Chapter

Genetic Alterations of Malignant Pleural Mesothelima

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Abstract

Malignant pleural mesothelioma (MPM) is a highly aggressive tumor that arises from the mesothelial cells lining the pleural cavity. Asbestos is considered the major factor in the pathogenesis of this malignancy, with more than 80% of patients with a history of asbestos exposure. MPM is characterized by a long latency period, typically 20–40 years from the time of asbestos exposure to diagnosis, suggesting that multiple somatic genetic alterations are required for the tumorigenic conversion of a mesothelial cell. In the last few years, advancements in next-generation sequencing and "–omics" technologies have revolutionized the field of genomics and medical diagnosis. The focus of this chapter is to summarize recent studies which explore the molecular mechanisms underlying this disease and identify potential therapeutic targets in MPM.

Keywords: pleural mesothelioma, next-generation sequencing, transcriptome, exome sequencing, tumor suppressor gene

1. Introduction

Malignant pleural mesothelioma (MPM) is a lethal cancer of the mesothelial cells lining the pleural cavity and, less frequently, the pericardium, peritoneum, and tunica vaginalis [1]. Many years after the peak of asbestos use in United States, 3200 cases of MPM continue to be diagnosed annually, indicating that the U.S. population remains at risk of exposure to asbestos and development of mesothelioma [2]. There are two major histological variants: epithelioid, which accounts for about 60% of cases and has the more favorable prognosis, and sarcomatoid, whose incidence is 10%. The remaining cases demonstrate histologic characteristics of both types and are classified as biphasic [3]. The prognosis for patients with MPM is poor, with a median survival of 5–15 months [3]. However, some patients with early MPM who undergo multimodality therapy including surgical resection and chemotherapy demonstrate longer-term survival of up to 25% at 5 years [4].

Many studies have shown a causal relationship between exposure to asbestos and mesothelioma (reviewed by Bianche et al. [5]). Although it has been suggested that brief asbestos exposure is sufficient to induce disease, MPM is the consequence of prolonged exposure in most cases. However, only a small percentage of individuals exposed to asbestos develop MPM, suggesting that genetic predisposition may modulate the effect of exposure to asbestos. In addition, 20% of MPM cases with unknown asbestos exposure have been related to other risk factors such as radiation therapy and thorotrast [6].

Studies conducted on large numbers of patients indicate that the time between asbestos exposure and diagnosis of MPM is generally more than 20 years. The molecular mechanisms for the transformation of mesothelial cells are unknown; it has been suggested that asbestos induces multiple chromosomal aberrations, particularly deletions, facilitating oncogenesis [7].

Investigations prior to the advent of next-generation sequencing (NGS) revealed the complexity of the genetic alterations observed in MPM tumors by using karyotypic and comparative genomic hybridization (CGH) analyses [8, 9]. Chromosomal losses were found to be more frequent than gains and particular chromosomal regions (1p22, 3p21, 4q, 6q, 9p21, 13q13–14, 15q11–15, and 22q12) were deleted at higher frequency in MPM tissues and cell lines [10–12]. Two tumor suppressor genes (TSGs) were identified by positional cloning approaches: *CDKN2A* at 9p21 and *NF2* at 22q12. In the last few years, the genetic landscape of MPM has been characterized using high-throughput technologies [13–15]. The focus of this chapter is to summarize the major genetic changes occurring in MPM as identified by high-throughput sequencing and to describe the novel insights obtained through transcriptomic studies.

2. Exome sequencing studies

NGS technologies have allowed the sequencing of DNA and RNA at unprecedented speed, uncovering potential driver genes and creating novel biological applications [16]. In the last decade, NGS has been used to detect driver genetic mutations in cancer and provide new insights into tumorigenesis.

Shotgun pyrosequencing was used to characterize RNA expression levels and mutations of four patients in the first effort to investigate MPM by NGS. Several different mutations were found in the four transcriptomes. In addition, RNA editing gene deletions and gene silencing were identified [17].

In 2010, the first whole genome sequence of one MPM tumor and matching normal tissue was conducted using a combination of sequencing-by-synthesis and pyrosequencing methodologies [18]. This study showed that aneuploidy and chromosomal rearrangements were more numerous than point mutations in this tumor. One large deletion in the dipeptidyl peptidase like 10 (*DPP10*) gene, altering the expression of the corresponding transcript, was further investigated in 53 additional MPM tumors. Patients expressing *DPP10* had statistically longer survival compared to patients lacking *DPP10* expression [18].

In 2016, Bueno et al. conducted an extensive analysis of the mutational landscape of MPM. Ninety-nine MPM tumors were examined by whole exome sequencing, whereas additional 103 samples were characterized by targeted exome sequencing [13]. *BAP1*, *NF2*, *TP53*, *SETD2*, *DDX3X*, *ULK2*, *RYR2*, *CFAP45*, *SETDB1* and *DDX51* were found to be significantly mutated (q-score ≥ 0.8), and recurrent mutations were found in *SF3B1* (2%) and *TRAF7* (2%).

In 2018, The Cancer Genome Atlas (TCGA) program performed a comprehensive molecular profiling of 74 primary MPM samples including exome sequencing, copy-number arrays, mRNA sequencing, noncoding RNA profiling, DNA methylation, and reverse-phase protein arrays [15]. The significantly mutated genes in this study were *BAP1*, *NF2*, *TP53*, *LATS2*, and *SETD2*. Furthermore, this study identified a new near-haploid molecular MPM subtype.

The TCGA study performed a comparison of the significantly mutated genes between the Bueno and TCGA cohorts [15]. This analysis identified five genes that were frequently mutated in both studies: BRCA1-associated protein-1 (*BAP1*), neurofibromin 2 (*NF2*), tumor protein P53 (*TP53*), SET domain containing 2, histone

lysine methyltransferase (*SETD2)*, and SET domain bifurcated histone lysine methyltransferase 1 (*SETDB1*). The large tumor suppressor kinase 2 (*LATS2*) gene was found frequently altered in the TCGA cohort alone, whereas four additional genes, DEAD-box helicase 3 X-linked (*DDX3X*), Unc-51-like autophagy-activating kinase 2 (*ULK2*), ryanodine receptor 2 (*RYR2*), and DEAD-box helicase 51 (*DDX51*) were identified as commonly mutated in the series from Bueno et al. (**Table 1**).

2.1 BAP1

BAP1 is located on the short (p) arm of chromosome 3, at position 21.1., a region frequently deleted in MPM [9]. This gene encodes for a deubiquitinase involved in cell cycle regulation, modulation of gene transcription, cellular differentiation, and DNA repair [19]. *BAP1* is one of the most commonly mutated genes in MPM [13, 15, 20, 21]. Germline *BAP1* mutations have been linked to the development of *BAP1* tumor predisposition syndrome, which includes uveal and cutaneous melanoma, atypical Spitz tumors, renal cell carcinoma, and MPM. In all these malignancies but MPM, *BAP1* mutations are associated with poor prognosis [22, 23]. In contrast, some studies have shown that patients with MPM carrying *BAP1* mutations have longer overall survival compared to patients with wild-type *BAP1* [24, 25]. In one study, BAP1 immunohistochemistry (IHC) was performed using tissue microarray including 229 MPM tumors. The results showed that loss of BAP1 nuclear staining was associated with longer median survival of 16.11 months (95% CI: 12.16–20.06) versus 6.34 months for patients with nuclear BAP1 staining (95% CI: 5.34–7.34) ($P < 0.01$) [24]. Baumann et al. compared the survival in 23 patients with MPM carrying germline mutations in *BAP1* with a control group of MPM patients from the Surveillance, Epidemiology, and End Results (SEER) database and found a 7-fold increase in long-term survival in patients with BAP1 mutation [25].

Given its prevalence in MPM, loss of nuclear BAP1 expression by IHC is commonly used as a diagnostic marker in MPM [26, 27].

Recently, *BAP1* status has been associated with drug response [28, 29]. I*n vitro* studies showed MPM cell lines carrying *BAP1* mutations were significantly less sensitive to gemcitabine compared to wild-type cells. Silencing of *BAP1* in MPM

Gene symbol	Gene ID	Chromosomal location	Number of mutations in Bueno's cohort	Number of mutations in Hmeljak's cohort	Total
BAP1	ENSG00000163930	3p21.1	55	17	72
NF2	ENSG00000186575	22q12.2	39	19	58
TP53	ENSG00000141510	17p31.1	17	10	27
SETD2	ENSG00000181555	3p21.31	18	8	26
SETDB1	ENSG00000143379	1q21	7	3	10
LATS2	ENSG00000150457	13q12.11	2	9	11
DDX3X	ENSG00000215301	Xp11.4	8	0	8
RYR2	ENSG00000198626	1q43	4	1	5
ULK2	ENSG00000083290	17p11.2	4	0	4
DDX51	ENSG00000185163	12q24.33	3	0	3
Total			157	67	224

Table 1.
Number of mutations in each gene in the two studies.

wild-type cells significantly increased resistance to gemcitabine, suggesting a role of *BAP1* in drug response [28]. Kumar et al. performed a retrospective study analyzing presence or absence of nuclear BAP1 by IHC in MPM tumors from 60 patients in the MS01 trial (NCT00075699) [29]. Nuclear BAP1 expression was associated with a small but statistically nonsignificant decrease in survival in patients treated with vinorelbine.

2.2 NF2

NF2 is located on the long (q) arm of chromosome 22 at position 12.2. Loss of chromosome 22 is a common alteration in MPM [9]. This gene codes for a protein known as merlin (moesinezrin-radixin-like protein) or schwannomin, which regulates key signaling pathways involved in cell growth, adhesion, and microtubule stabilization [30]. Germline mutation or chromosomal deletion of *NF2* causes the neurofibromatosis type 2 syndrome, which is associated with tumors of the cranial and peripheral nerves as well as meningioma and ependymoma [31]. Germline mutations in *NF2* have also been linked to MPM; however, patients with both neurofibromatosis type 2 syndrome and MPM are extremely rare [32]. Recent studies have shown that *NF2* mutations occur in 14–19% of MPM [13–15, 20]. In addition, karyotype and/or FISH analyses demonstrated that 56% MPMs have shown loss of chromosome 22q. Deletions of 22q are more frequently associated with epithelioid than non-epithelioid MPM ($p = 0.037$) [20].

In 2009, a study suggested that *NF2* may be inactivated by upstream regulators in MPM tumors where no *NF2* aberration can be detected [33]. In an investigation of 204 MPM patients, low cytoplasmic merlin expression was found to predict shorter recurrence interval and shorter overall survival [34]. Lopez-Lago et al. investigated the association between loss of merlin and mTORC1 activation in MPM cell lines and found that merlin-negative or merlin-depleted cell lines were more sensitive to the growth-inhibitory effect of rapamycin [35]. In 2014, low merlin expression was found to be associated to increased sensitivity of MPM cell lines to a FAK inhibitor, VS-471 [36]. However, in clinical trials, the FAK inhibitor defactinib did not improve progression free or overall survival in patients with MPM after first-line chemotherapy [37].

2.3 TP53

Located at 17p31.1, *TP53* codes for tumor protein p53 (p53), which is a sequence-specific DNA binding protein that regulates transcription and has a tumor suppressor function controlling cell apoptosis in presence of DNA damage [38]. Named "the guardian of the genome," p53 is involved in many cellular processes such as checkpoint control, cellular senescence, and BCL-2 mediated apoptosis [39]. *TP53* is, overall, the most frequently altered gene in human cancer [40]. The frequency of *TP53* mutations in MPM across different studies is variable, but overall it is much lower than in other solid tumors [13–15, 20]. *TP53* was significantly more frequently mutated in women (10/40; 25%) compared to men (17/169, 10%) (Fisher's exact $P = 0.044$) when all samples included in two large MPM studies [13, 15] were analyzed. In addition, Bueno et al. reported that MPM patients with mutations in *TP53* had shorter overall survival than those with wild-type *TP53* ($p = 0.0167$) [13].

2.4 SETD2

SETD2 maps to 3p21.31. It encodes a histone methyltransferase specific for lysine-36 of histone H3 which regulates transcription through epigenetic

mechanisms [41]. Inactivating *SETD2* mutations have been identified in multiple cancers [42]. In particular, targeted sequencing revealed *SETD2* bi-allelic inactivation in clear cell renal cell carcinoma tumors suggesting for the first time that *SETD2* may contribute to tumor formation [43]. In MPM, single nucleotide mutations in *SETD2* as well as 3p losses are frequently observed [13, 15, 44]. In the last few years, *SETD2* alterations have been linked to mechanisms of resistance to DNA-damaging chemotherapy in several cancers [45, 46].

2.5 SETDB1

SETDB1 is positioned at 1q21, another region frequently deleted in MPM [9], and codes for histone-lysine N-methyltransferase *SETDB1* which trimethylates Lys-9 of histone H3 [47]. As an epigenetic modulator, *SETDB1* has a critical role in several biological processes such as embryonic development, adipocyte differentiation, and inflammation, as well as providing regulation of several signaling pathways including the P13K-AKT axis, p53, the STAT1-CCND1/CDK6 axis, and gene promoter methylation [48].

Targeted deep sequencing has revealed somatic *SETDB1* mutations in 10% (7/69) patients with MPM [49]. No significant correlation between mutation in *SETDB1* and survival was found ($p = 0.351$). Mutations in *SETDB1* were also identified in 3% (7/202) of MPMs in a different cohort [13]. Hmeljak et al. found that *SETDB1* mutations were present together with *TP53* and extensive loss of heterozygosity in 3% of MPM. This rare genomic subtype was associated with female sex and younger age at diagnosis [15].

2.6 LATS2

LATS2, located on 13q12.11, encodes for a serine/threonine kinase which is involved in a broad array of programs such as cell cycle regulation, cell motility, and differentiation [50]. Loss of *LATS2*, either through copy number alteration or mutation, has been identified in several different cancer types [51], as well as in MPM [15, 52]. In a cohort of 266 MPM samples, mutations in *LATS2* were observed in 5% of the samples, with lower frequency in epithelioid compared to non-epithelioid samples. In addition, *LATS2* mutations were more frequent in patients without asbestos exposure (7%) than those exposed (2%) [53]. Another study identified a new molecular subgroup of MPM characterized by a co-occurring mutation in *LATS2* and *NF2*. MPM patients in this subgroup had poor prognosis compared to the cohort at large [54].

Several investigations have linked *LATS2* to the transcription regulator YAP involved in the Hippo pathways. Mizuno et al. found that inactivation of *LATS2* leads to YAP overexpression, which, when knocked down, inhibits cell motility and invasion *in vitro* [55]. Another study demonstrated that *LATS2* is a key binding partner of AJUBA, which suppresses YAP activity in mesothelioma [56].

2.7 DDX3X

DDX3X resides on Xp11.4 and encodes an ATP-dependent RNA helicase with RNA-independent ATPase activity stimulated by either DNA or RNA [57]. DDX3X has both cytoplasmic and nuclear functions including translation, regulation of transcription, pre-mRNA splicing, and mRNA export [58]. Its functions are complex and varied: *DDX3X* has been recognized as both an oncogene and a tumor suppressor, sometimes within the context of a single type of cancer [59]. An analysis of the COSMIC database found that 12% of genetic abnormalities in *DDX3* are typical for tumor suppressors, while 81% are more typical for gain of function [59].

2.8 RYR2

RYR2 is located at 1q43. It encodes a member of the ryanodine receptor family of calcium channels, highly expressed in cardiac muscle but also found in smooth muscle and the nervous system [60]. The release of calcium from the sarcoplasmic reticulum into the cytoplasm via RyR2 triggers contraction in myocytes, whereas in the brain, it aids in functions related to learning and memory [60]. Although mutations in *RYR2* have been reported in other cancers [61], *RYR2* mutations in MPM have been identified only in one study [13].

2.9 ULK2

ULK2 maps on 17p11.2. It codes for an Atg1 homolog and serine/threonine kinase which normally localizes to the membrane of autophagosomes and plays a key role in autophagy, particularly in the setting of nutrient deprivation or mTOR inhibition [62]. *ULK2* has been linked to the development of astrocytoma [63], and colorectal cancer [64]. Rare *ULK2* mutations have been identified in MPM [13]. In spheroid models of MPM, autophagy was successfully inhibited by the ULK1/2 inhibitor MRT 68921 [65].

2.10 DDX51

DDX51 resides on 12q24.33. It is a ribosome synthesis factor required for the formation of the 3′ end of 28S rRNA [66]. Abnormal function of *DDX51* has been linked to NSCLC, leukemia, and breast cancer [67–69]. Few *DDX51* mutations have been found in MPM [13].

3. Transcriptome sequencing studies

Since gene expression is linked to tumor behavior, bulk expression profiling of tumors has revolutionized our understanding of cancer by giving insight into the expression levels of thousands of genes measured at once. In addition, the allocation of cancer specimens into molecular clusters having similar biological and clinical characteristics has improved the understanding of the molecular biology of tumors and identified both actionable targets for therapies as well as biomarkers for prediction of response [70].

In 2005, Gordon et al. profiled 40 MPM tumors using microarray technologies [71]. Four normal pleura specimens and four normal lung tissues were included in the analysis as controls because MPM arises from mesothelial cells of the pleura and often involves the lung parenchyma [71]. Unsupervised cluster analysis revealed four distinct subclasses with two, named C1 and C2, consisting only of MPM samples. These two clusters had epithelial (88%) and mixed (78%) subtypes, respectively, showing a partial correlation with tumor histology. Differential gene expression analysis demonstrated genes related to cytoskeletal/support, such as keratins, cadherins, and other proteoglycans, were over-expressed in cluster C1, whereas genes associated with extracellular matrix and structural proteins such as collagen, actin, biglycan, and fibronectin were highly expressed in subclass C2 [71].

In 2014, a study from de Reynies et al. generated a transcriptomic classification of MPM using 38 primary cultures [72]. Consensus clustering of the expression profiles identified two groups of MPM, C1 and C2, which are partially related to histology. Epithelioid MPM were found in both clusters, whereas sarcomatoid

tumors clustered only in C2. In addition, tumor samples in C1 tended to have more frequent mutations in *BAP1* (P = 0.09) and deletions of the chromosomal region 3p21 (P < 0.01), where *BAP1* is located. Furthermore, 40 genes that discriminated the two groups were used to validate the molecular classification in 108 MPM tumors. Survival analyses showed that patients in C2 had shorter survival compared to the survival of patients in cluster C1 (*P* = 0.02). This difference persisted when only epithelioid samples were included (P < 0.01) [72]. Pathway analyses revealed that the most deregulated pathways were those related to the epithelial-to-mesenchymal transition (EMT) process [72].

In 2016, a seminal publication on genomics in MPM described unsupervised consensus clustering of RNA sequencing data from 211 MPM tumors. This analysis classified the samples into four distinct molecular clusters: epithelioid, biphasic-epithelioid (biphasic-E), biphasic-sarcomatoid (biphasic-S), and sarcomatoid [13]. The clusters were loosely associated with the spectrum from epithelioid to sarcomatoid histology. Epithelioid and biphasic samples were distributed in all four subgroups, whereas sarcomatoid tumors were only in one cluster. Biphasic samples clustered according to the proportion of epithelioid and sarcomatoid cells contained in the specimen; biphasic tumors with the highest portion of sarcomatoid cells grouped with the sarcomatoid samples. Notably, patients in the epithelioid cluster had longer overall survival compared to the survival of patients in the other three groups. Differential expression analysis of the sarcomatoid and epithelioid clusters revealed that genes related to the EMT process were differently expressed between the two groups, and that ratio of two genes *CLDN15* and *VIM* (C/V score) significantly differentiated the four clusters [13].

A different approach to classify MPM tumors was used by Hmeljak et al. [15]. To determine whether a multi-platform molecular profiling may offer additional power to identify subsets of MPM, two clustering algorithms, iCluster [73] and PARADIGM [74] were used to integrate somatic copy-number alteration, gene expression, and epigenetic data from 74 MPM samples. Both algorithms grouped the samples into four distinct clusters with high concordance between the two methods in the assignment of the sample into the groups. Survival analyses showed significant differences in survival across the four groups. In addition, the four clusters were significantly associated with histology: cluster 1 contained many epithelioid samples, whereas cluster 4 was enriched for sarcomatoid tumors as found in previous studies [13, 71, 72]. This study, using a small number of samples, mostly epithelial, confirmed that genes related to the EMT process were differentially expressed between the two most extreme clusters [15].

In 2019, unsupervised clustering of microarray profiles assigned 63 primary MPMs into four groups (C1A, C1B, C2A, and C2B) [75]. Then, a meta-analysis of mesothelioma expression profiles was conducted to compare these clusters with the groups from previous classifications [13, 15, 71, 72, 75, 76]. This analysis identified two highly correlated MPM clusters present in all expression profiles, which corresponded to the extreme epithelioid and the sarcomatoid phenotypes. The remaining groups did not associate closely suggesting that they may represent different points of a continuum or "histo-molecular gradient" of epithelioid and sarcomatoid components. A deconvolution approach was used to identify novel insights into the intra-tumor heterogeneity of MPM by dissecting whole tissue RNA-sequencing signatures into biologically relevant components. This analysis produced two molecular signatures of 150 genes, E-score and S-score, which were related to histology and recapitulated the molecular classification. These signatures reflected the proportion of epithelioid-like and sarcomatoid-like components within each MPM tumor. In addition, the proportions of these cellular components were significantly associated with prognosis [75].

Regardless of the metric used, the whole transcriptome studies indicate that MPM is characterized by a molecular gradient associated with the EMT process. Most recently, the relationship between the C/V score [18] and other published metrics [75, 77] associated with the EMT process has been investigated [78] demonstrating a significant correlation of the C/V score with other molecular signatures. These results indicate that the ratio of just two genes can be sufficient to determine the "EMT-component" in each MPM [78].

4. Clinical significance

While further work is needed before these data can be applied directly to patient care, an understanding of the molecular heterogeneity of MPM and the mutations that contribute to different subtypes can have a meaningful impact on the direction of clinical research in this field. In 2014, *in vitro* and tumor xenograft experiments suggested that low Merlin (NF2 protein) expression may predict increased sensitivity of MPM cells to a FAK inhibitor, VS-4718 [36]. Subsequently, the use of defactinib, a FAK inhibitor, was investigated in the neoadjuvant setting for surgically resectable disease (a "window of opportunity" study). The treatment was well tolerated and resulted in successful inhibition of FAK, as well as inhibition of multiple cancer stem cell markers such as CD133 and SOX2 (Bueno et al., 2018 personal communication, International Mesothelioma Interest Group (IMIG) Conference, 2016 Birmingham UK). The use of defactinib as maintenance therapy following first-line chemotherapy in advanced MPM was also assessed in the COMMAND trial, a phase II randomized placebo-controlled study. Three hundred forty-four patients were stratified by merlin expression and randomized; however, there was no significant improvement in progression-free survival (4.1 [95% CI: 2.9–5.6] versus 4 [95% CI: 2.9–4.2] months) or overall survival (12.7 [95% CI: 9.1–21] versus 13.6 [95% CI: 9.6 to 21.2] months) of patients treated with defactinib compared to placebo [37].

Knowledge of key mutations in MPM has guided investigations into other forms of targeted therapy, although many are still at the preclinical stage. For example, LaFave and colleagues found evidence that loss of Bap1 expression increases Ezh2 expression in xenograft and *Bap1* knock-out mice and enhances sensitivity to EZH2 inhibition *in vitro*. Szlosarek and colleagues studied arginine deprivation in 68 patients with advanced ASS1-deficient malignant pleural mesothelioma (defined by >50% low expressor cells on immunohistochemical analysis) [79]. Treatment with the deprivation agent ADI-PEG20 improved progression-free survival (3.2 vs. 2 months, $p = 0.03$) with no significant difference in life expectancy or adverse events.

Beyond identifying therapeutic targets, multi-omic data have enhanced the understanding of tumor biology, providing novel ways to stratify patients, determining prognosis and predicting sensitivity to existing treatments (reviewed in [80]).

We have developed a gene expression ratio-based method to translate expression profiling data into clinical tests based on the expression levels of a small number of genes [81]. This method uses standard supervised methods for microarray analysis to compare gene expression in two types of tissues differing by a single clinical parameter such as histology or outcome. Genes with the most significant difference in expression are selected and used in combination to calculate ratios of gene expression able to predict the clinical parameter associated with a random patient sample.

Using this method, a 6-gene 3-ratio test has been developed to distinguish MPM from adenocarcinoma using resection specimens and fine needle biopsies [81, 82]. A similar approach was used to generate a 4-gene 3-ratio prognostic test to identify

patients likely to benefit from tumor resection in the preoperative setting [83, 84], as well as a 4-gene 3-ratio signature to distinguish the epithelioid from the sarcomatoid MPM subtype [85].

Despite rapidly decreasing sequencing costs [86], there remain several barriers to introducing the use of NGS technology in clinical practice, especially in MPM. In many solid tumors, the development of targeted sequencing panels has led to targeted therapies and prediction of survival of cancer patients. MPM is rare, making large-scale validation studies difficult to perform, and heterogeneous, characterized by mutations highly variable among tumors. In addition, loss of TSGs is a common feature of MPM making potential treatments associated with these genes difficult to be applied to real life treatment. Clinical trials focused on specific mutated genes [29, 37] have been infrequent and the results never translated to practice. Transcriptome analyses have classified MPM patients into several groups stratifying patients into categories of risk; however, a substantial margin of error in these predictions persists because the sensitivity and specificity of these tests are difficult to define [87]. Precision medicine based on cancer genomics is still far from being applied in clinical practice in MPM. Nevertheless, we are confident in the value of NGS for personalized medicine and believe additional efforts are needed for the implementation of NGS in identifying patients who might benefit from targeted treatments.

5. Conclusions

NGS has revolutionized the study of human genetics by transforming our ability to analyze the causes of disease, develop new diagnostics, and identify potential therapeutic targets. NGS studies have led to the discovery of several commonly mutated genes in MPM [13, 15]. Although analyses of transcriptome data have contributed to the understanding of the molecular biology of MPM subtypes, these studies were based on bulk profiling where tumors were profiled as a single entity averaging the gene expression of all the cells in the specimen and ignoring the intra-tumor heterogeneity that regulates many critical aspects of tumor biology [88]. The importance of intra-tumor heterogeneity in MPM is becoming evident. Future single-cell RNA sequencing work will be able to elucidate molecular roles of immune infiltrates and stroma in MPM as well as to clarify whether the molecular mechanisms associated with the genetic heterogeneity are due to subclonal mutations, epigenetic programs, or other environmental factors such as cell-cell interaction or nutrient availability.

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