4 Genetics and Epigenetics of Mesothelioma

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4.1 Introduction

The definition of the malignant mesothelioma (MM) genome may have important endpoints, both in terms of pathobiology and translation to clinical practice. Generally, the identification of DNA changes within a tumor genome is useful to identify the molecular events that lead to carcinogenesis or tumor progression, i.e., the driver mutations. Early studies focused on the analysis of single genes, especially *TP53*. Looking at melanoma and lung cancer genomes, these studies achieved the important milestone of deciphering the mutational profile (signature) generated by two carcinogens, i.e., UV radiation and smoke carcinogens, respectively [1, 2]. The advent of next generation sequencing (NGS) and novel bioinformatic approaches allowed to explore systematically a large number of tumor types. The seminal studies by Stratton and co-workers allowed to identify several signatures, each associated with exposure to a specific carcinogen or due to key events in carcinogenesis, such as inactivation of specific DNA repair mechanisms or activation of deamination enzymes [2].

The identification of abnormalities in specific pathways shed light on shared carcinogenetic pathways in tumors with or without the same histological origin, paving the road to the creation of pathway-specific targeted drugs. In addition, tumor classification may be supported by looking at the tumor genome and transcriptome.

Furthermore, it is important to consider that the individual germline genome can modulate the response to carcinogens and hence transformation. Genetic risk factors are well known for several tumors and may have important translational output. For example, individuals carrying such risk factors may benefit from the implementation of screening programs aimed at early diagnosis of tumors. Additionally, the same risk factor may modify specific carcinogenic pathways and response to specific therapies.

Finally, it is well known that tumor suppressor genes may also be inactivated by epigenetic mechanisms. The term "epigenetic" refers to

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© Springer Nature Switzerland AG 2019
G. L. Ceresoli et al. (eds.), *Mesothelioma*, https://doi.org/10.1007/978-3-030-16884-1_4

heritable and reversible changes in the mechanisms that regulate gene activity without altering the genomic sequence. In recent years, there is increasing evidence of the major role of epigenetic mechanisms in tumorigenesis, as well as in drug-response. Much attention is also devoted to epigenetic changes as biomarkers of early disease detection, prognosis, and response to therapy.

In this review, different patterns of genetic and epigenetic signatures of the malignant pleural mesothelioma (MPM) genomes will be discussed, together with peculiar aspects of genetic predisposition and gene/environment interactions. The potential use of these genetic/epigenetic signatures for the development of future therapeutics will also be addressed.

4.2 Genetic Risk Factors of Mesothelioma

MPM carcinogenesis is caused in the large majority of cases by asbestos or asbestos-like fibers exposure. It is well known that the level of asbestos exposure directly correlates with the risk of MM ([3], more details are given in a different chapter of this book), but several epidemiological studies suggested that different individuals may respond differently to this carcinogen. An important observation is that only about 10% of the workers heavily exposed to asbestos develop MPM [4]. Additionally, several papers reported familial aggregations of MPM [5]. These observations suggested the hypothesis of an inherited predisposition that modifies the carcinogenic effect of asbestos.

Generally, inherited predisposition factors are DNA variants that occur in the germline genome and modify the function of a specific gene. They are divided into three classes, depending on the relative risk (RR) they carry: low-, moderate-, and high-risk factors.

Low-risk factors are DNA variants that subtly modify the function of a gene or a biochemical pathway. In this case, a single DNA variant does not have any substantial effect on human phenotypes, but many DNA variants affecting the same biochemical pathway may alter its functions, favoring disease development. Therefore, the disease risk does not follow the rules of Mendelian heredity, because each variant is inherited independently from the others.

Risk factors are identified using genome wide association studies (GWAS) on thousands of patients and controls [6]. Large numbers are required to obtain statistically significant results, because each variant confers a low risk. The aim of these studies is to identify DNA variants that are differently represented in patients versus controls. These studies are expected to increase the knowledge of asbestos carcinogenesis and improve risk evaluation.

So far, only two GWAS on MPM have been performed, both including several hundreds of patients and controls, but not enough to obtain statistically significant results [7, 8]. However, both studies identified a region associated with the MPM status, that included *FOXK1*, encoding for an interactor of BAP1 (BRCA1-associated protein 1), a well-known high-risk factor for MPM.

BAP1 codes for a tumor suppressor that is frequently deleted in the genomes of several tumors, including cutaneous melanoma, uveal melanoma, mesothelioma, and others [9].

Germline variants in *BAP1* characterize the *BAP1*-tumor predisposition syndrome (*BAP1*-TPDS, MIM#614327) [10]. Tumor predisposition syndromes are due to germline mutations in tumor suppressor genes and are inherited with an autosomal dominant pattern. The patients with these syndromes show a high or moderate risk for specific tumors during their whole life. Often they develop several independent tumors.

Individuals with *BAP1*-TPDS show a high risk of developing mesothelioma, cutaneous and uveal melanoma, clear cell renal carcinoma, and basal cell carcinoma [10]. Moreover, they develop peculiar nonmalignant skin tumors, called atypical Spitz tumors or MBAITs (BAP1-mutated atypical intradermal tumors) or bapomas [10, 11].

Patients with *BAP1*-TPDS and uveal melanoma have a poor prognosis [10, 12], whereas those with mesothelioma seem to have a longer survival than those without *BAP1*-TPDS [13].

Ninety-seven families with *BAP1*-TPDS have been identified so far, 48 of them included

patients with MM; thus, this syndrome is indeed very rare [11, 14–38]. Age at onset of mesothelioma in patients with *BAP1*-TPDS is earlier than that in patients without this syndrome [13, 26]. Most of the MM are MPM and show an epithelioid histotype, while peritoneal mesothelioma (PM) has been rarely reported [10]. The prevalence of *BAP1*-TPDS among patients with familial MPM varied between 6% (9/153) and 7.7% (3/39) [26, 31] and was higher than the prevalence observed in sporadic cases [23, 39, 40].

Other tumors have been reported in patients with *BAP1*-TPDS, i.e., breast cancer [12, 14, 21, 22], cholangiocarcinoma [12, 22, 41], meningioma [18, 25, 38, 41], neuroendocrine tumors [18, 19], non-small cell lung cancer (NSCLC) [12, 18, 19, 42], thyroid carcinoma [21, 43], and mucoepidermoid carcinoma of the tongue [23].

BAP1 (#MIM 603089) is located on 3p21.1 and encodes for a ubiquitin carboxy-terminal hydrolase, a nuclear enzyme that catalyzes the cleavage of a ubiquitin residue from its target proteins. The product of the gene, BAP1, has three domains: the ubiquitin C-terminal hydrolase domain and two nuclear localization sequences. The BAP1 protein together with FOXK1, HCFC1, ASXL1/2, and OGT [44] forms a multiprotein complex.

BAP1 has been implicated in DNA repair, chromatin modulation, transcriptional regulation, cell proliferation, cell death, and glucidic metabolism [45–49]. The mechanism of BAP1-dependent carcinogenesis is not known, but these functions are not mutually exclusive. *BAP1* is involved in DNA repair by the HRR (homologous recombination repair) pathway [49].

Bap1 (+/−) mice are more sensitive to asbestos compared with wild-type mice [50, 51]. Quantification of asbestos exposure has been reported only for four individuals with MPM and *BAP1*-TPDS: all showed very low exposure [31, 52].

BAP1 germline mutations cause loss of function, and only ten of the different mutations have been identified in patients within apparently non-consanguineous families [24]. Recurrent mutations could be due to mutable hot spots, such as CpG dinucleotides.

Eleven other genes were reported to confer predisposition to MPM: *CDKN2A*, *PALB2*, *BRCA1*, *FANCI*, *ATM*, *SLX4*, *BRCA2*, *FANCC*, *FANCF*, *PMS1*, and *XPC* [32, 53] (Table 4.1). All these genes but *PMS1* are tumor suppressors, responsible for cancer predisposition syndromes with specific tumor spectra. In particular, *BRCA1*, *BRCA2*, *ATM*, *SLX4*, and *PALB2* can predispose women to breast and ovarian cancer whereas *BRCA1* and *BRCA2* also to prostate and pancreatic carcinomas [61]; *CDKN2A* to melanoma and pancreatic cancer [54]; and *XPC* to basal cell carcinoma, squamous cell carcinoma, and melanoma [62]. *PMS1* is involved in MMR (DNA mismatch repair) and possibly in cancer predisposition [63, 64].

Homozygous germline variants in *BRCA1* (also called *FANCS*); *BRCA2* (*FANCD1*); *FANCC*,

Table 4.1 High- or moderate-risk predisposition genes

Gene	Function	Reference
BAP1	Deubiquitination enzyme, cell proliferation, DNA repair pathway (HRR)	[11, 12, 14–39, 41–43, 54–60]
CDKN2A	Cell cycle regulation	[32]
ATM	Cell cycle regulation, DNA repair pathway (HRR)	[53]
BRCA1	DNA repair pathway (HRR)	[53]
BRCA2	DNA repair pathway (HRR)	[53]
FANCC	DNA repair pathway (HRR)	[53]
FANCF	DNA repair pathway (HRR)	[53]
FANCI	DNA repair pathway (HRR)	[53]
PALB2	DNA repair pathway (HRR)	[53]
SLX4	DNA repair pathway (HRR)	[53]
XPC	DNA repair pathway (NER)	[53]

Only genes harboring germline PTVs in MM patients are included. *HRR* homologous recombination repair, *NER* nucleotide excision repair

FANCI, *FANCF*, and *SLX4* (*FANCP*); and *PALB2* (*FANCN*) are found in patients with Fanconi anemia, a recessive disease that predisposes to a variety of hematological and solid tumors. This disorder can be caused by at least 20 different genes [65], all acting in a specific signaling pathway activated in response to cross-linking agents.

Mutations in *XPC* cause the recessive disease xeroderma pigmentosum (MIM# 278720). *XPC* is involved in the NER (nucleotide excision repair) pathway, a DNA repair system that removes the pyrimidine dimers induced by exposure to ultraviolet radiation.

In most cases, the loss of the wild-type allele, due to a further acquired mutation, induces carcinogenesis in the target tissues of patients with a germline variant. Except for *CDKN2A*, which is involved in the control of cell proliferation, all these genes have a role in DNA repair.

Anecdotal studies allow to include two more genes involved in cancer predisposition syndromes, *NF2* and *TP53*, because MPM was reported in patients with neurofibromatosis Type 2 or Li-Fraumeni syndrome, due to germline variants in *NF2* or *TP53* [66, 67], respectively.

Interestingly, some of these genes are often somatically mutated in MPM, i.e., *BAP1*, *CDKN2A*, *NF2*, and *TP53* [55, 68, 69].

The involvement of DNA repair genes in MPM risk has been confirmed by others [70] and is in accordance with the observation that 12% of patients with different types of metastatic tumors were reported to carry germline variants, 75% of which in DNA repair genes [71].

Most probably, the development of a specific tumor type in patients with these germline mutations depends on the carcinogen to which they are exposed. If the carcinogen is asbestos, the tumor is likely MPM. Analysis of the genomic signature of the different cancers affecting these patients may confirm this hypothesis.

4.3 The Mesothelioma Genome

Deciphering tumor genomes is important both to gather information about the processes that induce carcinogenesis and to identify druggable pathways in the landscape of precision oncology.

Different methodologies are required to identify point mutations or large rearrangements and copy number variants (CNVs). Ideally, rearrangements and CNVs are studied on the whole genome by using CGH (comparative genomic hybridization) arrays, SNP (single nucleotide polymorphism) arrays, or whole genome sequencing. These methods simultaneously identify all copy gains and copy losses in a genome. Point mutations (also called single nucleotide variants, SNVs) are detected by NGS. Different approaches may be used. Targeted resequencing screens hundreds of known cancer genes that are usually analyzed in the regions corresponding to exons (panel NGS analysis). Exome analysis has the advantage of studying all the genes of the human genome, with a focus on exons. Using appropriate bioinformatic tools, CNVs and rearrangements may be identified in exomes, but not those affecting noncoding regions.

Whole genome analysis addresses the entire genome and could theoretically identify all variants, but management of big data may be time-consuming. In addition, the role of the majority of the genome noncoding regions is not known, so the functional interpretation of variants is difficult.

Usually the cancer and the blood cell genomes are sequenced at the same time to distinguish somatic from germline variants. It should be considered that a very large amount of mutations are generated in each tumor cell at every cell division because of its genetic instability. Therefore, most of these variants are passenger (neutral) variants; only a small number are driver mutations, those that confer a selective advantage to the cell. It has been calculated that only half of the driver mutations in tumors are located in known cancer genes, whereas the others reside in genes or regions whose effect on carcinogenesis is still unknown [72].

The first studies reporting copy gains and copy losses in the mesothelioma genome were published 20 years ago (Table 4.2) [9, 55, 56, 69, 73–80], but point mutations in mesothelioma have been addressed only after the implementation of NGS strategies (Table 4.3) [9, 55–59, 68, 69, 74, 76, 78, 79, 81–85]. Most studies are focused on

Table 4.2 Mesothelioma genome: genes harboring somatic CNVs

#	Gene	Aberration	Function	Reference
1	***NF2***	Loss, Chr rearrangements, fusion	Cell shape, cell growth, cell adhesion	[55, 56, 69, 73–77]
2	***BAP1***	Loss, Chr rearrangements, fusion	Deubiquitinating enzyme, cell proliferation, DNA repair pathway (HRR)	[9, 69, 74, 76, 77]
3	***CDKN2A***[c]	Loss, Chr rearrangements	Cell cycle regulation	[55, 56, 69, 73, 75, 77, 78], [79][a]
4	***TRAF7***	Loss	Ubiquitin-protein transferase activity	[76]
5	***LATS2***	Loss	Mitosis, cytoskeleton damage response	[69, 74, 76]
6	***CDKN2B***[c]	Loss	Cell cycle regulation	[55, 69, 78], [79][a]
7	***SETD2***	Loss, fusion	Regulation of chromatin	[69, 75–77]
8	***FGFR3***	Loss	Cell shape, cell growth, cell adhesion	[76]
9	*PBRM1*	Loss, fusion	Regulation of chromatin, DNA replication	[69, 75, 77]
10	***HUWE1***	Loss	Ubiquitination	[76]
11	*GRM8*	Loss	Transcription regulation	[76]
12	*PTEN*	Loss, fusion	Phosphatase activity	[69, 74]
13	***TP53***	Loss	Cell division, DNA repair pathway, senescence, apoptosis	[55, 69, 74, 76]
14	***LATS1***	Loss	Cell cycle regulation	[69, 74]
15	***STK11***	Fusion	Protein tyrosine kinase	[69, 74], [75][b]
16	*CDH5*	Loss	Cell adhesion, cytoskeleton organization	[74]
17	*ERRFI1*	Loss	Cell growth, cell stress, cell signaling	[74]
18	*SDHB*	Loss	Citric acid cycle regulation, respiratory chain regulation	[74]
19	*RAP1*	Loss	Signal transduction, cell adhesion, cell junction formation	[74]
20	***RASSF1***[c]	Loss	Cell cycle regulation, apoptosis, DNA repair pathway	[74]
21	*DUSP7*	Loss	MAPK pathway	[74]
22	*PTPN13*	Loss	Apoptosis, cell growth, differentiation, mitotic cycle	[74, 77]
23	*PTPRD*	Loss	Cell growth, differentiation, mitotic cycle	[74]
24	*RB1*	Loss	Cell cycle regulation	[74, 77]
25	*ING1*	Loss	Cell growth arrest, apoptosis	[74]
26	*SPRY2*	Loss	Protein translocation	[74]
27	*CDKN3*	Loss	Cell cycle regulation	[74]
28	***SMARCB1***	Loss	Regulator of chromatin	[74, 75, 77]
29	*CHEK2*	Loss	DNA repair pathway, cell cycle arrest, apoptosis	[74, 75, 77]
30	*DMC1*	Loss	Meiotic homologous recombination	[74]
31	*RICTOR*	Gain	Cell growth, cell proliferation	[74]
32	*TRIO*	Gain	Actin remodeling, cell migration, cell growth	[74]
33	*RHEB*	Gain	Cell cycle regulation, cell growth	[74]
34	*DPP10*	Chrom break	Potassium channels regulation	[80]
35	*EPHA6*	Chrom break	Transferase activity	[80]
36	*EYS/PRIM2*	Chrom break	Integrity of photoreceptor cells	[80]
37	*NRG3*	Chrom break	Neuroblast proliferation, migration, and differentiation	[80]

(continued)

Table 4.2 (continued)

#	Gene	Aberration	Function	Reference
38	*NOS2A*	Chrom break	Oxidoreductase activity, neurotransmission, antimicrobial activity	[80]
39	*RAB11FIP4*	Chrom break	Regulation of endocytic traffic	[80]
40	*CA10*	Chrom break	Brain development	[80]
41	*MAP2K6/ CA10*	Chrom break	Activating protein kinase	[80]
42	*ARSG*	Chrom break	Hormone biosynthesis, modulation of cell signaling, degradation of macromolecules	[80]
43	*CCDC123 (CEP89)*	Chrom break	Organelle biogenesis and maintenance, cell cycle progression	[80]
44	*CHODL*	Chrom break	Neurogenesis, motor axon growth, and guidance	[80]
45	*DLG2*	Chrom break	Regulation of synaptic stability	[80]
46	*GRK5/ KCNJ12*	Chrom break	Apoptosis, cell proliferation, cell cycle regulation/controlling the resting membrane potential	[80]
47	*CCDC46 (CEP112)*	Chrom break	Cell division, centrosome	[80]
48	*TANC2*	Chrom break	Morphogenesis of the optic cup	[80]
49	***TERT***	Gain	Telomerase maintenance	[77, 81]
50	***CUL1***	Loss	Ubiquitination, protein degradation	[55]
51	*NOSIP*	Fusion	Ubiquitination	[69]
52	*LIFR*	Fusion	Cell differentiation, cell proliferation, cell survival	[69, 77]
53	*CLTC*	Fusion	Intracellular trafficking	[69, 77]
54	*RRBP1*	Fusion	Protein transport, translocation, transport	[69]
55	*DTD1*	Fusion	DNA replication	[69]
56	*RPTOR*	Gain	Cell growth	[69]
57	*BRD4*	Gain	Regulation of chromatin, DNA repair pathway, DNA replication	[69]
58	*TNFRSF14*	Gain	Host-virus interaction	[75]
59	*DVL1*	Gain	Developmental protein, cell proliferation	[75]
60	*ACSL6*	Gain	Fatty acid metabolism	[75]
61	*RECQL4*	Gain	Chromosome segregation, DNA repair	[75, 77]
62	*MYC*	Gain	Cell cycle progression, apoptosis, cellular transformation	[75]
63	*KDM5A*	Gain	Regulation of chromatin	[75]
64	*HOXC11*	Gain	Morphogenesis, cell growth	[75]
65	*HOXC13*	Gain	Morphogenesis, cell growth	[75]
66	*TRIM33*	Loss	Transcription regulation, ubiquitination	[75, 77]
67	*UBE4B*	Loss	Ubiquitination	[75]
68	*MLL3 (KMT2C)*	Loss	Methylation, transcription regulation	[75]
69	*WRN*	Loss	DNA repair, replication, transcription, telomere maintenance	[75]
70	*BMPR1A*	Loss	Cell differentiation	[75]
71	*SUFU*	Loss	Developmental protein, cell proliferation	[75]
72	*PTPN11*	Loss	Cell growth, differentiation, mitotic cycle	[75]
73	*CASC5 (KNL1)*	Loss	Chromosome segregation, spindle elongation	[75]
74	*RABEP1*	Loss	Endocytosis, protein transport, apoptosis	[75]
75	*SUZ12*	Loss	Chromatin regulation, methylation	[75]

Table 4.2 (continued)

#	Gene	Aberration	Function	Reference
76	***ASXL1***	Loss	Chromatin regulation, transcription	[75]
77	*PDGFB*	Loss	Embryonic development, cell proliferation, cell migration, cell survival, chemotaxis	[75, 77]
78	*MKL1*	Loss	Smooth muscle cell differentiation	[75, 77]
79	*EP300*	Loss	Chromatin regulation, cell growth, cell division, cell differentiation	[75, 77]
80	*PATZ1*	Loss	Chromatin regulation	[75, 77]
81	***MYH9***	Loss	Cytokinesis, cell shape, cytoskeleton reorganization	[56, 75, 77]
82	*CLTCL1*	Loss	Chromatin modeling, transcription regulation	[75, 77]
83	*BCR*	Loss	Chemical signaling, migration	[75, 77]
84	*RAF1*	Gain	Cell proliferation, cell differentiation, apoptosis, survival	[75][b]
85	***KIT***	Gain	Cell growth, cell division, cell survival, cell migration	[75][b]
86	*CCND3*	Gain	Cell cycle regulation	[75][b]
87	*TFEB*	Gain	Transcription regulation	[75][b]
88	*ELN*	Gain	Extracellular matrix structural constituent	[75][b]
89	*HIP1*	Gain	Structural constituent of cytoskeleton	[75][b]
90	*RUNX1T1*	Gain	DNA-binding transcription factor activity	[75][b]
91	*NOTCH1*	Gain	DNA-binding transcription factor activity	[75][b]
92	*RALGDS*	Gain	GTPase regulator activity	[75][b]
93	*FGFR2*	Gain	Cell shape, cell growth	[75][b]
95	*CCDN1*	Gain	Cell cycle regulation	[75][b]
96	*KRAS*	Gain	Cell proliferation, cell differentiation, apoptosis, survival	[75][b]
97	*FUS*	Gain	Regulation of gene expression	[75][b]
98	*HERPUD1*	Gain	Protein processing in endoplasmic reticulum, unfolded protein response	[75][b]
99	*BRCA1*	Gain	DNA repair pathway (HRR)	[75][b]
100	*RARA*	Gain	Regulation of development, differentiation, apoptosis, transcription	[75][b]
101	*CANT1*	Gain	Pyrimidine metabolism	[75][b]
102	*ELL*	Gain	Transcription	[75][b]
103	*AKT2*	Gain	Metabolism, cell proliferation, cell survival, cell growth, angiogenesis	[75][b]
104	*APOBEC3B*	Loss	Deoxycytidine deaminase activity	[77]
105	*MN1*	Loss	Transcription regulator	[77]
106	*EWSR1*	Loss	Gene expression, cell signaling, RNA processing, and transport	[77]
107	*MAPK1*	Loss	Cell proliferation, differentiation, transcription regulation, development	[77]
108	*SEPT5*	Loss	Cell division, cytoskeletal organization	[77]
109	*LZTR1*	Loss	Transcriptional regulator	[77]
110	*NCKIPSD*	Loss	Signal transduction, stress fiber formation	[77]
111	*SDHA*	Gain	Complex of the mitochondrial respiratory chain	[77]
112	*DROSHA*	Gain	miRNA synthesis	[77]
113	*ILR7*	Gain	VDJ recombination (lymphocyte)	[77]
114	*FCGR2B*	Gain	Phagocytosis, regulation of antibody production	[77]

(continued)

Table 4.2 (continued)

#	Gene	Aberration	Function	Reference
115	*CDC73*	Gain	Cell division, cell cycle	[77]
116	*PTPRC*	Gain	Cell growth, differentiation, mitosis	[77]
117	*MDM4*	Gain	p53 regulator	[77]
118	*ELK4*	Gain	Chromatin regulation, transcription	[77]
119	*SLC45A3*	Gain	Transmembrane transport	[77]
120	*HLF*	Gain	Transcription regulation	[77]
121	*MSI2*	Gain	Transcription regulation	[77]
122	***RNF43***	Gain	Ubiquitination	[77]
123	*PPM1D*	Gain	Cell stress response	[77]
124	***BRIP1***	Gain	DNA repair pathway (HRR)	[77]
125	*CD79B*	Gain	Transmembrane signaling receptor activity	[77]
126	*DDX5*	Gain	Coregulator of transcription, regulator of splicing, processing of small noncoding RNAs	[77]
127	*AXIN2*	Gain	DNA repair pathway (MMR), cell proliferation, cell death, ubiquitination	[77]
128	*PRKAR1A*	Gain	Ubiquitination	[77]
129	*ROS1*	Loss	Cell growth, differentiation	[77]
130	*CACNA1D*	Loss	Muscle contraction, hormone, or neurotransmitter release	[77]
131	*FLT3*	Loss	Hematopoiesis	[75, 77]
132	*FOXO1*	Loss	Myogenic growth, differentiation	[77]
133	*EPS15*	Loss	Cell growth	[77]
134	*WHSC1*	Loss	Transcriptional regulation, developmental transcription factors	[77]
135	*RAP1GDS1*	Loss	Proton-transporting ATPase activity	[77]
136	***FBXW7***	Loss	Cell cycle regulation, ubiquitination	[77]
137	*FAT1*	Loss	Cell proliferation	[77]
138	*NFIB*	Loss	Transcriptional activator	[77]
139	*MLLT3*	Loss	Chromatin regulation, transcription	[77]
140	*BRCA2*	Loss	DNA repair pathway (HRR)	[77]
141	*LHFP*	Loss	Transmembrane protein	[77]
142	*LCP1*	Loss	Actin-binding protein	[77]
143	*PMS2*	Gain	DNA repair pathway (MMR)	[77]
144	*EIF4A2*	Gain	Translation regulation	[77]
145	*HNRNPA2B1*	Gain	mRNA metabolism and transport	[77]
146	***EGFR***	Gain	Cell growth	[75, 77]
147	***MET***[c]	Gain	Cell survival, cell migration, embryogenesis, invasion	[77]
148	*RAD21*	Gain	DNA double-strand breaks pathway	[77]
149	*KLF6*	Gain	Transcriptional activator	[75][b], [77]
150	*NAB2*	Gain	Transcriptional regulator	[77]
151	*MLLT6*	Gain	Histone-binding protein	[77]
152	*CIC*	Gain	Transcriptional regulator	[77]
153	*FAM131B*	Gain	Cell proliferation, differentiation	[77]
154	*PLAG1*	Gain	Transcriptional activator	[77]
155	*CHCHD7*	Gain	Metabolism of proteins, mitochondrial protein import	[77]
156	*NUTM2B*	Gain	Intracellular protein	[77]
157	*NUTM2A*	Gain	Intracellular protein	[77]
158	*ETNK1*	Gain	Transferase activity	[77]
159	*DICER1*	Gain	Metabolism of RNA	[77]

Table 4.2 (continued)

#	Gene	Aberration	Function	Reference
160	***ZNF521***	Gain	Protein domain-specific binding	[77]
161	*ABL1*	Gain	Cell differentiation, cell division, cell adhesion, stress response	[79][a]
162	*COL1A1*	Gain	Member of group I collagen	[79][a]
163	*PITCH1*	Loss	Embryonic development	[78]

HRR homologous recombination repair, *MMR* mismatch repair
Genes underscored and in bold also harbor PTVs
[a]Gene that can also be lost by epigenetic mechanisms
[b]Tumor type not specified
[c]Peritoneal mesothelioma

Table 4.3 Mesothelioma genome: genes harboring somatic point mutations or small indels

#	Gene	Function	Reference
1	***BAP1***	Deubiquitinating enzyme, cell proliferation, DNA repair pathway (HRR)	[9, 55–57, 59, 68, 69, 74, 76, 78, 81–85], [79][a]
2	***NF2***	Cell shape, cell growth, cell adhesion	[55, 56, 68, 69, 74, 76, 81, 84], [78][d], [58][c]
3	***TP53***	Cell division, DNA repair pathway, senescence, apoptosis	[55, 56, 68, 69, 76, 81, 84, 85], [79][a], [58][c]
4	***LATS2***	Mitosis, cytoskeleton damage response	[76, 84]
5	***TERT***[b]	Telomerase maintenance, senescence	[81]
6	*RIF1*	DNA repair pathway, regulation of chromatin, regulation of replication timing	[76]
7	***CUL1***	Ubiquitination, protein degradation	[55]
8	*RDX*	Cytoskeleton	[55]
9	*TAOK1*	Transferase activity	[55]
10	*PIK3C2B*	Cell proliferation, cell survival, cell migration, and intracellular protein trafficking	[55]
11	***EGFR***	Cell proliferation, apoptosis, angiogenesis, cell migration, cell adhesion, invasion	[68], [79][a]
12	***LATS1***	Cell cycle regulation	[55, 84]
13	***SMARCB1***	Regulator of chromatin	[68, 74]
14	***CDKN2A***[e]	Cell cycle regulation	[69, 78, 81, 84], [58][c]
15	***CDKN2B***[e]	Cell cycle regulation	[78, 81, 84]
16	*PIK3C2A*	Cell proliferation, cell survival, cell migration, and intracellular protein trafficking	[68]
17	*PDGFRA*	Growth factors receptor	[68]
18	*HRAS*	Cell transduction, cell growth, cell division	[68]
19	***KIT***	Cell growth, cell division, cell survival, cell migration	[68]
20	*KDR*	Transferase activity	[68]
21	***STK11***	Protein tyrosine kinase	[68, 78]
22	***MET***[e]	Cell survival, cell migration, embryogenesis, invasion	[68]
23	***FBXW7***	Cell cycle regulation, ubiquitination	[68]
24	*SMAD4*	Cell proliferation	[68]
25	*ERBB4*	Cell growth	[68]
26	*CSF1R*	Cytokine involved in production, differentiation, and function of macrophages	[68]
27	*APC*[e]	Cell division, cell adhesion, cell polarization	[68]
28	*RET*	Cell proliferation	[68]

(continued)

Table 4.3 (continued)

#	Gene	Function	Reference
29	***FGFR3***	Cell shape, cell growth, cell adhesion	[68, 76]
30	***TRAF7***	Ubiquitin-protein transferase activity	[78]
31	*DDX3X*	ATP-dependent RNA helicase activity	[78]
32	*RYR2*	Calcium regulation	[78]
33	*CFAP45*	Cell migration	[78]
34	*SETDB1*	Methyltransferase activity	[69], [58][c]
35	*SETD5*	Methyltransferase activity	[69]
36	*ULK2*	Axonal elongation	[69]
37	*DDX51*	Nucleic acid binding and hydrolase activity	[69]
38	***SETD2***	Regulation of chromatin	[69], [78][d], [57][c]
39	*APOBEC2*	Cytidine deaminase, RNA editing	[56]
40	***MYH9***	Cytokinesis, cell shape, cytoskeleton reorganization	[56]
41	*PTPRT*	Signal transduction, cellular adhesion	[56]
42	***RNF43***	Ubiquitination	[56]
43	*SCRN2*	Dipeptidase activity	[56]
44	*CENPE*	Chromosome movement, spindle elongation	[56]
45	*RHOA*	Signal transduction pathway, cell adhesion	[56]
46	*SAV1*	Protein degradation, transcription, RNA splicing	[84], [58][c]
47	***RASSF1***[e]	Cell cycle regulation, apoptosis, DNA repair pathway	[84]
48	*STK3 (MST2)*	Apoptosis	[84]
49	*MST1*	Ciliary motility (lung cells), cell signaling	[84]
50	***HUWE1***	Ubiquitination	[76]
51	*NF1*	MAPK pathway	[79][a]
52	*PREX2*	GTPase activator	[79][a]
53	*KDM5C*	Chromatin remodeling	[79][a]
54	*KDM6A*	Demethylation	[78][c]
55	***ASXL1***	Chromatin regulation, transcription	[78][c]
56	***BRIP1***	DNA repair pathway (HRR)	[78][c]
57	*SMPD4*	Response to DNA damage, cellular stress, and tumor necrosis factor	[58][c]
58	*ARPC1A*	Actin filament binding	[58][c]
59	*PLA2G5*	Inflammatory response	[58][c]
60	*INTS4*	Transcription	[58][c]
61	*PIBF1*	Steroid hormone progesterone	[58][c]
62	*ATP1B2*	Electrochemical gradient establishing and maintaining	[58][c]
63	*PMSD3*	Embryonic development, growth control, homeostasis	[58][c]
64	*TTYH*	Ion transport	[58][c]
65	*LACE1*	Mitochondrial protein homeostasis	[58][c]
66	*ORM1*	Acute inflammation	[58][c]
67	*RHBDF1*	Cell survival, cell proliferation, cell migration	[58][c]
68	*KCNJ2*	Potassium channel	[58][c]
69	*P2RY12*	Platelet aggregation, blood coagulation	[58][c]
70	*ANKRD65*	Intracellular protein	[58][c]
71	*OIT3*	Liver development and function	[58][c]
72	*EED*	Histone methyltransferase activity, cellular senescence, embryonic development	[58][c]
73	*FOXM1*	Transcriptional activator, cell proliferation	[58][c]
74	*ICAM2*	Intercellular adhesion molecule	[58][c]
75	*KNCJ2*	Chondrocyte differentiation	[58][c]

Table 4.3 (continued)

#	Gene	Function	Reference
76	***ZNF521***	Protein domain-specific binding	[58][c]
77	*NLRP9*	Innate immune response	[58][c]
78	*PLXNB2*	Axon guidance, cell migration	[58][c]
79	*MSH5*	DNA repair pathway (MMR)	[58][c]
80	*EPBH2*	Developmental processes in the nervous system	[59][c]
81	*GTPBP3*	Mitochondrial tRNA modification	[59][c]
82	*STYK1*	Transferase activity	[59][c]
83	*TMEM18*	Transmembrane protein	[59][c]

HRR homologous recombination repair, *MMR* mismatch repair
Genes underscored and in bold also harbor CNVs
[a]Tumor type not specified
[b]Both in peritoneal and pleural mesothelioma
[c]Peritoneal mesothelioma
[d]GOF (gain of function)
[e]Gene that can also be lost by epigenetic mechanisms

MPM and show that MPM genomes include a large number of chromosomal abnormalities, such as CNVs and chromosomal translocations often leading to gene fusion, but a relatively low number of protein altering mutations compared with most tumors [60]. These alterations involve mostly tumor suppressor genes. A great inter-individual heterogeneity is also typical.

A recent study on CNVs in MPM was performed by Hylebos et al. [77]. They used information obtained using CGH arrays on 85 MPM patients and stored within The Cancer Genome Atlas (TCGA). Data were validated on a panel of 21 patients using low-pass whole genome sequencing. Both datasets showed losses on chromosomes 1, 3, 4, 6, 9, 13, and 22 in 25% of tumors. These losses included *CDKN2A*, *NF2*, *BAP1*, *EP300*, *SETD2*, and *PBRM1*. Copy number gains were less represented compared to losses. They were located on chromosomes 1, 5, 7, and 17 and occurred in 15% of tumors. Genes affected by these gains were *TERT*, *FCGR2B*, *CD79B*, and *PRKAR1A*. In conclusion, recurrent CNVs were detected in both datasets, occurring in regions harboring known MPM-associated genes and genes not previously linked to MPM.

The first studies addressing the MPM mutational landscape were reported by Lo Iacono et al. and Guo et al., independently in 2015, using different NGS approaches [55, 68]. A limit of both studies is that they included patients who had been subjected to chemotherapy; thus, it is possible that a portion of the mutations was due to the mutagenic effect of the drugs [60]. Lo Iacono et al. investigated 52 cancer genes in FFPE (formalin-fixed, paraffin-embedded) tumor samples of 123 MPM patients [68]. Mutated genes included *TP53*, *SMARCB1*, *BAP1*, *PDGFRA*, *KIT*, *KDR*, *HRAS*, *PIK3CA*, *STK11*, and *NF2*. The most represented pathways were the p53/DNA repair and the phosphatidylinositol 3-kinase-AKT. Guo et al. performed whole exome sequencing in fresh tumor samples from 22 patients [55]. These samples showed frequent genetic alterations in *BAP1*, *NF2*, *CDKN2A*, and *CUL1*. The MAPK and the Wnt signaling pathway frequently carried alterations.

Bueno et al. reported data on 216 MPM genomes, 99 of which were studied by whole exome and 103 by panel sequencing (344 genes) [69]. These data were paralleled by RNAseq, an approach that investigates all the RNA species transcribed and allows to validate the functional effect of genetic anomalies. They identified the following genes that are often mutated or lost in MPM: *BAP1*, *NF2*, *TP53*, *SETD2*, *DDX3X*, *ULK2*, *RYR2*, *CFAP45*, *SETDB1*, *DDX51*, *TRAF7*, and *SF3B1*. The pathways that were more frequently affected were Hippo, mTOR, histone methylation, RNA helicase, and p53 signaling [69].

De Rienzo et al. performed whole genome sequencing of 10 MPM patients [56]. The identified mutations and copy number aberrations were validated by targeted resequencing of 9 genes in 147 additional samples (*BAP1*, *NF2*, *TP53*, *MYH9*, *MYH6*, *MYH10*, *PIK3C2A*, *RHOA*, *TNFRSF1A*). A further 136 patients were analyzed for *TP53*, *BAP1*, *NF2*, and *CDKN2A*, which were the most frequently mutated genes. *TP53* variants were more often found in women. Interestingly, three patients showed germline PTVs (protein-truncating variants) in *BAP1* [56].

Exome NGS was also performed on cells from pleural effusions from 27 patients with MPM. Mutations in *BAP1*, *CDKN2A*, and *NF2* and loss of *TRAF7*, *LATS2*, *SETD2*, and *TP53* were identified [76], suggesting that analysis of pleural effusions might be used to monitor the MPM molecular evolution.

Looking at 61 primary mesothelioma cultures, Tranchant et al. identified a subgroup of tumors harboring both *LATS2* and *NF2* mutations [84]. Co-occurring mutations in these genes were associated with a poor prognosis. These cell lines showed abnormalities both in the Hippo signaling pathway and mTOR protein expression suggesting specific therapeutic strategies.

FFPE portions from 11 patients (7 MPM and 4 PM) were studied by Ugurluer et al. using a NGS panel including 236 cancer genes [78]. In MPM samples the mutations most commonly found were in *BAP1*, *CDKN2A/B*, and *NF2*. Other PTVs were found in *PTCH1*, *SETD2*, *STK11*, *KDM6A*, *ASXL1*, and *BRIP1*.

Two PM reported by Ugurluer et al. showed mutations in *BAP1* or *NF2*, whereas the other two did not show PTVs. The whole genome of two PM was reported by Sheffield et al. in 2015 [58]. The two patients reported different histology and different response to chemotherapy. The first had an epithelioid histology, a high disease burden, and did not respond to chemotherapy, whereas the second showed minimal clinical symptoms; histology was poor-prognosis sarcomatoid MM but responded well to treatment. The two tumors shared PTVs in *NF2* but were elsewhere very different. The first had only 18 variants, whereas the second had more than 260 variants in each of the 2 samples that were studied, corresponding to a status called somatic hypermutation. Another study focused on 12 patients with PM [59]. They used copy number analysis and exome sequencing and targeted sequencing and found a low number of CNVs (mostly losses) and SNVs. The gene that was more frequently affected was *BAP1*, whereas *NF2* and *CDKN2A* were not affected. One of the patients carried a nonsense germline variant paired to gene loss in the tumor; thus, he had *BAP1*-TPDS.

Overall, PM seems to have a mutation rate lower than MPM, but driver mutations in PM seem to affect the same genes that are often involved in MPM.

A limit of these studies is that they do not generally consider the hypothesis of intra-tumor heterogeneity, which may be an important issue in mesothelioma considering that there are hints of a polyclonal origin of carcinogenesis [86]. The paper by Zhang et al. focused on testicular MM is a good example of intra-tumor heterogeneity and rapid molecular evolution [87]. They performed whole genome sequencing using DNA obtained from FFPE samples of four successive tumors from a single patient. The first sample was obtained from the primary tumor, whereas the other samples were from a local recurrent tumor, an inguinal lymph node metastasis and a recurrent tumor from the same localization. This study evaluated the tumor progression looking at molecular events. The signature of molecular lesions and also the mutated genes were different from those reported for MPM. Other patients should be studied to evaluate whether this testicular MM is different from the other MM [87].

Tumor exome sequencing may give important information about carcinogenesis in individuals who develop multiple independent tumors. This approach was followed in the case of a 73-year-old male who developed two independent lung cancers (adenocarcinoma and squamous cell carcinoma) and a malignant PM with an epithelioid histology. The patient was a heavy smoker and did not report asbestos exposure. The somatic mutational signatures of the two lung tumors were in agreement with the smoking carcinogen effect, and the mutated genes corresponded to

those reported for the tumor types. Conversely, the PM showed a very low number of somatic events, including one PTV in *BAP1* and one in *SETD2*. Several low-risk variants in DNA repair genes could account for the PM predisposition in this patient.

The mutation types prevalent in the tumor genome may be identified in large studies [69]. In particular, Bueno et al. analyzed the mesothelioma exome for transitions (C > T, T > C) and transversions (C > A, C > G, T > A, T > G), taking into account the flanking base immediately 5′ and 3′ of the somatic base (so-called triplets). They identified five distinct signatures (S1, S2, S4, S5, and S6) that are operative in MPMs, two of them being the most represented (S1 and S2). The patterns of contribution of these signatures were different between MPM and lung cancers, in agreement with epidemiological studies that revealed that MPM is not related to smoking like lung cancer. For example, signature S3, characterized by C > A transversions, caused by bulky adducts, is not shown by MPM but is typical of cigarette smoking, an exposure that is not epidemiologically associated with MPM.

The S1 signature is characterized by no predominant transition or transversion and is considered indicative of a base-agnostic mutagen such as reactive oxygen species (ROS) [88, 89]. The S2 signature is represented by C > T transitions at NpCpG trinucleotides and is attributed to an endogenous mechanism, the deamination of 5-methylcytosine to thymine in CpG dinucleotides. The S4 signature is characterized by C > T transitions and is typical of repair errors at UV-induced pyrimidine dimer sites observed in melanoma. Signature S5 shows C > T transitions or C > G transversions at TpCpN nucleotides, considered as indicative of the function of APOBEC enzymes responsible for cytidine deamination and frequently activated in cancer [88, 89].

In conclusion, the study of Bueno et al. identified a mutational pattern concordant with the effect of asbestos exposure (i.e., S1 signature) [69]. The authors did not observe a significant difference of this signature in samples with ($n = 69$) or without (17) asbestos exposure, but this may depend on the fact that asbestos fiber quantification in the lung was available only for 64/217 patients, whereas asbestos exposure of the other patients was reported, but not quantified.

Overall it is expected that asbestos causes DNA damage in two ways, first by inducing chromosomal breaks by interfering with spindle fibers during cell division and second by inducing inflammation and ROS production. The first mechanism may explain some of the chromosomal rearrangements whereas the second some of the point mutations.

4.4 Translation to the Clinics: Druggable Targets

The identification of driver mutations in mesothelioma is expected to pave the way to precision oncology. In general, this task may be particularly difficult in MPM, considering the wide inter-individual and possibly intra-tumor heterogeneity. Moreover, MPM driver mutations in protein-coding genes are rarer than in other tumors [72]. On the other hand, it is important to note that all these studies reported a frequent involvement of *BAP1*, *NF2*, *CDKN2A*, and *SETD2*.

A thorough evaluation of possible translational steps is beyond the scope of this review, and we refer to other chapters of this book and specific literature [90, 91]. We only mention that PARP or EZH2 inhibitor drugs have been considered for tumors characterized by *BAP1* loss, CDK4/6 or PRMT5 inhibitors for tumors with *CDKN2A* mutations, FAK inhibitors for tumors with *NF2* mutations, and PI3K-AKT inhibitors for tumors with *PI3K-AKT* abnormalities [90]. More in detail, a phase II clinical trial in *BAP1*-deficient patients with the EZH2 inhibitor, tazemetostat, was recently opened to accrual (NCTO02860286); a phase II clinical trial to evaluate the CDK4/6 inhibitor, ribociclib, in solid tumors carrying relevant CDK4/6, cyclinD1/3, or $p16^{INK4A}$ aberrations, including MPMs, has been designed (NCT02187783); while after a randomized switch maintenance, clinical trial (NCT01870609) with the FAK inhibitor defactinib (VS-6063) versus placebo was discontinued

in late 2015, in 2016 a new single-center clinical trial tested defactinib before surgery for MPM (NCT02004028); at last, the modest response obtained in a phase I study of apitolisib (GDC-0980), dual phosphatidylinositol-3-kinase, and mammalian target of rapamycin kinase inhibitor (NCT00854152) indicated that combination regimens must be explored.

Conversely, predisposing factors may also give some therapeutic opportunities to the patients that carry them. Patients with ovarian cancer and germline variants in *BRCA1* or *BRCA2* respond to PARP1 inhibitors drugs, through a mechanism called synthetic lethality [92, 93]. This mechanism is induced when two (or more) variants are not lethal singularly but are lethal when both are present in a cell [94]. PARP1 is a nuclear enzyme that functions in three DNA repair systems, i.e., SSBs (single-strand breaks), BER (base excision repair), and alt-NHEJ (alternative nonhomologous end joining) [95]. PARP1 binds to SSBs and causes the formation of polymers of ADP-ribose (PAR) on its target proteins (this phenomenon is called PARylation). PARs are required for the recruitment of SSBs repair scaffolding proteins. PARP1 auto-PARylation is followed by its release from DNA and inactivation [94]. PARP1inhibitors traps PARP1 to the site of DNA damage and interfere with the progression of the replication fork causing the accumulation of SSBs that evolve to DSBs (double-strand breaks), following replication fork collapse. Both HRR and NHEJ (nonhomologous end joining) are used to repair DSBs and restart replication forks stalled by PARP1 inhibitors. When HRR is deficient, because of loss of *BRCA1* or *BRCA2*, the damage cannot be repaired by alt-NHEJ, because this system requires PARP1. If these systems are not functional, cells can only use classical NHEJ, which causes chromosomal anomalies, genomic instability, and cell death [96].

PARP1 inhibitors could inhibit growth of cells that have lost both *BAP1* alleles either because of a germline and a somatic variant or because of two somatic variants. Tumor cells in patients with a germline variant in *BAP1* have a very high likelihood of a second somatic variant on the wild-type allele. Thus, theoretically, in patients with a germline variant in *BAP1* MPM, tumor tissue could have a more homogeneous *BAP1* loss than in sporadic patients and may better respond to this treatment. Patients with germline mutations in other HRR genes may also show such behavior.

4.5 Tumor Epigenetics

The mechanisms underlying tumor initiation and progression involve also epigenome aberrations that share an intricate relationship with genetic instability in the tumor evolution process.

Epigenetic includes three main regulatory mechanisms: histone modifications, DNA methylation, and microRNA (miRNA)-mediated gene regulation.

Histones are members of a highly conserved family of proteins that associate with DNA to organize chromatin in the nucleus. Several post-translational modifications may occur at N-terminal histone tails, including the addition or removal of methyl and acetyl residues. Histone modification is associated with the transcriptional regulation of genes, promoting the transition between open and close chromatin conformation.

DNA methylation consists in the addition of a methyl residue (–CH3) to the cytosine residues within the dinucleotide CpG. DNA methylation mainly occurs at the carbon-5 position of the cytosine ring [97], even though a small fraction (~2%) may occur at cytosines in any context of the genome, or also in a non-CpG context in embryonic stem cells [98]. CpGs DNA methylation may occur in gene promoters, where a high concentration of CpGs dinucleotides can be seen in the so-called CpG islands. Promoter DNA methylation is a well-known mechanism to repress gene transcription, leading to gene silencing through inhibition of transcription factor binding to DNA [99]. Deregulation of the DNA methylation levels may result in cell transformation. Diffuse genome-wide hypomethylation is frequently seen in cancer cells, together with site-specific hypermethylation [100, 101].

miRNAs are a class of small noncoding RNAs involved in gene silencing through a posttran-

scriptional mechanism that requires miRNA binding to 3′-UTR regions of mRNAs and leads to translation inhibition or mRNA degradation [102]. Dysregulation of miRNAs has been associated to cancer development [103–105], and they have been proposed as tools for cancer diagnosis, classification, prognosis, and treatment [106–109].

Epigenetic alterations may be critical determinants of malignant transformation of pleural mesothelial cells following asbestos exposure. The relationship between DNA methylation modifications and *in vitro* asbestos exposure in MeT5A mesothelial cell lines was recently described [110]. The authors report slight DNA methylation in MeT5A cells after both crocidolite and chrysotile treatments, mainly in genes involved in the regulation of cellular matrix and adhesion, which are mechanisms for mesothelial infiltration and injury, facilitating epithelial-to-mesenchymal transition (EMT) in MPM. This finding may suggest an involvement of methylation changes as potential modulators of asbestos-induced pleural injury.

Evidence of relationship between asbestos burden and promoter methylation of selected tumor suppressor genes (*APC*, *CCND2*, *CDKN2A*, *CDKN2B*, *HPPBP1*, and *RASSF1*) was also reported in lung tissue from MPM patients. Moreover, the increase in methylation of these genes correlates with asbestos body counts [111]. Inactivation of *CDKN2A* by methylation was also reported by Kobayashi et al. [112].

The examination of over 6000 CpG islands in MPM and lung adenocarcinomas showed that 387 genes (6.3%) and 544 genes (8.8%) were hypermethylated in MPM and adenocarcinoma, respectively, and that the two malignancies have characteristic DNA methylation patterns, likely a result of different pathologic processes [113]. Moreover, Goto et al. suggest that *KAZALD1*, *MAPK13*, and *TMEM30B* genes, which were specifically methylated only in MPM, could serve as potential diagnostic markers.

In a larger study of 158 mesothelioma specimens and 18 normal pleura samples, Christensen et al. reported that the DNA methylation profile of 803 cancer-associated genes was able to discriminate normal pleura from mesothelioma and was a predictor of shorter survival [114].

Aberrant promoter methylation of *WIF-1* and *SFRP1*, *2*, *4* genes was found in MPM tissue and mesothelioma cell lines [115]. The analysis of 52 MPM samples and 38 histologically non-tumor lung samples identified higher methylation levels of *ESR1*, *SLC6A20*, and *SYK* genes in MPM [116]. The combination of *SLC6A20*, *SYK*, and *APC* yielded a sensitivity of 92% and a specificity of 73% as positive markers for MPM. The inclusion of *ESR1* methylation as a third positive marker increased sensitivity but reduced specificity.

Cheng et al. [117] reported downregulation of the *ZIC1* gene via promoter methylation in MPM. This gene acts as a tumor suppressor, targeting apoptosis-related miRNAs. In particular, miR-23a and miR-27a are expressed at higher levels in epithelioid MPM patients with shorter survival. These studies highlight that epigenetic silencing through promoter hypermethylation is a frequent event in MPM.

Other studies looked for miRNAs involved in MPM development. Guled et al. [118] identified a number of miRNAs that were differentially expressed between MPM tissue and normal pericardium.

With an *in vitro* study, Pass et al. reported that miR-29c-5p may be a tumor suppressor in MPM and thus a potential therapeutic target [119].

Several miRNA-targeted therapeutics have reached clinical testing. For example, miR-16 is involved in a phase I clinical trial, MesomiR 1. The trial is based on the work by Reid et al. who reported the downregulation of miR-15-16 in MPM tissue and cell lines associated with increased levels of the target oncogenes *CCND1* and *Bcl-2* [120]. Restoring miRNA expression, cell growth is inhibited, and cells acquire sensitivity to gemcitabine and pemetrexed. miR-16 is also a regulator of programmed death ligand 1 (*PD-L1*) in MPM and may therefore contribute to immune system evasion [120].

In MPM, miR-34b/c and miR-126 are regulated by methylation and oxidative stress [121, 122]. Several studies showed that miR-34b/c is a regulator of C-*MET* and *BCL*-2 oncogenes, and

its downregulation promotes transformation of mesothelial cells [122–124]. *In vivo* studies showed that during oxidative stress, miR-126 compromises mitochondrial function, induces autophagy by altering cell metabolism, and inhibits cell growth and tumor formation, showing that increased autophagy has a protective role in MPM [121, 125].

The identification of miRNA target genes is of paramount importance for understanding how these small noncoding RNAs regulate MPM cell function. A recent approach [126] identified miR-21-5p as a candidate regulator of *MSLN* (mesothelin). The increased expression of miR-21-5p reduced MSLN expression and inhibited MPM cell proliferation, uncovering a potential tumor suppressing miRNA in MPM.

A single miRNA can regulate many genes, and one gene may be targeted by many miRNAs. *MCL-1* is overexpressed in MPM and is associated with the resistance to apoptosis and chemotherapy [127]. Khodayari et al. reported that the transfection of MPM cells with miR-302b reduced MCL-1 expression, decreasing cell and tumor growth and inducing apoptosis [128]. The same antitumor activity has been observed for miR-193a-3p, suggesting that miRNA replacement therapy to target *MCL-1* may provide an effective treatment for MPM [129].

4.6 Epigenetic as a Potential Diagnostic Biomarker

Epigenetic markers are considered potential biomarkers for early diagnosis and prognosis in cancer research [130].

DNA methylation is rather stable but may change across time [131], and it can be modified by several factors during lifetime [132], such as lifestyle, environmental exposures, aging, and diseases [133, 134]. The DNA methylation asset of each individual is thus considered as an adaptive phenomenon potentially linking environmental factors and development of disease phenotypes [135]. Aberrant DNA methylation is found as an early event in tumor development and has been suggested as a tool for early cancer detection and prognosis [136, 137], including MPM [138].

Whereas tumor tissue DNA methylation is widely investigated in MPM, only few studies addressed the relationship between DNA methylation in blood-derived specimens and MPM.

With a targeted study focused on free serum DNA of mesothelioma patients, Fischer et al. [139] investigated the methylation status of the promoter region of nine candidate genes that were previously shown to be epigenetically altered in MPM tissue and cell lines. The authors reported hypermethylation in the promoter region of *FHIT* and the gene encoding for E-cadherin and to a lower extent *ACP1A*, *RASSF1A*, and *DARK* genes. Intermediate values were observed for *CDKN2A*, *APC1*, *ARF*, and *RARβ* [139]. The same study reported a correlation of the methylation levels of *DAPK*, *RASSF1A*, and *RARβ* genes with overall survival, though the effect was only seen in combination.

A recent study [140] investigated for the first time the whole genome DNA methylation levels in peripheral blood cells to assess the potentiality of DNA methylation profiles in blood to discriminate MPM cases from asbestos-exposed controls without MPM. The authors report significant case/control differential DNA methylation (>800 CpG sites) with consistent hypomethylation in MPM cases with respect to controls. Moreover, a small panel of seven differentially methylated CpGs was able to significantly increase discrimination between cases and controls (AUC = 0.81 vs AUC = 0.89) when considering DNA methylation together with asbestos exposure vs asbestos exposure alone.

miRNAs have been also suggested as promising candidates for the development of noninvasive techniques for early cancer detection and as therapeutic targets [141, 142]. Specific miRNA profiles have been suggested as diagnostic/prognostic biomarkers also for MPM [143–146]. Aberrant miRNA profiles have been already described in MPM tissue and biological fluids [145, 146]. Weber at al. [147], in a pilot study, identified miR-103a-3p in peripheral blood cells as a potential marker for the discrimination of mesothelioma patients from both asbestos-

exposed controls and general population. The use of miR-103a-3p improved the discrimination power of serum mesothelin, reaching a sensitivity of 95% and a specificity of 81% when the two biomarkers were combined [147].

More recently, Cavalleri et al. further validated the suitability of miR-103a-3p as a MPM biomarker. A miR-103a-3p/miR-30e-3p signature of plasma-derived extracellular vesicles distinguished MPM patients from subjects reporting a past asbestos exposure with a sensitivity of 95.5% and a specificity of 80.0% [148]. While miR-103a-3p is a potential biomarker, several other studies that investigated miRNA deregulation in plasma/serum yielded heterogeneous and inconclusive results.

miR-200 family members have been suggested as potential candidates for discriminating MPM from lung cancer [144, 145, 149, 150]. Gee et al. reported downregulated miRNAs as potential biomarkers to distinguish MPM and lung adenocarcinoma [149]. Also Benjamin et al. identified a panel of three deregulated miRNAs (miR-193-3p, miR-200c, miR-192) reaching a sensitivity of 100% and a specificity of 94% to discriminate MPM from carcinoma of epithelial origin that may invade the pleura [145, 150]. High diagnostic accuracy was also reached by using a panel of four miRNAs (miR-126, miR-143, miR-145, and miR-652) that were significantly downregulated in MPM compared with nonneoplastic pleura [151]. Santarelli et al. quantified the levels of 88 miRNAs reported to be associated with cancer in 10 samples of MPM and 1 sample of healthy mesothelial tissue using a customized PCR Array [146]. The study identified three miRNAs (miR-335, miR-126, and miR-32), but only miR-126 replicated in 27 FFPE MPM samples and 27 adjacent healthy pleural tissues. Limits of these studies were the small number of miRNA investigated and the different methods used to preserve samples (RNA later in discovery and FFPE in replication phase).

The downregulation of miR-126 is also a significant prognostic factor associated with poor survival [152]. Andersen et al. showed an epigenetic downregulation of miR-126 and its host gene *EGFL7*. Silencing of *EGFL7* is associated with a poor clinical outcome in epithelioid subtype [152]. Understanding DNA hypermethylation of *EGFL7* and miR-126 may provide potential avenue for therapeutic intervention.

The first study suggesting that miRNA can be used to predict survival outcomes identified miR-29c-5p as an independent prognostic factor for time to disease progression [119]. Pass et al. identified a signature as a potential tool for predicting survival, based on the expression of let-7c-5p and miR-151a-5p in 52 MPM tumors [153].

4.7 Conclusions and Future Developments

The identification of driver mutations in MPM is a prerequisite for precision medicine, and the results are expected in the long run. The presence of germline predisposing mutations in tumor suppressor genes may be useful to identify the driver genes in cancers and address their specific therapy. miRNAs are also attractive therapeutic targets because of their powerful regulatory functions.

Additionally, different epigenetic profiles, which include miRNA and DNA methylation, in peripheral blood might be a useful tool to monitor exposed subjects.

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