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Mesotheliomas in Genetically Engineered Mice
Unravel Mechanism of Mesothelial Carcinogenesis

Didier Jean 1,2,3,4 and Marie-Claude Jaurand 1,2,3,4,*

Abstract: Malignant mesothelioma (MM), a rare and severe cancer, mainly caused as a result of past-asbestos exposure, is presently a public health concern. Current molecular studies aim to improve the outcome of the disease, providing efficient therapies based on the principles of precision medicine. To model the molecular profile of human malignant mesothelioma, animal models have been developed in rodents, wild type animals and genetically engineered mice harbouring mutations in tumour suppressor genes, especially selecting genes known to be inactivated in human malignant mesothelioma. Animals were either exposed or not exposed to asbestos or to other carcinogenic fibres, to understand the mechanism of action of fibres at the molecular level, and the role of the selected genes in mesothelial carcinogenesis. The aim of the manuscript was to compare mesothelioma models to human malignant mesothelioma and to specify the clue genes playing a role in mesothelial carcinogenesis. Collectively, MM models recapitulate the clinical features of human MM. At least two altered genes are needed to induce malignant mesothelioma in mice. Two pathways regulated by Cdkn2a and Trp53 seem independent key players in mesothelial carcinogenesis. Other genes and pathways appear as bona fide modulators of the neoplastic transformation.

Keywords: malignant mesothelioma; mesothelium; mineral fibres; gene mutations; tumor suppressor genes; signalling pathways; carcinogenesis

1. Introduction

Human malignant mesothelioma (HMM) is a cancer with current poor outcome, which is diagnosed with advanced non-curable disease. HMM has a strong association with asbestos exposure, a natural mineral fibre. The present researches mainly aim to find efficient therapeutics. Many of the current studies focus on target therapy to counteract the physio-pathological mechanisms allowing mesothelioma cells to grow in and invade their microenvironment, and to escape from the immune survey. For that purpose, mesotheliomas are developed in so called “mesothelioma models”, which include orthotopic or heterotopic xenografts of human mesothelioma cell lines and patient-derived xenografts in immunodeficient mice [1,2]. Moreover, experimental mesotheliomas models have been developed for different purposes. Malignant mesotheliomas (MM) models have been generated to understand the carcinogenic mechanism induced by asbestos fibres or to identify the most relevant genes and important signalling pathways associated to mesothelial cell transformation. This aim was developed with both in vitro studies on mammalian cells, including mesothelial cells, and in vivo studies in animals [3]. Efforts have been also made to generate MM in animals treated or not treated with asbestos fibres. More recently, recombinant inbred mouse lines were designed to
determine the genetic bases of the disease. In this context, genetically engineered mice (GEM) carrying genes modified to mimic the human disease were chosen and exposed or not to carcinogenic fibres. These experiments allow comparison between mesotheliomas developed in different genetic context and possibly emphasise specific clinical and molecular features.

The application of target therapy needs a deep knowledge of the tumour microenvironment characteristics to permit an appropriate way to suppress tumour cell proliferation, survival, migration, invasiveness and impair the interactions with the microenvironment as final outcome to eradicate the tumour. The different animal models may bring some relevant knowledge of the specific molecular pattern of the tumours and of the disease. In this review, we will discuss the features of mesothelioma induced in animals and to what extent they are close to the HMM.

2. Human Malignant Mesothelioma

2.1. Human Malignant Pleural Mesothelioma

The clinical and pathological features of pleural MM will be briefly summarised here. Several reviews can be suggested to the reader [4–6].

2.1.1. Natural History

The major risk factor for malignant pleural mesothelioma (MPM) is a past exposure to asbestos fibres, and more than 80% of MM are located in the pleura as a result of inhalation exposure. MPM occurs after a long delay, up to 40 years, after the beginning of exposure. However, malignant peritoneal mesothelioma (MPeM) is also found in asbestos-exposed patients, exceptionally in the testis [7]. MPM can be found in populations not exposed for occupational reasons, but showing domestic or environmental exposures [8–11].

2.1.2. Histological Classification

On the basis of histological morphology, MM is divided into three major histologic types, epithelioid, sarcomatoid, or mixed (biphasic) categories. Epithelioid and sarcomatoid categories have several secondary growth patterns as reported by Hussain et al. [12].

2.1.3. Physiopathology

Mesothelial cells form a monolayer at the surface of the mesothelium. Their cellular morphology is not uniform, depending on the regional location with flattened, intermediate, cuboidal and microvilli-rich mesothelial cells. Mesothelial cells play an important role in maintaining pleural homeostasis [13]. Pleuro-lymphatic communication is made through stomas [14]. In human, stomas open at the mesothelial surface and extend into a lymphatic capillary connected to the submesothelial lymphatics [15]. Inhaled asbestos fibres are deposited in the respiratory airways, reach the lung and are translocated into the pleura. The presence of fibres has been demonstrated both in the human pleura and in animals [16,17].

2.1.4. Molecular Alterations in MPM

Many publications have reported molecular alterations in MPM (see for review [6,18]). They concern copy number alterations (CNAs) of chromosome regions, gene mutations and epigenetic modifications. One recurrent finding is the numerous chromosome rearrangements, with several specific chromosomal gains on 1, 5, 7 and 17 or losses on 1, 3, 4, 6, 9, 13 and 22 [19]. Losses in 3p21, 9p21, 14q and part or whole chromosome 22 were recurrently observed. These loci contain many tumour suppressor genes (TSGs) such as BAP1, CDKN2B, CDKN2A, and NF2 which are frequently inactivated. Other genes of interest, LATS2, SETD2 and TP53 are inactivated at a lower extent [20–22]. A loss on the chromosome region 14q11.2–q21 was the only difference detected between patients exposed (loss) and not exposed (no loss) to asbestos [23,24]. Gene alterations consisted in base substitution,
apuric or apurinic base losses, deletion of one or several exons, or the whole gene. Gene fusions and splice alterations were also described mostly in NF2, BAP1 and SETD2 genes [22]. So far, no recurrent oncogene was found altered in MM, but an oncogenic hotspot mutation was reported in the promoter of TERT in 15% MPM [25]. However amplification of oncogenes such as PDGFRB, MYC or VEGFR could play a role in mesothelial neoplastic transformation [26–28].

Investigation of epigenetic changes demonstrated changes in gene methylation, and differential expression in non-coding RNAs such as microRNAs and long non-coding RNAs in comparison with normal cell [29]. It is known that miRNAs interact with the regulation of oncogenes and TSGs and can work either as oncogenes or TSGs [30]. Methyloyme analyses have shown a variety of methylation profiles in MPM, and an association with asbestos exposure [31]. Analysis of promoter methylation of cell cycle control genes showed that the number of methylated genes was a predictor of asbestos exposure [32]. MiRNome analyses also revealed differential expression between MPM and normal counterparts, between MPM and reactive pleural cells and between histological categories [33–35].

2.1.5. Alterations in Regulatory Pathways

Whole genomic and transcriptomic analyses have emphasised the regulatory pathways activated or inactivated in MPM. Hippo and PI3K/AKT/mTOR are deregulated either because of the mutation in critical genes of the pathway and/or inappropriate activation of members of the pathway [36–38]. Other regulatory pathways that play a role in development and cell differentiation are reported to be differentially activated in comparison with normal cells, Hedgehog that is associated with the maintenance of cancer stem cells, and Wnt, a pathway, which plays a role in intracellular signal traffic [39–41]. Important deregulation of the mitotic spindle assembly checkpoint pathway (MSAC) and microtubule network has been reported in MPM, although no mutation was detected in these genes [42]. Highest levels of expression of genes of the MSAC pathway, notably in sarcomatoid MPM [42].

2.1.6. Molecular Classification of MPM

In addition to histological classification, molecular classification of MPM was performed from transcriptomic analyses. Studying primary MPM cultures and tumour samples by transcriptomic microarray resulted in the definition of two molecular classes (C1 and C2) [43]. Gene mutations were investigated in selected genes BAP1, CDKN2A, CDKN2B, NF2 and TP53. Briefly, BAP1 alterations were more frequent in C1 and epithelioid MPM were found in both groups, with a worse survival prognosis in the C2 subgroup. Pathway analysis revealed that EMT was differentially regulated between MPM subgroups [43].

In an extensive study, transcriptomes, whole exomes (n = 99) and targeted exomes were analysed in MPM tumours [22]. Using RNA-seq data, four molecular subtypes were defined, sarcomatoid, epithelioid, biphasic-epithelioid (biphasic-E) and biphasic-sarcomatoid (biphasic-S). In this study, genes significantly mutated were identified: BAP1, NF2, TP53, SETD2, DDX3X, ULK2, RYR2, CFAP45, SETDB1 and DDX51, and a multitude of mutations in several genes. These mutations result in the alteration of several signal pathways such as Hippo, mTOR, histone methylation, RNA helicase and p53 pathways. Hippo pathway was altered in all molecular subtypes, mTOR more in biphasic-S. Histone methylation and BAP1 alteration were more frequent in epithelioid MPM. Six mutation signatures were identified, but none was associated to asbestos exposure [22].

Gene expression was also investigated to differentiate MPM cells and benign mesothelial hyperplasia (MH) using NanoString technologies in tumour tissues [33]. One hundred and seventeen genes were selected. An unsupervised cluster analysis defined two clusters, one composed only of MPM and one only of MH samples. Interestingly, this approach identified already known mesothelioma genes, BAP1 and NF2 being downregulated, and MSLN, which encodes mesothelin, upregulated in MPM in comparison with MH. In contrast, CDKN2A was not statistically deregulated
in MPM in comparison with MH [33]. This suggests different roles of these genes in the neoplastic progression of mesothelial cells.

2.1.7. MPM Response to Treatments

There is agreement that globally, MPM survival is dependent on the histological subtype; epithelioid mesothelioma having better prognosis than sarcomatoid mesothelioma. The recent molecular analyses have shown that the outcome of MPM is also related to the molecular group with differential outcome within epithelioid mesothelioma [43].

2.2. Malignant Peritoneal Mesothelioma

MPeM also found as a result of asbestos inhalation, is reported as slightly different from MPM. As in MPM, the major histologic types of MPeM as in MPM are found, with the epithelioid type being the most frequent. Histologic variants comprise heterologous (osteosarcomatous, chondrosarcomatous, and rhabdomyosarcomatous) elements and desmoplastic mesothelioma [44]. However, MPeM shows differences with MPM in terms of survival, which is longer than MPM. The main risk factor remains asbestos exposure in about 50% of the cases, lower than in MPM [45].

Genome wide analysis of epithelioid MPeM and MPM showed similarities in CNAs [24]. Overall, regions of copy number gain were more common in MPeM, whereas losses were more common in pleural MPM. Losses occurring in 3p, 9p and 22q genomic regions carrying the TSGs BAP1, CDKN2A and NF2, respectively were seen at a statistically significant higher rate MPM than in MPeM [24]. The authors studied CNAs in groups of different exposures and found different results. Patients with history of medical radiation exposure showed multiple regions of gain, including 1q, 3p, 3q and 5p. Region of losses in 6q, 14q, 17p and 22q and gains 7q, 10p, 10q, 17q were found in tumours from asbestos-exposed patients [24]. Recurrent mutations are also found in similar genes than MPM [46], even if specific alterations were described in subgroup of MPeM such as ALK rearrangement [47].

2.3. Conclusions on Human Malignant Mesothelioma Biology

HMM appears to have a spectrum of different features. First, MM can grow in the serosa of the pleura, peritoneum, pericardium or tunica vaginalis. The MM tumour morphology is heterogeneous. MM cells in different tumours differ by their physiological and genomic status, and relationship with their microenvironment. Although some physiological and molecular alterations are recurrently found in mesothelioma cells, sometimes at a high rate, given tumours have specific features that need to be known to more precisely define groups of tumours and perform precise therapeutics. In the following, it is discussed to what extent models of MM are close to HMM.

3. Models of Malignant Mesothelioma

Mesotheliomas have been developed in rodents by injection of asbestos fibres in wild type (WT) rats or in mice and GEM mice, exposed or non-exposed to asbestos, refractory ceramic fibres (RCF) or carbon nanotubes (CNT).

3.1. Spontaneous Mesotheliomas in Wild Type Rodents

Spontaneous mesotheliomas that occur in control or sham cohorts in toxicological studies using rats are rare events. An incidence of 4.3% (7/395) of genital and serosal mesotheliomas, and only one pleural mesothelioma has been reported in male rats, with a variety of morphological patterns [48]. More recently, 0.2–5% mesotheliomas of the tunica vaginalis (MTV) were classified as epithelioid, sarcomatoid of mixed, consistent with the histologic classification in HMM [49]. Spontaneous mesotheliomas were reported in male F344/N rats controls in a summary over 5 decades from 2-year National Toxicology Program carcinogenicity bioassays. The frequency was 0.2–5% MTV [49]. Spontaneous mesothelioma is also rare in mice [50–52].
3.2. Mesothelioma in Animal Experiments

3.2.1. Asbestos-Induced Mesotheliomas in WT Animals

These studies were carried out mainly in rats, less in mice. The aim was to investigate the carcinogenicity of different types of fibres [53]. Rats were exposed by inhalation, intra-tracheal instillation or intra-serosal administration. Lung tumours and mesotheliomas were observed at different rates, depending on the route of exposure and fibre type [54]. The natural history of mesotheliomas showed similarities with HMM, they occurred after a long delay and ascites developed after exposure via the intra-peritoneal route. Histological analyses reported similar features as found in HMM, but epithelioid is not the most frequent histologic category. For instance, after administration in the pleural cavity of rats, reported histologic types were tubulo-papillary (a category of pleural epithelioid, 8.2%), mixed (74.8%) and spindle (16.9%) MM [55].

Recently, a whole exome sequencing of asbestos-induced murine mesotheliomas (MuMM) was performed in 3 different strains of WT mice stains, BALB/c, CBA and C57BL/6, and 15 MM cell lines were analysed, obtained from 4, 4 and 6 ascites, respectively [56]. In all but one cell line, recurrent genomic changes included homozygous (Hom) loss of Cdkn2a (this gene encodes two proteins, p16\textsuperscript{Ink4a} and p19\textsuperscript{Arf}) and deletion in Lats2 and Setd2, but no mutation in Bap1 or N\textsuperscript{2}. Hom loss of Trp53 was found in one cell line. Mutation signature was principally C to T, as found in HMM, and G to A transitions were also found. BALB/c cell lines carried more mutations than the others. Several pathways were affected by mutations such as Wnt, Hedgehog, Notch, mTOR, MAPK and p53 pathways, but not Hippo [56]. These results suggest a unique key role of Cdkn2a in murine mesothelial carcinogenesis. Moreover, mesotheliomas arose in the absence of alteration of Bap1 and N\textsuperscript{2}, as in HMM, consistent with a role of other pathways affected by the genes mutated at low frequency, or epigenetic mechanism.

An epigenetic mechanism of inactivation of Cdkn2a locus was suggested to be an initial step of MuMM induction, leading later to allelic deletion of Arf, in WT mice exposed to CNT by intrapleural instillation [57].

3.2.2. Mesothelioma in GEM

Spontaneous MuMM

GEM heterozygous (Htz) or homozygous (Hom) in N\textsuperscript{2}, Bap1, Cdkn2a (Ink4a and/or Arf), Trp53 or Bap1 genes, either alone or in combination, were generated, based on the knowledge of the TSGs genes playing a role in mesothelial carcinogenesis. GEM in Rb, Tsc1 and Pten were also generated despite the absence of mutation in HMM [58–61]. Tables 1 and 2 summarize the different studies carried out with GEM.

One MuMM was reported (6%) in N\textsuperscript{2}K\textsuperscript{O3}/+ carrying the loss of N\textsuperscript{2} exon 3 [8]. Jongsma et al. [59] injected AdCre in the pleural cavity of mice carrying conditional mutant alleles in N\textsuperscript{2}, Cdkn2a, Trp53 or Rb, and Htz Ink4a mutant [59]. The highest rate of thoracic MuMM was observed in double mutants N\textsuperscript{2} and Cdkn2a, Trp53 or Rb and triple mutants N\textsuperscript{2}, Trp53 and Ink4a. Mutations in Cdkn2a, Ink4a or Trp53 were the most pejorative in term of MuMM incidence. Rb inactivation induces the lowest incidence of MuMM. Hom N\textsuperscript{2} enhanced tumours rate in Rb mutants [59]. A majority of epithelioid mutants was only found in Hom N\textsuperscript{2}/Htz Trp53 mice. Guo et al. [58] injected AdCre in the peritoneal cavity or in the bladder, in conditional mutants Trp53 and Tsc1. High rate of MuMM was found in double Hom Trp53/Tsc1 mutants, but none in Htz/Hom mutants. MM were mostly of epithelioid type [58]. Hom Pten leads to MuMM with a frequency of 7% in mice, but when coupled with Hom Trp53, 56% of mice developed pleural MuMM. The histologies of Hom Pten and Trp53 MuMM were sarcomatoid and biphasic [61].

Three types of Htz Bap1 mutants were generated in mice, one was knockout in exons 6 and 7 of Bap1, and the two others with point mutations identical to germline mutations found in two human
families (W and L, respectively) with a BAPI cancer syndrome presenting mesothelioma in several family members [60]. Htz germline mutations in BAPI predispose to a range of benign and malignant tumours, including mesothelioma. In Htz mice, although numerous types of cancers were developed, mesothelioma was absent or rare (2/93 Htz mice) and developed after a long delay (19 and 29 months). The tumour type with the highest incidence was ovarian sex cord stromal tumours, 38 of 60 (63%) in Bap1 mutant mice.

Collectively, the results show a differential role of the altered genes. Data from Jongsma et al. [59] suggest a prominent role of Cdkn2a and Trp53, compared to Nf2, as mice harbouring Hom Nf2 and either Htz Cdkn2a or Htz Trp53 have longer survival than mice with Hom Cdkn2a or Trp53 and Htz Nf2. However, Htz Trp53 in association with Htz or Hom Tsc1 did not induce MM, contrary to its association with Nf2, but consistent with a bona fide role of Nf2 in MM [58,59]. Results also showed that Bap1 Htz mutations are not sufficient to induce MuMM [60]. All histologic types of mesotheliomas were observed, with a majority of mixed and sarcomatoid types, with the exception of epithelioid type in Tsc1/Trp53 mice. Despite the different genetic background of mice, these studies underline several key genes for MM, consistent with findings in HMM, and that MM can develop with a variety TGS mutations, and likely with more than one TSG.

MuMM in Mice Exposed to Carcinogenic Fibres

Mice harbouring Htz genes Nf2, Cdkn2a (Ink4a and/or Arf), Trp53 or Bap1 and their WT counterparts were exposed to carcinogenic fibres administered intra-peritoneally [60,62–68]. In one study, both Nf2 and Cdkn2a were Htz [69]. MuMM arose in both WT and Htz mice, more frequently and with a shorter survival in Htz mice than in their WT counterparts, showing the role of these genes in enhancing sensitivity to fibres. MuMM generally arise after a long delay, often preceded by the occurrence of ascitic fluid. MuMM were detected several months after exposure, 18 and 27 weeks in Htz Nf2 mice [66,69] and 21 to 37 weeks in Htz Cdkn2a, Ink4a or Arf [63]. Median survivals were around one year or more. From the number of MuMM or lag time after fibre exposure in different genetic situations, it is difficult to establish a hierarchy between genes, because of the variety of protocols between studies (mice strains, dose and schedule of exposure, fibre type). Htz Trp53 mice were also developed high rate of MuMM when exposed to asbestos or to CNT [68,70].

Additionally, genes other than TSG such as Asc, relevant of inflammatory process, was also Hom- or Htz-inactivated in GEM [71]. Inactivation of Asc in GEM non-significantly reduced the percentage of mice with MuMM, but the disease-free survival was significantly lowered. These results suggest a role of inflammation in disease progression and the authors showed a relation with IL1b/IL1R signalling [71].

Asbestos induces MuMM in MexTAg transgenic mice that carry a fragment of the Simian Virus 40 (SV40) TAg open reading frame [72]. These MuMM replicate many aspects of MM at the molecular level, but MuMM development was not dependent on Cdkn2a, likely attributable to the Tag expression [73].

3.2.3. Mutation Profiles in MuMM of Mice Exposed to Fibres

Genetic alterations have been studied in MM cells cultured from ascitic fluids in fibre-exposed GEM. In MM cells from Htz Nf2 mice, a loss of heterozygosity (LOH) of Nf2 was found in all or a majority of MM cell lines from Htz Nf2 mice, 85% (6/7), 83.5% (10/12) and 100%, respectively [64–66]. Inactivation of Cdkn2a and Cdkn2b was predominant, and resulted from biallelic deletions. Otherwise, co-deletion of Cdkn2a (Ink4a and Arf) and Cdkn2b was predominant [64,65,74]. Rates of Trp53 mutations were less frequent, about 20% as in HMM [64,65,74]. Two cell lines with alteration of Trp53 were Cdkn2a (Ink4a and Arf) and Cdkn2b WT, suggesting two different pathways of carcinogenesis [74]. A role of the hippo pathway is suggested by the activation of Yap/Taz in tissue from asbestos-exposed Htz Nf2 mice, as shown by its nuclear localisation [75].

Altomare et al. [62] reported biallelic inactivation of Arf in all cell lines from Htz Arf mice, in 3/7 from WT mice, and no deletion of Ink4a or Ink4b (Cdkn2b) in all but one cell line from these mice,
and no loss of p53 protein. However, one WT cell line showed loss of Trp53 and p53 and retention of both Cdkn2a and Cdkn2b. Most of MM cells from Htz Arf mice showed hemizygous loss of Faf1 and down-regulation of its protein, which regulated TNF-α-mediated NF-κB signalling pathway in these cells. Accordingly, in Htz Cdkn2a (Ink4a and Arf) mice, a biallelic loss of both Ink4a and Arf was observed, with protein loss of p16Ink4a and p19Arf, and in Htz Ink4a mice, there was a biallelic inactivation of Ink4a, loss of p16Ink4a or p53, and frequent loss p15Inkba and p19Arf, but one cell line from Htz Ink4a mice expressed p19Arf but did not express p53 [63]. In the three configurations of Htz Ink4a, Arf or Cdkn2a (Ink4a and Arf), nearly all cell lines expressed Nf2 and p53 [63]. The reciprocity between retention and loss of Cdkn2a (Ink4a and Arf) and Trp53 expression of p53 consistent with an alternative role of the p53 pathway independently of hippo pathway and Ink4a regulation. These results suggest a major role of Arf in a context of fibre exposure and the role of alternative pathways in mesothelial carcinogenesis, as suggested above from the results obtained in Htz Nf2 mice.

Molecular analyses of cell lines from Htz Bap1 mice showed Bap1 LOH, but no alteration of Ink4a, Ink4b and Arf, in contrast to WT mice that retain WT Bap1, but were deleted in Ink4a, Ink4b and Arf, suggesting two alternative mechanisms of MM development despite the fact that CDKN2A and BAP1 mutations are not exclusive in HMM [76]. Rb protein was down regulated in cells from Htz Bap1 mice due to aberrant epigenetic of the Rb promoter, suggesting a role of Bap1 on Rb expression [76]. Fifty per cent of MM cell lines from ascites in asbestos-exposed Htz Trp53 mice had loss of the WT allele. In addition while cell lines with no loss of WT allele were diploid, those with LOH were tetraploid, consistent with a genetic instability related to checkpoint.

In tissues from asbestos-exposed Htz Nf2 mice, Rehrauer et al. [75] reported a higher number of mutations determined by RNA-seq, with an increase in A to G mutations, but not T to C, as compared to sham. This may be due to hydrolytic deamination of adenosine (Ada), as Ada expression is significantly increased, and linked to an Adar downstream activity [75].
Table 1. Induction of murine mesotheliomas (MuMM) in genetically engineered mice (GEM) (Injection of AdCre in GEM).

| Gene(s) Affected | Gene(s) Status | MuMM % | Epi 1 % | Sarco 2 % | Mixed % | Survival 3 Weeks | Reference |
|-----------------|----------------|--------|---------|-----------|---------|------------------|----------|
| Nf2             | Htz            | 34 4   | 28.6    | 21.4      | 50      | 58 5             | [59]     |
| Nf2             | Hom            | 34.6 4 | 22.2    | 27.8      | 50      | 71 5             | [59]     |
| Nf2             | Htz            | 79 4   | 2.2     | 68.9      | 28.9    | 31 5             | [59]     |
| Nf2             | Hom            | 5.9 4  | 0       | Primarily sarco | Some mixed | ND 7             | [59]     |
| Nf2             | Htz            | 13.3 4 | 0       | Primarily sarco | Some mixed | ND             | [59]     |
| Nf2             | Hom            | 26.3 4 | 0       | Primarily sarco | Some mixed | ND             | [59]     |
| Nf2             | Htz            | 6.75 6 | 0       | Primarily sarco | Some mixed | ND             | [59]     |
| Nf2             | Htz            | 13.3 6 | 0       | Primarily sarco | Some mixed | ND             | [59]     |
| Nf2             | Hom            | 20 6   | 0       | Primarily sarco | Some mixed | ND             | [59]     |
| Nf2             | Htz            | 25 4   | 25      | 25        | 50      | 29 5             | [59]     |
| Nf2             | Htz            | 25 4   | 60      | 40        | 0       | 86 5             | [59]     |
| Nf2             | Hom            | 82 4   | 15.5    | 46.7      | 37.8    | 19 5             | [59]     |
| Nf2             | Hom            | 93.7 4 | 0       | 40        | 60      | ND               | [59]     |
| Nf2             | Htz            | 91.1 4 | 0       | 76.6      | 23.4    | 11               | [59]     |
| Tsc1            | Hom            | 85 6   | Mostly  |           |         | 37               | [58]     |
| Tsc1            | Htz            | 0 6    | NA 7    |           | >57     | [58]             |
| Tsc1            | WT             | 0 6    | NA      |           | >57     | [58]             |
| Tsc1            | Htz            | 0 6    | NA      |           | >57     | [58]             |
| Tsc1            | WT             | 0 6    | NA      |           | >57     | [58]             |
| Tsc1            | Htz            | 73 8   | Mostly  |           | 44      | [58]             |
| Tsc1            | WT             | 0 8    | NA      |           | >57     | [58]             |
| Tsc1            | WT             | 0 8    | NA      |           | >57     | [58]             |
| Tsc1            | WT             | 0 8    | NA      |           | >57     | [58]             |

1 Strain of mice: FVB/N [59]; Hybrids [58]; 2 Epi.: Epithelioid; Sarco: Sarcomatoid; 3 Median survival of the series; 4 After intrathoracic injection of AdCre; 5 Mice with thoracic tumours; 6 After intraperitoneal injection of AdCre; 7 ND: Not done; NA: Not applicable; 8 After injection of AdCre in the bladder.
Table 2. Induction of MuMM in GEM (Induction of MuMM by injection of fibres).

| Mice Strain | Gene(s) Affected | Gene(s) Status | Treatment | MuMM % | Epi ² % | Sarco ² % | Mixed % | Survival ³ Weeks | Reference |
|-------------|------------------|----------------|-----------|---------|---------|-----------|---------|------------------|-----------|
| FVB/N       | Nf2              | Htz            | Asbestos  | 47      | 30.4 Hz + WT | 65.2 Hz + WT | 4.3 Hz + WT | 65 | 129/Sv/Jae       | [66]     |
| FVB/N       | None             | WT             | Asbestos  | 15      | 30.4 Hz + WT | 65.2 Hz + WT | 4.3 Hz + WT | 65 | 129/Sv/Jae       | [66]     |
| FVB/N       | Nf2              | Htz            | Saline    | 0       | NA       | NA        | NA      | NA               | [66]     |
| FVB/N       | None             | WT             | Saline    | 0       | NA       | NA        | NA      | NA               | [66]     |
| 129Sv/Jae   | Nf2              | Htz            | Asbestos  | 85      | 6.25    | 18.75     | 75      | 43               | [66]     |
| 129Sv/Jae   | None             | WT             | Asbestos  | 59      | 31      | 27.6      | 41.4    | 52               | [66]     |
| FVB/N       | Nf2              | Htz            | RCF       | 55      | 27      | 38.4      | 34.6    | 68               | [66]     |
| FVB/N       | None             | WT             | RCF       | 7.1     | 0       | 0         | 100     | 80               | [66]     |
| C57/Bl6     | Nf2              | Htz            | Asbestos  | 10      | ND      | ND        | ND      | ND               | [75]     |
| C57/Bl6     | Arf              | Htz            | Asbestos  | 96.2    | 68      | 12        | 20      | 42               | [66]     |
| C57/Bl6     | None             | WT             | Asbestos  | 81.5    | 68.2    | 18.2      | 13.6    | 56               | [66]     |
| Hybrids     | Ink4a/Arf        | Htz            | Asbestos  | 88      | Occasional | Prevalent | Occasional | 29.6  | [63]     |
| Hybrids     | Ink4a/Arf        | Htz            | TiO₂      | 0       | NA       | NA        | NA      | NA               | [63]     |
| Hybrids     | Ink4a/Arf        | Htz            | Asbestos  | 66      | Occasional | Prevalent | Occasional | 34.6  | [63]     |
| Hybrids     | Ink4a/Arf        | Htz            | Asbestos  | 65      | Occasional | Prevalent | Occasional | 38    | [63]     |
| Hybrids     | Nf2              | Htz            | Asbestos  | 50      | Occasional | Prevalent | Occasional | 49.4  | [63]     |
| Hybrids     | Ink4a/Arf        | Htz/Htz        | Asbestos  | ND      | ND       | ND        | ND      | ND               | [63]     |
| Hybrids     | None             | WT             | Asbestos  | ND      | ND       | ND        | ND      | ND               | [63]     |
| 129/Sv on a 75% C57/Bl6 background | Trp53 | Htz | Asbestos | 76 (after 44 weeks) | ND | ND | ND | ND | [68] |
| 129/Sv on a 75% C57/Bl6 background | Trp53 | Hom | Asbestos | ND | ND | ND | ND | ND | [68] |
| 129/Sv on a 75% C57/Bl6 background | None | WT | Asbestos | 32 (after 67 weeks) | ND | ND | ND | ND | [68] |
| FVB         | Bap1             | Htz            | Asbestos  | 73      | ND      | ND        | ND      | 43               | [76]     |
| FVB         | None             | WT             | Asbestos  | 32      | ND      | ND        | ND      | 55               | [76]     |
| FVB         | Bap1             | Htz (L)        | Asbestos  | 71      | ND      | ND        | ND      | 46               | [60]     |
| FVB         | Bap1             | Htz (W)        | Asbestos  | 74      | ND      | ND        | ND      | 48               | [60]     |
| FVB         | None             | WT             | Asbestos  | 35      | ND      | ND        | ND      | 60               | [60]     |
| C57BL/6     | Bap1             | Htz            | Asbestos low dose | 36 | all or part | 57 | all or part | 57 | [67] |
| C57BL/6     | None             | WT             | Asbestos low dose | 10 | all or part | 57 | all or part | 57 | [67] |
| C57BL/6     | None             | WT             | Saline    | 0       | NA       | NA        | NA      | 67               | [67]     |
| C57BL/6     | Bap1             | Htz            | Asbestos std dose | 60 | all or part | 39 | all or part | 39 | [67] |
| C57BL/6     | Bap1             | WT             | Asbestos std dose | 28 | all or part | 57 | all or part | 57 | [67] |
| C57BL/6     | Asc              | Hom            | Asbestos  | 55      | 0       | 75        | 25      | 66.2             | [71]     |
| C57BL/6     | Asc              | Htz            | Asbestos  | 65      | 0       | 68        | 32      | 69.4             | [71]     |
| C57BL/6     | None             | WT             | Asbestos  | 80      | 0       | 67        | 33      | OK               | [71]     |

² Epi.: Epithelioid; Sarco.: Sarcomatoid; ³ Median survival of the series.
4. Discussion

Literature data on MM in rodents led us to consider several issues concerning the molecular mechanism of mesothelial cell transformation, and its relationship with exposure to mineral and synthetic fibres. Most studies showed remarkable similarities between human and rodent MM. In both species, MM is a rare spontaneous cancer that is found in the similar sites, pleura, peritoneum and tunica vaginalis. When exposed to carcinogenic fibres, MM occurs after a long delay post-exposure, and all histological categories are observed. From studies carried out in GEM, no single gene predisposes to MM since MuMM are only in fibre-exposed mice, but asbestos is a powerful agent to facilitate the development of MM. MuMM were developed in mice harbouring Htz and Hom inactivation of TSG, or Hom and Hom inactivation.

In WT animals, exposure to fibres induces a significant incidence of MPM or MPeM, depending on the route of administration, respectively, in both rats and mice. The animals were symptomatic, showing ascites after intra-peritoneal administration of fibres. When reported, early MM appeared after several months, and were further detected during the whole life time of the species. In mice, the median survival in animals was about more than one year, except in Hom Nf2/Trp53 and Hom Nf2/Trp53/Ink4a. The survival was lower in asbestos-exposed GEM mice.

Although no precise quantitative data in the distribution of histological categories are given, the epithelioid type is not the most frequent in WT rodents and in GEM. In GEM the most frequent categories are sarcomatoid or mixed MPeM. In contrast, the epithelioid type is the most frequent human MPeM. However, a prevalence of epithelioid MPeM was reported in GEM Hom Trp53/Tsc1 not exposed to asbestos [58], and in both WT and Htz Arf asbestos-exposed mice [62].

Investigations of spontaneous MM in GEM harbouring co-mutations in TSG showed that two genes, Cdkn2a and Trp53 are predominant for MM development, as biallelic inactivation generates the highest rate of MM [58,59]. This is found despite the biallelic inactivation of Nf2, suggesting a cooperative but not predominant role of this gene [59]. Accordingly, in asbestos-exposed Htz Nf2 mice, Nf2 LOH is associated to loss of Cdkn2a and/or Cdkn2b. A key role of Cdkn2a and Trp53 is also seen when using cell cultured from ascites fluids from Htz Cdkn2a, Ink4a and Arf GEM. Among genes encoded at the Ink4 locus (Ink4a, Ink4b and Arf), Trp53 biallelic inactivation is an alternative mechanism to carcinogenesis via genes inactivated at the Ink4 locus. Of note, TP53 mutations are found in 11% of HMM (Cosmic database v85, https://cancer.sanger.ac.uk). Interestingly, in Htz Bap1 mice, Cdkn2a or Cdkn2b are not inactivated in MM, in contrast to MM with WT Bap1 where both genes are lost, but Rb down regulation was evidenced [76]. Independently of the inactivation of TSG already known to be involved in MM, mutations in genes involved in other regulatory pathways act as complementary mechanism accounting for mesothelial carcinogenesis.

As a whole, these studies brought information on the molecular changes in MM. A few genes are key players in the carcinogenic process. Others are bona fide modulators, which may be requested to favour the progression of the tumour, due to their involvement in signal or metabolic pathways. The diversity of mutated genes, the complex combination of altered genes, and the variety of associated deregulated pathways, lead to the heterogeneity of the tumour molecular profiles and is in agreement with the inter-tumour heterogeneity observed in HMM.

5. Conclusions

The data on asbestos-exposed mice do not bring significant information on the mechanism of genotoxicity of asbestos fibres. A better identification of the mutation signatures, characterisation of deleted regions and break points localisation and epigenetic changes, in both MM tumours and MM cell lines could help understanding the mechanism genome damage [77]. Inflammation is thought to act as a contributor, but it is not known whether it is the driving force for DNA damaging at lower doses than required in experiments [78]. Events entailing gene deletion and rearrangements should be considered. The contribution of gene methylation is not enough documented, but Rb is regulated by DNA methylation in Htz Bap1 mice [76]. Jongema et al. [59] reported that epigenetic
inactivation of *Ink4a*, although enhancing the malignancy, does not contribute to the development of pleural MuMM in *Htz Nf2/Trp53*, in agreement with the evidence of deletions of this gene demonstrated in several studies [59]. Nevertheless, hypermethylation of *Cdkn2a* locus preceding allelic *Arf* deletion was suggested to be a mesothelial carcinogenesis step in pleura of mice exposed to CNT [57]. These studies have emphasised the diversity of the molecular events entailing the development of MM in experimental animals, and their consistency with the molecular status of HMM, in term of key genes and pathways, and potent modulators of tumour progression.

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**Abbreviations**

- **CNT:** Carbon Nanotubes
- **GEM:** Genetically Engineered Mice
- **HMM:** Human Malignant Mesothelioma
- **MM:** Malignant Mesothelioma
- **MPeM:** Malignant Peritoneal Mesothelioma
- **MuMM:** Murine Malignant Mesothelioma

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