 immunocompromised mice [161-163].
645 Cell cycle control can be affected in MM cells by the loss of other negative regulators, CDK (cyclin–
646 dependent kinases) inhibitors or by the overexpression of CDKs and cyclins (CCNs), and regulators of
647 the mitotic checkpoints [85, 88]. The expression profile of 60 genes involved in cell cycle has been
648 investigated in forty-five MM tumor samples and normal pleural tissue [78]. Among genes
649 overexpressed in MM, several were involved in cell cycle checkpoints such as CDK1/CDC2 (cyclin-
650 dependent kinase 1), CDC6 (cell division cycle 6, a regulator of replication), CDKN2C (cyclin-
651 dependent kinase inhibitor 2C, p18), CCNH (cyclin H), CCNB1 (cyclin B1, controling the cell cycle
652 at the G2/M transition), CHEK1 (Chk1 is required for checkpoint-mediated cell cycle arrest in
653 response to DNA damage) and FOXM1 (forkhead transcription factor, a regulator of gene expression
654 in the G2 phase). In contrast, CCND2 (cyclin D2, a regulator of Cdk4 and Cdk6, controls the cell
655 cycle at the G1/S transition) was underexpressed [78]. Aurora kinases are involved in microtubule
656 formation and are important regulators of the mitotic spindle checkpoint system, controlling
657 progression of mitosis until all chromosomes are properly aligned during metaphase. An
658 overexpression of aurora kinases has been reported in different studies [25, 85]. Aurora B levels increase
659 after gamma irradiation, and MM cells arrest at the G2/M checkpoint of the cell cycle to repair DNA
660 damage before proceeding through mitosis [164]. Stathmin is also important for the evolution of mitosis
661 as it is involved in the regulation of the microtubule dynamics, by inhibiting the formation of
662 microtubules and/or promoting their depolymerization. Kim *et al.* [165] identified potential genes
663 involved in pathogenesis of MPM. They investigated seven MM cell lines, fresh mesothelioma tissues
664 and adjacent normal pleural tissues using cDNA microarray chips. Multiple genes were overexpressed
665 in MM cell lines compared to the human mesothelial cell strain LP-9 derived from the ascitic fluid of

666 a patient with an ovarian carcinoma, and stathmin was one of the most strongly overexpressed genes
667 [165-166]. Protein expression of stathmin was observed in MPM tissues but not in matched normal pleural
668 samples [165].
669 Because of these different alterations, response to DNA damage can be impaired in MPM cells
670 entailing chromosomal instability. Well-controlled cell cycle progression is necessary for cells to
671 respond to both endogenous and exogenous DNA damage. Although MPM cell cycle may be arrested
672 in response to DNA damaging agents, it may be assumed that MPM cells recover, likely due to their
673 inability to trigger the apoptotic mechanism. Moreover, a heterogeneity exists between different
674 tumors. After exposure to gamma-radiation, human MPM cells were arrested either in one or more
675 phases of the cell cycle, demonstrating a heterogeneity in cell cycle control. G1 arrest was
676 p21WAF1/CIP1- and p53-dependent [167]. As mentioned in chapter 3.1 p53 can be inactivated in MPM,
677 and its inactivation will facilitate chromosomal instability, in relation to loss of cell cycle control,
678 especially in response to DNA damage. Regulation of p53 function occurs via post-translational
679 mechanisms and interaction with several protein. MDM4 was recently shown to control p53 function
680 in a human MM cell line [168].
681 Overall, these studies demonstrate that cell cycle dysregulation occurs in all phases, at the level of
682 checkpoint control and related factors, encouraging the search for stimulation of death pathways in
683 MPM cells.
684 ***Apoptosis.*** Malignant MM responds poorly to standard therapy [169]. Mesothelioma tissue usually has a
685 lower apoptotic index than other carcinomas [170], suggesting major defects in the apoptotic machinery.
686 Apoptosis is mediated by two signaling pathways, the extrinsic and intrinsic pathways. The extrinsic
687 pathway is initiated by death receptors, while the intrinsic pathway is triggered by internal apoptotic
688 signals and involves the release of cytochrome c from the mitochondrial intermembrane space. These
689 two pathways merge and share mechanisms of the caspase cascades [171]. In the extrinsic pathway, the
690 death receptor agonist TRAIL can induce apoptosis with a high specificity toward tumor cells and is
691 currently being tested in clinical trials in a variety of human cancers. In mesothelioma, TRAIL has
692 been to shown to enhance the chemosensitivity of tumor cells to various therapeutic agents, such as
693 doxorubicin, gemcitabine, cis-platinum or etoposide. However, most MM cells are resistant to

694 apoptosis induced by TRAIL alone [172]. This resistance can be explained notably by overexpression of
695 the caspase-8 inhibitor, *FLIP*/*CFLAR*, and by the methylation of TRAIL receptors in MM cells [173].
696 Several multimodal approaches have subsequently been applied to sensitize MM cells to TRAIL. Heat
697 stress, as well as subtoxic doses of alpha-tocopheryl succinate or anisomycin can sensitize MM cells
698 to TRAIL and induce apoptosis *in vitro*, via Bid-dependent mitochondrial amplification of the
699 apoptotic signal [174-176]. Inversely, the multikinase inhibitor sorafenib showed synergistic effects with
700 TRAIL in cells resistant to TRAIL, independently of caspase activation [177]. Interestingly, in contrast
701 with mesothelioma cell monolayers, tumor fragment spheroids exhibit higher resistance to apoptosis
702 and notably to TRAIL-combined treatments, and this resistance is mediated by the mTor/S6K pathway
703 [160, 178]. In the intrinsic pathway, the mitochondrial membrane potential and permeability are regulated
704 by the Bcl-2 family of proteins. Members of this family include both proapoptotic proteins such as
705 Bax, Bak, Bad, Bid or Bim, and antiapoptotic proteins, such as Bcl-2, Bcl-xL and Mcl-1. Bcl-2 is
706 rarely expressed in mesothelioma [170], while high levels of Bcl-xL are commonly observed [179]. Several
707 studies have shown that downregulation of Bcl-xL could decrease baseline tumor cell viability and
708 improve sensitivity to chemotherapeutic agents, both *in vitro* and *in vivo* [180-182]. Mcl-1 has also been
709 implicated in the apoptotic resistance of mesothelioma cells [158, 179]. Recently, Varin *et al.* showed that
710 Bcl-xL and Mcl-1 cooperated to protect mesothelioma cells from cell death and that their concomitant
711 targeting was sufficient to induce apoptosis [183]. Most members of the proapoptotic Bcl-2 family appear
712 to be expressed in mesothelioma with functional integrity, suggesting that the loss of their apoptosis-
713 inducing properties is due to sequestration by Bcl-xL or Mcl-1 [184]. In particular, functional inhibition
714 of Bim contributes to survival in the spheroid model of mesothelioma cells [138].
715 The inhibitor of apoptosis protein (IAP) survivin, encoded by the *BIRC5* gene, was highly expressed
716 in all MM primary tumors (12 samples) and cell lines (7/8) compared with normal pleura [185]. Survivin
717 expression in 34 MM tumors was confirmed by immunohistochemistry and linked to an apoptotic
718 defect [170]. Downregulation of survivin with anti-survivin oligonucleotides induced apoptosis when
719 tested in one cell line [185]. Inhibition of survivin expression has been shown to decrease tumor cell
720 growth and enhance drug response [186]. XIAP is also frequently expressed in malignant mesothelioma,
721 and is notably upregulated in mesothelioma effusions and peritoneal mesothelioma [187]. Moreover,

722 XIAP inhibition has been shown to increase the sensitivity of mesothelioma cells to TRAIL-induced
723 apoptosis [188]. Together, these results suggest that combined approaches, triggering the extrinsic and
724 intrinsic pathways or the caspase cascade, are promising for the treatment of mesothelioma.
725
726 ***Telomere.*** Human telomeres progressively shorten during cell division, and critical shortening is
727 believed to limit the cellular life span, and is involved in conferring growth-promoting properties to
728 tumor cells. Telomere lengthening is due to telomerase (*TERT*) activity, which was found in a large
729 proportion of the 22 primary pleural MM and the 4 MM cell lines in comparison with mesothelial cells
730 from normal pleura using the telomeric repeat amplification protocol (TRAP) [189]. These findings were
731 confirmed in a more recent study carried out in peritoneal MM and another mechanism, alternative
732 lengthening of telomeres, was also demonstrated to maintain telomere length [190]. Interestingly, in their
733 series of 44 MM peritoneal lesions from 38 patients, these authors found that telomerase activity was a
734 significant prognostic factor for 4-year relapse and disease-free survival. Telomerase activity was
735 reduced in MM cell lines in comparison with normal cells by inhibition of *MetAP2* (methionine
736 aminopeptidase) with angiostatic agents fumagillin and ovalicin. This enzyme is overexpressed, in
737 MM cells [191].
738
739
740 **3. Conclusions**
741 Molecular studies have identified somatic genetic and epigenetic alterations in MPM cells, associated
742 with altered expression, activation or inactivation of critical genes in oncogenesis. Deregulation of
743 signaling pathways related to differentiation, survival, proliferation, apoptosis, cell cycle control,
744 metabolism, migration and invasion has been demonstrated in complementary studies. These changes
745 were found by investigating individual gene status in genomic and transcriptomic studies, and were
746 supported by immunohistological studies. MPM cells show a large spectrum of abnormalities shared
747 with other malignancies, or more specific alterations such as those of the *NF2* gene. Comparative
748 studies of series of MPM have usually demonstrated that both alterations in a given gene and
749 combined genetic and epigenetic alterations are present in MPM subsets, consistent with inter-

750 individual variations of molecular alterations. There are therefore at least two levels of heterogeneity,
751 at the genome level and at the gene level, suggesting that identification of patient subgroups would be
752 essential in order to develop more specific therapies. Moreover, the tumor microenvironment,
753 consisting of a large number of different cell types, adds another level of complexity to identify the
754 best strategy to improve the outcome of this disease. This tumor heterogeneity could explain
755 differences in patient survival and response to treatments.
756 This review provides insight into a limited number of genes known to be frequently altered in MPM,
757 *INK4* locus and *NF2*, and a larger number of candidates that may play a role in MPM carcinogenesis,
758 especially those involved in various signaling pathways. Further studies should define the clustering of
759 these genes in specific MPM subsets. These findings have already been the basis for several studies
760 testing various targeted therapeutic approaches on specific RTKs, but mostly with limited success.
761 Demonstration of the multiple alterations present in the tumor should encourage research into
762 combined or more global therapies. Other studies have emphasized deregulation of signaling
763 pathways, but no pathway seems to be specific or a particularly relevant target, as certain
764 discrepancies have been observed concerning the response of MPM cells to specific inhibitors, and
765 key regulatory players in one pathway may interact with another pathway. Focusing on apoptosis is
766 probably an interesting strategy to counteract or trigger the activity of several of these pathways. More
767 recent data have indicated the presence of alterations that could be targeted at a global level
768 (methylation). Studies are ongoing to take advantage of these abnormalities for MPM treatment.
769 Prediction of a positive response in MPM would avoid a rapidly unfavorable course and avoid wasting
770 time and resources with inappropriate treatments. The critical issue concerning targeted therapy is to
771 focus on the most relevant target(s). Some molecules, pathways and/or epigenetic changes should be
772 selected, provided they are key factors in MPM. This is not an easy task regarding the interplay
773 between the various regulatory pathways, and the diversity of genomic alterations. Molecular studies
774 must be developed to identify and classify genomic alterations in MPM cells and correlate these
775 alterations with disease outcome in order to avoid random testing of therapies already used in other
776 cancers, but with unknown relevance in MPM. In recent years, several studies have been designed to
777 evaluate the predictive role of microarray data for MM outcome. Various authors have developed

778 predictors of survival, but in some studies the accuracy was lower than that of prognosis based on the
779 usual methods comprising clinicopathological variables and morphology. Other authors have proposed
780 innovative predictors based on gene expression ratios. These procedures are of great interest and
781 deserve further validation.
782 Our improved understanding of MPM development and treatment is partly based on well designed
783 preclinical studies. Numerous *in vitro* investigations are currently underway to suppress MPM cell
784 growth and/or induce apoptosis by interacting with proteins regulating proliferation and survival, or by
785 silence gene expression (RNA interference). These methods benefit from the data provided by
786 molecular analyses providing preclinical proof of concept for the feasibility of such strategies.
787 However, these studies were carried out in MPM specimens that do not necessarily present the same
788 genomic status as the tumors of patients selected for the relevant therapy. In the context of preclinical
789 investigations, animal models must be combined with studies prior to translation to humans. An
790 important point to be emphasized here is the paramount importance of frozen and paraffin MPM tissue
791 banks to allow better characterization and annotation of MPM, as well as panels for diagnostic
792 certification. Databases and panels are already available, such as the Mesothelioma Virtual Bank
793 (http://www.mesotissue.org) [192] or the International Mesothelioma Excellence Center (IM@EC).
794 Over recent years, considerable methodological progress has been made in the field of molecular
795 approaches to study cancer biology and this progress has been applied to MPM. Improvements are still
796 in progress. Other methodologies have not yet been applied to MPM, such as proteomics, cell
797 imaging, integrative biology and will likely be useful in the future, in order to identify MPM
798 biomarkers, exposure markers and MPM subgroups.
799 Various clinical studies have shown that future treatment strategies must not be based on
800 monotherapy, but must comprise multi-site and multimodal treatment. As this disease is particularly
801 aggressive, it requires a specific treatment strategy. Investigation of the tumor genome and related
802 pathophysiological events has therefore become a key step to a better understanding and possible cure
803 of this dreadful incurable cancer.
804

805 **Table 1. Genomic and epigenetic changes of potential interest for MPM histology, diagnosis and**
806 **prognosis.**
807

Genes	Significance	Reference
Diagnosis		
Chromosomal alteration	Frequency different between MPM and lung carcinoma and others splindle tumors of the pleura	[21-22]
DNA methylation status of specific gene loci	Frequency different between MPM and lung adenocarcinoma and non-malignant pulmonary tissue	[33-36, 43-44]
MiRNA expression level	Difference between MPM and lung adenocarcinoma	[49]
MiRNA expression level	Difference between MPM and various carcinoma	[52]
Histology		
Chromosomal alteration	Frequency different between epithelioid and sarcomatoid MPM	[6]
DNA methylation status of specific gene loci	Frequency different between between epithelioid and sarcomatoid MPM	[35, 42-44]
Prognosis		
Chromosomes and chromosome 7p	Inverse correlation between copy number and survival	[16, 23]
CDKN2A locus (9p21.3) homozygous deletion	Correlation with shorter survival or shorter time to relapse	[9, 25]
Number of chromosomal alteration	Correlation with shorter time to relapse	[9]
Number of chromosomal region loss	Correlation with shorter survival in deciduoid MPM	[26]
DNA methylation status of *HIC1*, *PYCARD*, *LZTS1* and *SLC6A20* gene loci	Potential association with survival	[43, 45]
Occurence of DNA methylation	Correlation between low frequency and longer survival	[34]
DNA methylation profile	Prognostic prediction depending of specific profiles	[33]
MiR-17 and mIR-30c	Correlation between reduced expression and better survival in sarcomatoid MPM	[48]
MiR-29c	Correlation between increased expression and better survival in epithelioid MPM	[51]

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Table 2. Recurrent regions of chromosomal alterations in MPM.

Alteration	CGH (90 tumors) [6]	CGH array (17 tumors) [7]	CGH array (26 tumors) [8]	ROMA (22 tumors) [9]	SNP array (23 tumors) [10]	SNP array (22 cultured cells) [11]	CGH array (33 cultured cells) [12]
Gain	1q23-q32 (16%)	1q (44%)			1q23 (35%) 1q32 (22%)		
		5p (44%)		5p14 (55%)	5p (22%)		5p15.3-p11 (51%)
	7p14–p15 (14%)	7p (44%)			7p14-p15 (22%)		7p22-p11.2 (37%)
	8q22–q23 (18%)	8q24 (56%)		8q23-q24 (36%)	8q22-q23 (20%) 8q24 (22%)		
	15q22–q25 (14%)				15q22-q25 (17%)		
			17q21.32-q25 (27%)	17q21-q23 (24%)		17q23.2 (55%)	
				18q12.1 (36%)			
		20p (33%)			20p (9%)		
							20q11.2-q13.1 (34%)
Loss		1p36.33 (11%) 1p36.1 (33%)		1p36.22-p36.23 (36%) 1p36.11-p36.12 (55%)	1p36.1 (30%) 1p36.33 (39%)	1p36.3-p36.2 (55%)	1p36.3-p35 (51%)
	1p21 (21%)	1p21.3 (56%)	1p31.1-p13.2 (42%)	1p13.2-p13.3 (36%)	1p21.3 (30%)	1p22.3-p22.1 (82%)	1p31-p12 (40%)
	3p21 (16%)	3p21.3 (33%)	3p22.1-p14.2 (42%)	3p21.31 (27%) 3p14.3-p14.2 (32%)	3p21.3 (44%)	3p22.1-p21.31 (77%)	3p23-p14 (63%)
	4q31–q32 (29%) 4p12–p13 (25%)	4q22 (56%) 4q34-q35.2 (33%)			4p12 (26%) 4q22 (30%) 4q31-q32 (35%)	Chr4 (53%)	Chr4 (54%)
	6q22 (16%)	6q25 (44%)	6q22.1 (58%)		6q22 (26%) 6q25 (39%)		6q14-q27 (57%)
							8p23-p12 (31%)
	9p21 (34%)	9p21.3 (100%)	9p21.3 (65%)	9p21.3 (32%) 9p21.1 (36%)	9p21 (39%)	9p21.3 (100%)	9p24-q21 (91%)
				9q34.11 (41%)			
	10p13–p15 (16%)	10p (44%)			10p13 (9%)		10p15-p12 (37%)
							10q23-q26 (37%)
						11q23.2-q23.3 (64%)	
							12p13 (54%)
	13q13–q14 (19%)	13q33.2 (44%)	13q11-q14.12 (35%)		13q13-14 (17%)	13q12.2-q13.2 (73%)	13q (60%)
	14q12–q24 (23%)				14q12-q24 (22%)		14q11.2-q21 (40%)
		14q32.13 (56%)	14q22.1-32 (38%)		14q32.13 (17%)	14q32.2 (73%)	14q24-q32 (40%)
						15q15.1 (55%)	15q13-q21 (40%)
	17p13–p12 (16%)			17p13.1 (46%)	17p12 (17%)		17p13-p11.2 (34%)
				17q21.31 (32%)			
		18q (33%)			18q (13%)	18q12.3 (59%)	18q12-q23 (46%)
				19p13.2 (55%)			19p13.1-p12 (31%)
				19q13.32 (55%)			19q13.2-q13.4 (31%)
	22q (32%)	22q (33%)	22q11-q12.3 (35%)	22q12.2 (74%)	22q (43%)	Chr22 (78%)	22q (80%)

813 **Table 3. Genes of potential interest for MPM characterization**
814

Genes	Reference
Overexpressed in comparison with normal cells	
MAP3K14/NIK, *JAG1/JAGGED1*, *CCND1*, *CCND3*, *CDC25B*, *FGF3*, *FGF12*, *PDGFRB*, *XRCC5/Ku80*	71
CFB, *FTL*, *IGFBP7*, *RARRES1*, *RARRES2*, *RBP1*, *SAT*, *TXN*	72
COL1A2, *COL6A1*, *tPA*, *MMP9*, *CDH3*, *L1CAM*, *ITGB4*, *PLXNA3/PLXN3*, *KRT14/K14*, *SEMA3C*, *CXCL10/INP10*	74
Genes involved in glycolysis	76
HSP90B1, *LRP*, *LGALS3BP*	76
Members of the condensin complex and of the kinesin family	77
CDK1/CDC2, *CCNA2*, *CCNB1*, *CCNB2*, *CCNL2*, *DLG7*, *CHEK1/CHK1*, *BUB1*, *MAD2L1*	77
CHEK1/CHK1, *CCNH*, *CCNB1*, *p18-CDKN2C*, *CDC2*, *FOXM1*, *CDC6*	78
Underexpressed in comparison with normal cells	
FGF1, *FGF7*, *CCND2*, *KDR/VEGFR2*, *RARβ*	71
ALOX5AP, *CLNS1A*, *EIF4A2*, *ELK3*, *DF2/REQ*, *SYPL*	72
UBE1L, *CCND2*	78
FUS1/TUSC2, *OSM*, *PL6/TMEM115*	9, 79

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816

817 **Table 4. Genes of potential interest for characterization of MPM subtypes**
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Genes	Significance	Reference
Histology		
ST14	Overexpressed in epithelial MM in comparison with sarcomatoid and biphasic MM	[80]
SEMA3C, *ITGB4*, *CDH3*, *COL6A1*	Overexpressed in epithelioid MM in comparison with normal cells	[74]
L1CAM, *K14*, *INP10*	Overexpressed in biphasic MM in comparison with normal cells	[74]
MMP9, *PLXN3*	Overexpressed in sarcomatoid MM in comparison with normal cells	[74]
UPK1B, *UPK3B*, *KLK11*	Overexpressed in epithelioid vs non epithlioid MM	[25]
TFDP2, *ABL1*	Overexpressed in epithelioid vs non epithlioid MM	[78]
TWIST11	Overexpressed in non epithelioid vs epithlioid MM	[78]

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823 **Table 5. Genes of potential interest for MPM characterization and predictive prognostic value**
824

Genes	Significance	Reference
Patients' outcome		
P16/CDKN2A	Gene loss or no protein expression associated with low survival	[25, 64-67]
KIAA0977/GDIA1, *L6/CTHBP*, *L6/GDIA1*	Gene ratios predict outcome	[81]
CD9/KIAA1199, *CD9/THBD*, *DLG5/KIAA1199*, *DLG5/THBD*	Gene ratios predict outcome	[82]
TM4SF1/PKM2 *TM4SF1/ARHDDIA* *COBLL1/ARHDDIA*	Gene ratio discriminate high risk and low risk patients	[83]
Gbx2, *KI67*, *CCNB1*, *BUB1*, *KNTC2*, *USP22*, *HCFC1*, *RNF2*, *ANK3*, *FGFR2*, *CES1*	Expression associated with poor prognostic	[84]
CDH2	Overexpressed in the short-term recurrence group	[9]
DNAJA1	Underexpressed in the short-term recurrence group	[9]
AURKA, *AURKB*	Expression associated with poor outcome	[85]
MELK	Upregulation associated with poor survival	[25]
BIRC5, *KIF4A*, *SEPT9*	Upregulation associated with poor prognosis	[77]
HAPLN1	Expression negatively correlated with survival	[86]
DNAJA1	Underexpressed in the short-term recurrence group	[9]
MMP14	High expression associated with lower survival	[77]
LELK1	Upregulation associated with poor survival survival	[25]
Thirteen genes involved in ECM, regulators of ECM assembly, angiogenesis	High expression associated with poor survival	[87]

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829 **Bibliography**

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