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Malignant Pleural Mesothelioma

Advances in Pathogenesis, Diagnosis, and Treatments

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Preface

Mesothelioma is an infrequent, aggressive malignant tumor that develops in the mesothelial cell layer of the pleural and peritoneal cavities, as well as the pericardium and the tunica vaginalis testis. Its global incidence has steadily risen along with widespread exposure of individuals to asbestos throughout the twentieth century. Those who have a history of asbestos exposure perceive worries about being high-risk group of developing fatal mesothelioma in future. Exposure to asbestos is a well-known cause of mesothelioma; however, there are many difficulties in understanding mesothelial cell carcinogenesis seen in patients exposed to no known or quite low-dose environmental exposure. A small number of mesothelioma patients with familial aggregation are associated with constitutional germ line *BAP1* mutation, who also develop other tumors such as melanoma and clear cell renal carcinoma. Recent marked progress in basic and clinical researches on this neoplasm elucidates some crucial evidence relating to molecular pathogenesis and perspectives on potential therapeutic approaches leading to promising treatment.

This book is designed to provide a state-of-the-art overview of the most recent scientific researches on mesothelioma, encompassing epidemiology, pathology, genetics, carcinogenesis, imaging, and treatment, together into a single publication. Mesothelioma is a complex scientific tumor and remains a public health issue; therefore, this book also has a significant impact on medicolegal aspects of this disease. All the authors are world-renowned experts in their field and present updates of their research on mesothelioma. We hope that the contents of this book will encourage the readers not only to learn more about these topics but to digest the rapid growth of knowledge of scientific mesothelioma research and multidisciplinary treatment approach. This book is aimed to be used by respiratory physicians, pathologists, medical oncologists, radiation oncologists, surgical oncologists, and cancer researchers, as well as by residents and fellows in training.

We, chapter editors, are grateful to all of the authors who took time from their incredibly quite busy schedules, especially in the situation of the SARS-CoV-2 pandemic in 2020, to contribute to this book. Finally, we are indebted to Ms. Chihiro

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Osaka, Japan — Takashi Nakano
Hyogo, Japan — Takashi Kijima

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Part I
Epidemiology

Chapter 1
Trend in the Global Incidence of Mesothelioma: Is There Any Changing Trend After Asbestos Regulation and Ban?

Diana Arachi, Matthew Soeberg, Odgerel Chimed-Ochir, Ro-Ting Lin, and Ken Takahashi

Abstract Asking "does an asbestos ban lead to a decrease in malignant mesothelioma?" is not a simple question as it may seem. This is largely because the phrase "asbestos ban" refers to a wide range of national situations and processes. It also reflects that countries have varied widely in their speed of reducing asbestos consumption in relation to, or independent of, adopting a ban. Thus, it is analytically complex to address an asbestos ban in relation to mesothelioma incidence, and few such studies have been conducted. The first study to directly address this question compared national-level data of changes in pleural mesothelioma mortality rates versus changes in asbestos use across a range of countries; the authors found correlations between these changes and suggested that there may be an early effect. The second study, which was a birth cohort analysis conducted in Sweden, showed that a later birth cohort (one active in the workforce after the decrease in asbestos use) had a decreased risk of pleural mesothelioma relative to an earlier birth cohort, regardless of gender. These studies implicated a causal effect, wherein an asbestos ban leads to a decrease in mesothelioma incidence. Given the very long latency period for mesothelioma, it can be expected that a much clearer effect will soon become evident in countries that adopted an early ban on asbestos. Furthermore, given the ongoing use of asbestos by many industrializing countries, it is also perti-

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nent to ask: “Did an increase in mesothelioma incidence lead to an asbestos ban in some countries?”.

Keywords Mesothelioma · Incidence · Mortality · Asbestos · Global burden of disease (GBD)

1 Introduction

1.1 Incidence and Mortality

Incidence data are compiled for malignant mesothelioma, along with other cancers, in the monograph series published by the International Agency for Research on Cancer (IARC) [1]. The metrics used to describe incidence include the number of cases and the crude and age-adjusted rates per 100,000 person-years grouped by gender and 5-year periods. Utilizing this dataset, Soeberg and van Zandwijk [2] identified the regions with the world’s highest age-standardized incidence rate (SIR) for mesothelioma (period 2003–2007) as being Bremen (Germany) at 6.0, Genoa (Italy) at 5.6, and Western Australia (Australia) at 4.5 per 100,000 person-years among men. They also noted that the population-level mesothelioma incidence rate rarely exceeded 1.0 per 100,000 (or 10 per million) person-years. A major limitation of this dataset is that data are confined to the catchment areas of the respective cancer registries (mostly regional, some national) and are only updated every 5 years. Nonetheless, actual data reported to and compiled by cancer registries worldwide provide additional insight. For example, although China lacks nationwide statistics on mesothelioma mortality, the SIR for mesothelioma in Beijing City was reportedly 0.3 and 0.2 per 100,000 person-years among men and women, respectively (period 2003–2007) [1].

Here, the *reported* data, such as those for incidence and mortality, should be clearly distinguished from *estimated* values. For example, Zhao et al. [3] reported the following estimates for all mesothelioma in China in 2013: 2041 incident and 1659 death cases; 1.50 per million (M) person-years (crude incidence rate); and 1.22 per M person-years (crude mortality rate). Although these are likely to be underestimates, the report represents an important first step in clarifying the mesothelioma situation in China.

Mesothelioma has one of the poorest survival rates of all cancer types, with a 5-year survival rate <10% and a median survival time <1 year. The incidence, therefore, approximates mortality and can be surrogated by mortality data. Mortality data are generally more available and accessible than incidence data, especially for mesothelioma, so the global situation (or “burden”) of mesothelioma is commonly expressed in terms of mortality (the number of deaths).

1.2 WHO Mortality Database

The WHO Mortality Database is a compilation of mortality data by age, gender, and cause of death, as reported annually by countries (i.e., WHO Member States) from their civil registration systems [4]. In general, the data are most complete for developed countries (though coverage of years are variable), whereas developing countries often have incomplete or missing data [5].

Delgermaa et al. [6] conducted a descriptive analysis of all mesothelioma deaths in the WHO Mortality Database, which recorded 92,253 mesothelioma deaths in 83 countries during 1994–2008. The crude and age-adjusted mortality rates (AAMR) were 6.2 and 4.9 per M person-years, respectively, with an AAMR increase of 5.4% per year during the 15-year period. The trends varied substantially by continent: the AAMR increased significantly in the countries of Europe (3.7% per year, $p < 0.05$) and Asia (3.4% per year, $p < 0.05$) but not the Americas (7.9% per year, not significant [NS]) or Oceania (−0.5% per year, NS). The AAMR increased significantly in countries of the high-income group (5.5% per year, $p < 0.05$) but not the middle-and-low income groups (2.2% per year, NS). The mean age at death was 70 years and the male-to-female ratio was 3.6:1. The three countries with the highest number of deaths were the USA, the UK, and Japan, and those with the highest AAMR were the UK (17.8 per M person-years), Australia (16.5 per M person-years), and Italy (10.3 per M person-years).

When viewing global mortality statistics, some limitations must be considered. For one, mesothelioma is technically difficult to diagnose: malignant pleural mesothelioma (MPM) can be misdiagnosed for lung cancer arising in peripheral areas and peritoneal mesothelioma can be difficult to differentiate from ovarian cancer in women. For another, malignant mesothelioma was first classified as category C45 in the tenth edition of the International Classification of Disease (ICD-10, 1994), but the reporting of mesothelioma based on ICD-10 varies widely by country: developing countries usually lack the infrastructure and/or expertise to diagnose mesothelioma; developed countries have only gradually established the expertise to accurately diagnose mesothelioma, and misdiagnosis is still not uncommon compared to other cancer types. This becomes a source of bias, especially regarding data for earlier years and countries with few reporting years.

Two reports utilizing the WHO Mortality Database revealed a clear contrast between the mesothelioma situation in Asia [7] and Europe [8]. Mesothelioma deaths were reported to the WHO by 17 (36%) of 47 countries in Asia and 37 (70%) of 53 countries in Europe (the observed periods were 1994–2008 in Asia and 1994–2012 in Europe). When combined with asbestosis, the continental burdens of asbestos-related deaths (ARDs), relative to the world, were 13 and 60% for Asia and Europe, respectively [7, 8].

1.3 GBD Estimates of Mesothelioma

The Global Burden of Disease (GBD) study, which is a comprehensive regional and global research program that assesses mortality and disability from major diseases, injuries and risk factors [9], is one of the largest scientific collaborations in the world. It is widely and often cited as a reliable source for global public health data and trends.

An earlier GBD study (conducted for the year 2010) estimated that 33,160 cancer deaths were caused by occupational exposure to asbestos but did not report a specific estimate for mesothelioma [10]. In later GBD studies, mesothelioma was estimated as either "all mesothelioma" or "mesothelioma caused by occupational exposure to asbestos." The GBD study for 2013 substantially upgraded the estimate to 194,000 cancer deaths caused by occupational exposure to asbestos [11], with 33,700 due to all mesothelioma [12] (Table 1.1).

Several reports/studies on national-level trends have suggested that the mesothelioma incidence may have peaked (or is peaking) in several developed countries but did not provide evidence for a substantial decrease of the disease burden in those countries [19–21]. Even in the absence of an actual increase of incidence, the diagnosis and reporting of mesothelioma are improving widely across developed countries and beginning in some industrializing countries. Thus, at least a nominal increase should be observable due to improved reporting alone. The chapter authors, therefore, believe that the GBD estimates indicating a global *decline* of mesothelioma incidence/mortality (Table 1.1) are unlikely to be correct.

The chapter authors further surmise that the GBD estimates for mesothelioma are underestimated and/or biased because: (1) the rates and numbers estimated for China and India (not shown here) are low given their relatively long and heavy use of asbestos, which would substantially decrease the global estimate due to their population size (underestimation); (2) the female-to-male ratio found in most GBD estimates (not shown here) is much higher than common knowledge (bias); and (3) a separate study to project GBD estimates from the year 2016 to the year 2040 forecasted a substantial increase from 30,200 to 50,600 mesothelioma deaths per year [22] (not shown in Table 1.1). However, annual GBD estimates from 2017 to 2019 show a steady decrease (Table 1.1), which contradicts the long-term forecast.

Table 1.1 Estimates of mesothelioma by the global burden of disease (GBD) study

Year of GBD study	All mesothelioma (A)	Mesothelioma due to occupational exposure to asbestos (B)	Other mesotheliomas (C)
2013	33,700 [12]	Data not found	Not applicable
2015	32,400 [13]	23,000 [14]	9,400
2016	30,200 [15]	27,600 [16]	2,600
2017	29,900 [17]	27,000 [18]	2,900

A: GBD Causes of Death Collaboration; B: GBD Risk Factors Collaboration; C: Calculated by chapter authors as A − (minus) B

1.4 Other Global Estimates of Mesothelioma

In 2005, prior to the GBD study, Driscoll et al. [23] estimated the global burden of mesothelioma at 43,000 deaths per year (Table 1.2). This was derived by combining estimates of the proportion of exposed workers and of exposure levels (based on workforce data and the Carcinogen Exposure [CAREX] database) with absolute risk measures for mesothelioma. The study provided a breakdown of the estimated number by WHO regional groupings, but not by countries or continents. This estimate has been referenced by many studies, as well as by position papers of the WHO and other United Nation agencies.

More recently, Odgerel et al. [25] estimated the global burden of mesothelioma at 38,400 deaths per year by extrapolating national-level "quality data" for mortality rates and asbestos usage data (Table 1.2). This estimate is lower than that of Driscoll et al. but substantially higher than recent estimates of the GBD study (Table 1.1). Odgerel et al. applied objective criteria to judge the "quality" of mesothelioma data in the WHO Mortality Database, with "insufficient" data defined as: (1) a crude period mortality rate of 0.5 per M per year or less (i.e., less than half the widely accepted background level); (2) two or fewer reported years of data; or (3) 10 or fewer total reported deaths for the entire period. Of the 230 studied countries, 104 (45%) countries reported data and 126 (55%) did not; for the former, the data quality was sufficient for 59 (57%) countries and insufficient for 45 (43%). Thus, the global status of reports on mesothelioma deaths and their data quality can be conservatively summarized as follows: less than half of all countries have national statistics, of which only half is of sufficient in quality.

2 Asbestos Bans and Mesothelioma

2.1 Global Situation of Asbestos Bans

According to Kazan-Allen [26], since Iceland totally banned asbestos in 1983, a total of 67 countries/regions have adopted a total (compared to a "partial") on asbestos. Table 1.3 shows that five European countries, particularly those of northern Europe (with the notable exception of Finland, which banned asbestos in 2005), independently banned asbestos in the 1980s, followed by Liechtenstein in 1990 and

Table 1.2 Global estimates of mesothelioma by studies other than the GBD study

Author	Estimated global mesothelioma deaths	Year/period
Driscoll et al. (2005) [23]	43,000 deaths per year	2000
Park et al. (2011) [24]	174,300 deaths for 56 countries with data 38,900 deaths for 33 countries without data	1994–2008
Odgerel et al. (2017) [25]	38,400 deaths per year 9.9 deaths per million population/year	1994–2014

Table 1.3 Countries that adopted total asbestos bans during 1982–2019 [26]

Continent/ group	Up to 1989	1990–1999	2000–2009	2010–2019	Total
Europe	Denmark, Iceland, Norway, Sweden, Switzerland	Poland	*EU*[a]: Austria, Belgium, Bulgaria, Croatia[b], Cyprus[b], Czech Republic[b], Estonia, Finland, France, Germany, Greece[b], Hungary[b], Ireland, Italy, Latvia, Lithuania[b], Luxembourg, Malta[b], Netherlands, Portugal[b], Romania, Slovakia[b], Slovenia, Spain *Non-EU:* Gibraltar, Liechtenstein, New Caledonia[c], UK	Israel, North Macedonia, Monaco, Serbia, Turkey	39
Middle East		Bahrain, Kuwait, Saudi Arabia	Egypt, Jordan, Oman	Iraq, Qatar	8
Asia/ Oceania		Brunei	Australia, Japan, South Korea	New Zealand, Taiwan	6
Americas			Chile, Honduras, Uruguay	Argentina, Brazil[b], Canada, Colombia	7
Africa		Djibouti	Algeria, Gabon[b], Mauritius[b], Seychelles[b], South Africa	Mozambique	7
Total	5	6	42	14	**67**

The chapter authors constructed this table based on data by Kazan-Allen [26]
[a]Partial adoption of the asbestos ban was adopted in several European countries prior to 2005; where information on total ban is not available, the 2005 EU-wide total asbestos ban year is noted
[b]Total ban compliance not verified or not strictly enforced: Brazil, Croatia, Cyprus, Czech Republic, Greece, Gabon, Hungary, Lithuania, Malta, Mauritius, Portugal, Slovakia, Seychelles
[c]New Caledonia is a French territory situated in Oceania

Poland in 1997. By the end of the first decade of 2000, all EU member states had to comply with EU Directive 1999/77/EC [27] to ban all types of asbestos beginning in January 2005.

A range of countries of non-EU Europe, Asia/Oceania, and the Americas followed suit mostly after the turn of the century, with the notable exceptions of Canada (ban in 2018) and the USA (no ban at present). Russia, Kazakhstan, China, Zimbabwe, and Brazil are still mining asbestos (i.e., "asbestos-producing"), and the majority of industrializing countries in Asia/Oceania, Africa, and the Middle East have not adopted total bans on asbestos. Moreover, even under a "total" ban, certain items and/or situations may be exempted; their status may vary by country, as can the implementation of and compliance with the law (Table 1.3, footnote).

Such variation in the definition of "ban" makes it difficult to assess the possible relationship between an asbestos ban and mesothelioma incidence. Furthermore,

the speed at which countries have tapered off consumption, in relation to, or independent of, adopting a ban, has increased over time [28]. Therefore, for the purpose of data analysis, the status of the ban may be more adequately represented by the speed of reduction of asbestos consumption as a continuous variable than a binomial or categorical variable.

2.2 Does an Asbestos Ban Lead to a Decrease in Mesothelioma Incidence?

By applying a straightforward ecological study design, Lin et al. [29] showed that there is a clear correlation between the level of asbestos use and subsequent mesothelioma rates, implicating that countries using more (less) asbestos will subsequently shoulder higher (lower) burdens of mesothelioma. Using the conventional steps of statistics [i.e., moving from assessing a cross-sectional correlation to a delta (Δ; change over time) correlation], Nishikawa et al. [30] conducted a natural extension of the study of Lin et al. by asking whether a substantial reduction in asbestos use (as often caused by an asbestos ban) affected a reduction in the mesothelioma burden.

Specifically, Nishikawa et al. [30] assessed the interrelationship between mortality from pleural mesothelioma and the adoption of national bans on a global scale. Age-adjusted period mortality rates (MRs) in men for pleural mesothelioma during 1996–2005 were calculated for 31 countries. "Trends" were characterized by calculating the annual percent changes (APCs) of the MRs. The APCs were further grouped by whether they reflected "increase (↑)," "equivocal (→)," or "decrease (↓)," and then compared with historical patterns of asbestos use and the national ban status. Trends in mortality showed significant increases (↑) in five countries and marginally significant increases (↑) in two countries and were equivocal (→) in 24 countries. Whereas the global median APC was 4.5% per year, non-significant negative APC values were recorded in five countries of northern and western Europe: Austria (APC, −5.9% per year; year of ban, 1990), Finland (−0.3% per year, 1992), France (−1.0% per year, 1996), Iceland (−1.4% per year, 1983), and Norway (−2.7% per year, 1984).

Importantly, the change in asbestos use during 1970–1985 was a significant predictor of APC in male mortality for pleural mesothelioma, with an adjusted R^2 value of 0.47 ($p < 0.0001$). Moreover, a graph plotting the change (Δ) in asbestos use on the x-axis and APC (also "Δ") on the y-axis showed that all of the above countries recorded reductions in asbestos use and thus contributed substantially to the overall correlation. Although the study could not establish the direct effect of a ban, it suggested that an asbestos ban leads to a decrease in mesothelioma incidence.

The authors conservatively noted that the study period was inadequate (i.e., too short) to depict trends in many countries and the observed relationship may have reflected only early effects of the ban on mesothelioma rates. Given the long latency

time required for mesothelioma, the full consequences of such effects would require a longer observation period. Nevertheless, the study took advantage of the earliest opportunity to analyze the relationship based on national-level data from a range of countries.

Jarvholm and Burdorf [31] argued that it is difficult to evaluate the impact of an asbestos ban based on population-level trends in mesothelioma mortality rates and that such an evaluation must consider age-specific mortality rates for consecutive birth cohorts. They thus utilized Swedish data (Sweden having adopted an early national ban) to assess how a ban influenced age-specific mortality rates over time. The authors noted that although Sweden banned asbestos in 1982, the use of asbestos was already substantially reduced in this country by around the mid-1970s. Therefore, the authors compared the incidence of pleural mesothelioma in birth cohorts who started to work before and after the mid-1970s. The age of starting work was assumed to be 15–20 years and, due to an increase in immigration over time, the birth cohort analysis was restricted to persons born in Sweden.

The analysis showed that the later birth cohort (active in the workforce *after* the decrease in asbestos use) had a decreased risk of pleural mesothelioma relative to the earlier birth cohort for both genders: the relative risks (RR) of men and women born 1955–1979 versus men and women born 1940–1949 were 0.16 (95% CI 0.11–0.25) and 0.47 (95% CI 0.23–0.97), respectively. This finding was clearly illustrated by a line graph showing incidence rates (*y*-axis) versus age (*x*-axis): the lines of the earlier birth cohort were almost always positioned higher than those of the later birth cohort when compared for the same age. In contrast, a line graph depicting the trend over time (*x*-axis) in *overall* incidence (*y*-axis) showed only a minimal change. The authors rightly highlighted that the decrease in actual exposure is more important than a ban per se, but concluded that although their findings were for Sweden, similar interventions in other countries will reduce the occurrence of pleural mesothelioma.

2.3 Does a High Mesothelioma Incidence Lead to an Asbestos Ban in Some Countries?

When considering the relationship between an asbestos ban and mesothelioma incidence, one should bear in mind the various effects that can arise from the long latency period (in the order of decades) of mesothelioma. For example, a straightforward comparison between asbestos-banned and no-ban countries will often reveal *higher* mesothelioma incidence in the asbestos-banned countries, relative to the no-ban countries. This trend is evident in the findings of studies on mesothelioma mortality in Asia [7] and Europe [8].

This likely reflects that countries with high mesothelioma incidence *consequently* adopted asbestos bans, the process of which took some time. In turn, after

adopting an asbestos ban, the expected reduction in the incidence of mesothelioma can manifest only after several decades. Meanwhile, current asbestos-using (i.e., no-ban) countries continue to do so, mostly because the health effects of asbestos use (e.g., the increased incidence of mesothelioma) are not observable until several decades later.

The following two contrastive questions, therefore, are not mutually or self-contradictory:

- Q_A: Does an asbestos ban lead to a decrease in mesothelioma incidence?
- Q_B: Does a high mesothelioma incidence lead to an asbestos ban?

Q_A was tested by the studies introduced in Sect. 2.2, although the timing was too early to observe the full effect in a global analysis. However, Q_B remains to be investigated in both a global analysis and by analyzing national-level data.

3 Conclusion: The Need for International Cooperation

A wide consensus holds that mesothelioma is caused specifically by exposure to asbestos. Although researchers continue to study other possible causes and contributing factors, including the role of genetics, mesothelioma is currently unique among cancers in having one confirmed cause. It has been established that contact with asbestos occurs via occupational exposure, non-occupational exposure to building/industrial/natural sources, and household exposure. If these statements are true, and if asbestos exposure can be eliminated via banning asbestos, it will naturally follow that mesothelioma will be eliminated, or at least substantially reduced, by a ban.

Given that around 70 countries have already banned asbestos (the earliest being Iceland in 1983) [26], it is theoretically possible to construct an analytical framework to examine the effect. In reality, however, only a few analytical studies have addressed asbestos bans in relation to mesothelioma incidence, because researchers may have considered it difficult to detect such an association. This, in turn, could reflect that: (1) it may be too early to observe the full effect in view of mesothelioma's long latency period; (2) it is uncertain how an asbestos ban relates to the elimination of exposure; (3) the term "asbestos ban" covers a wide range of national situations and processes; and (4) asbestos usage can be reduced in relation to, or independent of, an asbestos ban. Nevertheless, two studies [30, 31] have suggested that asbestos bans have caused subsequent decreases in mesothelioma incidence.

Today many industrializing countries continue to use asbestos despite the abundant science on ARDs. It is thus important to share the experience and expertise of asbestos-banned countries. It will also be important to answer the yet-unaddressed question of "Did an increase in mesothelioma incidence lead to an asbestos ban in some countries?" High priority should be given to promoting asbestos bans while simultaneously improving mesothelioma diagnosis in the scheme of international cooperation with developing countries.

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Part II
Pathogenesis

Chapter 2
Asbestos and Mesothelioma: What Is Recent Advance in Research on Asbestos-Induced Molecular Carcinogenesis?

Marie-Claude Jaurand, Clément Meiller, and Didier Jean

Abstract The relationship between asbestos exposure and malignant mesothelioma is established since the middle of the twentieth century. From this time, scientific researches have progressed investigating the mechanism of action of asbestos on mesothelial cells, and more intensively during the beginning of the twenty-first century the analysis of the molecular changes in mesothelioma. Indeed, asbestos fibers were reported to induce chromosomal and genetic damage in mammalian cells. Mesothelioma is characterized by chromosomal alterations, which include numerous chromosome rearrangements, gene mutations, and gene deletions. Recent studies have enhanced our knowledge of the molecular landscape of mesothelioma, emphasizing mutations targeting more specifically tumor suppressor genes, differential gene expression, and DNA methylation in comparison with normal cells and between mesotheliomas, expression of noncoding RNAs, and alterations of regulatory pathways. Researches also provided knowledge of susceptibility factors in malignant mesothelioma families and relationships with asbestos exposure. It is time to review the recent advances in asbestos-induced molecular changes related to mesothelial carcinogenesis.

Keywords Asbestos · Genetic damage · Genetic susceptibility · Molecular heterogeneity · Pleural mesothelioma

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1 Introduction

The role of asbestos exposure in human mesothelial carcinogenesis is well established, but our knowledge on the mechanism of mesothelial carcinogenesis needs to be enhanced, as well as on the link between the molecular changes in malignant mesothelioma (MM) and the mechanism of action of asbestos on mesothelial cells. Over about 10 last years, progresses have made in the field of MM molecular characterization. Some pathological and molecular changes were ascertained and other established. These findings encouraged us to review the recent advances in asbestos-induced molecular changes related to mesothelial carcinogenesis.

2 Researches on Malignant Mesothelioma

2.1 Molecular Characteristics of Malignant Mesothelioma

Our knowledge of the molecular characteristics of MM and its pleural form has recently progressed. Earlier, chromosome rearrangements and mutations in tumor suppressor genes were reported in MPM. Rearrangements concerned numerous chromosomes, especially chromosomes 9 (9p21), 3 (3p21), and 22q, with more frequent losses than gains. Gene mutations, especially in the tumor suppressor genes *CDKN2A*, *CDKN2B,* and *NF2* mostly occur via partial or complete deletions, and low rates of mutations were detected in *TP53*, one gene frequently mutated in other cancers [1, 2]. Further studies confirmed these findings and increased the list of frequently mutated genes, especially adding *BAP1* (BRCA1-associated gene) and other genes with a lower rate of mutations such as *SETD2* (SET domain containing 2) and *LATS2* (large tumor suppressor kinase 2) [3–6]. A few genes have been inconsistently reported as altered in mesothelioma, *CUL1* [7], or at a lower rate such as *DDX3X*, *ULK2*, *RYR2*, *CFAP45*, *SETDB1* and *DDX51*, or genes from the *SMARC* family (SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily C), *PBRM1*, *COPG1*, *MLRP1*, *INPP4A*, *SDK1*, and *SEMA5B* [4, 8–10].

Gene expression profiles in MPM revealed the differential expression of specific genes in comparison with normal mesothelial cells or lung tissues, or other thoracic cancers and provided a variety of information on the mechanism of mesothelioma carcinogenesis and the prognostic value of the expression level of specific genes [4, 11–15].

Recently, three comprehensive genomic studies demonstrated the molecular heterogeneity of MPM and allowed to distinguish molecular subtypes of MPM according to their gene expression profiles [4, 6, 16]. The molecular classifications were partially related to the histological types. Although MPM is classically defined at the histological level as epithelioid, mixed, and sarcomatoid types, the gene expression profiles allowed to define histology-independent or partly dependent subtypes,

discriminating especially within epithelioid morphologies. Importantly, molecular subtypes were linked to patients' survival [4, 6, 16].

MPM heterogeneity was further investigated by transcriptome analyses using deconvolution methods [17]. This approach allowed to define a set of genes that define epithelioid-like and/or sarcomatoid-like types of MPM. Then, an MPM tumor can be decomposed as epithelioid-like and sarcomatoid-like components and can be defined by an E- and S-score, which refers to the proportion of these components. Interestingly, the S-score is strongly associated with prognosis [17]. Besides, this study also revealed that markers of the adaptive immune response were predominant in tumors with a high S-score, whereas markers of the innate immune response are found in tumors with a high E-score, consistent with an impact of the tumor microenvironment on survival [17]. The interest of associating molecular investigations and histological analysis was later proposed in a review recommending to update the histologic classification of MPM by a more multidisciplinary approach to support clinical practice, research investigation, and clinical trials [18]. An influence of the microenvironment on patients' outcome was further suggested using deep learning based on MPM histology slides [19]. Contribution of histone methyltransferases can be illustrated by the overexpression of *EZH2*, a component of the polycomb complex PRC-2, which silent histone H3 by trimethylation [20]. Recent studies highlighted the strong contribution of epigenetic regulation through DNA methylation or miRNA expression deregulation in MPM. Integration of miRNome and methylome data revealed the contribution of epigenetic regulation in the epithelioid-like and sarcomatoid-like components of the tumors [17, 21]. Some genes such as *WT1* and *PI3KR1*, or *RUNX1* and *PBRM1* were hypermethylated and underexpressed in tumors with a high E-score or S-score, respectively [17]. Next-generation sequencing analyses linked alterations of histone methylation pathway to inactivation of histone lysine methyltransferases, mainly *SETD2* and *SETDB1* [4].

Long noncoding RNAs (LncRNAs) also play a role in epigenetic regulation mechanisms. A number of LncRNAs have been identified as potential regulators of MPM, several of them being involved in EMT [22]. Their expression may be modulated by key genes in MPM, such as *NEAT1*, whose expression is dependent on *BAP1* expression, or *HOTAIR* which regulates E-cadherin expression through the recruitment of PRC2 chromatin remodeling complex [22].

A few data are available on protein expression in MM. Mass spectrometry analyses were carried out to compare differentially expressed proteins in biphasic MM and benign tumors [23]. Pathways analysis revealed a decrease of activation state in pathways of reactive oxygen species (ROS), respiratory system and cell death, and an increase of activation of phagocytes in MM tumors [23]. Großerueschkamp et al. [24] compared epithelioid and sarcomatoid MM using a method integrating FTIR (Fourier Transform InfraRed spectroscopy) imaging and laser capture microdissection, and proteome analysis of the dissected tissue. Laser capture is interesting as it allows the selection of specific regions within the tumor. Epithelioid MM overexpressed calretinin (CALB2) and several cytokeratins (CKs), and collagen A1 was overexpressed in the sarcomatoid form, consistent with the EMT. CKs and CALB2 are markers of epithelioid MM [25].

Proteomic approaches were also used to characterize MM secretome and exosome. MM secretome was analyzed in six cell lines by iTRAQ® mass spectrometry and compared to non-malignant cell lines. Results showed differential expression of proteins involved in metabolic energetic pathways, upregulation of proteins involved in cancer invasion and metastasis, and downregulation of proteins involved in cell adhesion [26]. The protein content of MM-derived exosomes was investigated in the four MPM cell lines studied in the previously quoted paper. A majority of proteins detected are expressed in various types of cancer, but specific proteins were identified in MM, either shared with all MM of differential between the MPM [27]. The proteomic findings correlated with gene expression reported in transcriptomic studies of MPM and identified biomarkers known to be expressed from immunohistochemical studies, as well as immunomodulatory components and tumor-derived antigens [27].

2.2 State of Signaling Pathways in Malignant Mesothelioma

Several signaling pathways are deregulated in human MM, leading to an unmaintained mesothelial cell homeostasis. Pathways analyses from transcriptomic data have revealed alterations in cell proliferation control, apoptosis, differentiation, cell migration, and survival [28, 29]. In cancer, both the MAPKs and PI3K/AKT/mTOR pathways are often affected by activating oncogenic mutations in genes involved in these signaling pathways, but these mutations are rare in MM [30]. In MM, these pathways are activated as assessed with the use of specific inhibitors that reduce cell growth or cell viability, and their activation may result from overexpression of specific growth factors or receptors such as EGFR and MET [29, 30]. Pathway analyses carried out in recent comprehensive integrative genomic studies highlighted P53and mTOR pathways as deregulated in MPM [4, 6, 17]. Other pathways were identified as differentially activated between MPM tumors, depending on the E/S-scores (angiogenesis, EMT, immune checkpoints, and metabolic pathways) [17].

One prominent feature in MM is the deregulation of Hippo, an evolutionarily conserved pathway involved in the development and control of organ size. When turned on, this pathway negatively controls cell proliferation, partly maintaining cell–cell contacts. Protein players of the pathway are merlin (*NF2*), LATS1 and LATS2 that silence YAP and TAZ by phosphorylation, and consequently avoid the transcription of downstream genes such as *CTGF*, *CYR61,* or *c-MYC* [31]. In MPM, several members (*NF2*, *LATS2*, *LATS1*, *SAV1*, etc.) of the Hippo pathway are inactivated due to gene mutations and/or deletions [5, 32]**.** This pathway crosstalks with other pathways, Hedgehog, Wnt, and P53. This last cross is of particular interest regarding the different rates of mutations of *NF2* and *TP53* in MM, with a possible repercussion of alteration of one pathway on the other. A recent review sheds light on the interactions between Hippo and P53 pathways, which show both mutated member genes in MPM [33]. YAP and P53 can bind to the *TP53* and *YAP* promoters, respectively. Moreover, LATS1/2 binds to MDM2, a negative regulator of P53,

and YAP1 can bind to mutant P53 and members of the P53 family [33]. Finally, these two pathways may coordinately maintain genomic stability in response to stress by the modulation of cell senescence, apoptosis, and growth.

2.3 Gene Susceptibility Factors

The possible role of genetic susceptibility in MM was suggested by recurrent familial MPM cases in cancer families. They reported increased susceptibility related to asbestos exposure [34, 35]. Some polymorphisms were found in genes involved in oxidative metabolism such as *GSTM1* or participating in base excision repair (BER) pathway, *XRRCC1* and *XRCC3* [36]. Two genome-wide association studies were carried out to identify the genetic risk factors that may contribute to the development of MPM. In an Australian study, no single nucleotide polymorphisms (SNPs) was of statistical significance when compared to Australian resident controls or asbestos-exposed control population without MM [37]. However, suggestive results for MPM risk were identified in the *SDK1*, *CRTAM,* and *RAS-GRF2* genes, and in the 2p12 chromosomal region [37]. In a case-control Italian study, with a known history of asbestos exposure, SNPs were identified in genes *SLC7A14*, *THRB*, *CEBP350*, *ADAMTS2*, *ETV1*, *PVT1,* and *MMP14* in MPM cases, but without significant threshold [38]. All these genes appeared as low risk-predisposing factors for MPM, with possible synergistic effect with asbestos exposure [39]. In contrast, *BAP1* was reported as a high-risk genetic factor for MPM [39]. Germline *BAP1* mutations were observed in families developing MM [40]. Although not occupationally exposed to asbestos, the family members were exposed in their indoor environment [40].

The frequency of germline mutations was also investigated in 198 MM patients, by targeted capture and NGS. Among 85 cancer susceptibility genes analyzed, mutations were identified in 12% of patients, and in 13 genes. A significant enhancement of the frequency of mutations in *BAP1*, *BRCA2*, *CDKN2A*, *TMEM127*, *VHL,* and *WT1* was found in MM cases in comparison with a non-cancer control population (Exome Aggregation Consortium) [41]. This study, which collected MM from peritoneum, pleura, and tunica vaginalis reported higher germline mutation frequencies in peritoneal MM, in patients with no known asbestos exposure, with a second cancer, and in tumors of epithelioid histology, when compared to pleural MM, definite exposure, no cancer, and biphasic and sarcomatoid histology, respectively. Other studies identified germline mutations in MPM patients in genes such as *PALB2*, *FANCI*, *ATM*, *SLX4*, *BRCA2*, *FANCC*, *FANCF,* and *PMS1* [39, 42–44].

Although germline mutations in *BAP1* are susceptibility factors in the induction of MM in individuals exposed to asbestos, they do not seem to lead to MM in the absence of exposure. This hypothesis is supported by experimental studies using heterozygous $Bap1^{+/-}$ mutant mice not treated with asbestos showing no or a low rate of spontaneous mesotheliomas, despite a high incidence of other types of malignant tumors, and an increased incidence $Bap1^{+/-}$ asbestos-exposed mice in

comparison with their *Bap1*$^{+/+}$ counterparts [45, 46]. Moreover, homozygous conditional knockout mice *Bap1*$^{-/-}$ generated by the injection of Adeno-*Cre* in the pleural cavity also developed a low rate of pleural mesothelioma (1/32 mice) [47, 48].

3 Asbestos Fibers and Mesothelial Carcinogenesis

Literature data have demonstrated that in addition to asbestos fibers, other types of fibers, erionite or fluoro-edenite induce MM due to environmental exposure [11, 49]. Additionally, it should be mentioned that some synthetic fibers were classified as probably (carbon whiskers) or possibly carcinogenic (some type of carbon nanotubes) by IARC [50].

3.1 Global Mechanism of Action of Mineral Fibers

Many papers reviewed the mechanism of action of asbestos fibers. Schematically, they focused either on the physicochemical properties of asbestos that may trigger toxic effects related to their fibrogenic and carcinogenic potency or on the consequences on the cell state in terms of cytotoxicity (cell growth, cell death) and genotoxicity (see for review [51–56]). Important discriminating physicochemical fiber parameters for asbestos effects are dimensions, surface reactivity, and biopersistence [56].

Hypotheses on the mechanisms accounting for the asbestos effects are based on studies with in vitro cell systems and on animal experiments. They will be briefly reminded here. Following asbestos inhalation, the mechanism first includes the clearance mechanism, which eliminates some fibers from the airways, leaving others to deposit in the lung and translocate to the pleura [57–60]. Early effects in the mesothelial microenvironment are suggested to be linked to an inflammatory reaction, as in the presence of foreign particles [58, 61, 62]. As reported in several publications, this reaction produces molecules deleterious for the cells and their microenvironment, and potentially carcinogenic such as ROS and nitrogen–oxygen species (NOS). Endogenous ROS can be also produced by normal cellular metabolism [63]. Asbestos fibers also induce genomic damages such as DNA and chromosome alterations, chromosome missegregation, and mitosis impairment [15]. Accordingly, fiber uptake, inflammation, DNA repair, and cell death are processes that play a role and modulate the effects and the consequences of asbestos–cell interactions on cell homeostasis. At present, one can ask how the molecular features identified in MPM can be linked to the mechanism of action of asbestos. We will briefly suggest some clues.

3.2 Molecular Features of MPM Possibly Related to the Mechanism of Action of Mineral Fibers

3.2.1 Genetic Damage in MPM

Remembering that carcinogenesis is a multistep process, the effects observed on cultured cells, and in short-term animal experiments can tell us on the initial damages from early effects, inflammatory response of cells, and genotoxicity of asbestos fibers. In that context, the production of ROS and NOS play a role, inducing base oxidation and nitration [53]. Inflammation is thought to play a key role in genotoxicity, due to the production of ROS by macrophages and neutrophils. Based on studies of the relationship between dose-dependent inflammation and genotoxicity of particles in animal lungs, no direct experimental evidence suggests that inflammation is a prerequisite for oxidative damage of DNA in the lung, but the association might be due to the use of high doses of particles [53]. In MPM, transversions C > A, which are lesions resulting from unrepaired 8-oxo-7,8-dihydroguanine (8-oxoGua) oxidation by ROS are not the most frequent lesions, but C > T transitions occurring by deamination of 5-methylcytosine in CpG islands [4]. This does not demonstrate a predominant role of ROS to account for gene alterations. It is noteworthy that alterations of genes frequently inactivated in MPM, such as *BAP1*, *CDKN2A*, *CDKN2B*, *SETD2*, consist often in partial or complete large deletions of exons, likely linked to other types of damage and repair systems [6, 32]. DNA alterations may occur in later stages, as a result of chronic inflammation, which can be induced by many physical and chemical [64].

DNA double-strand breaks (DSB) are other forms of DNA damage that can be caused by different sorts of clastogenic agents, by mechanical stress on chromosomes or in case of replication stress, and also promoted by abnormal mitosis [65, 66]. Several experimental works carried out with different types of cultured cells, including mesothelial cells, have shown that asbestos may interfere with mitosis [67–69]. Abnormal mitoses are revealed by various observations including the occurrence of aneuploidy, chromosome and chromatin damages, defects in spindle formation, lagging chromosomes, centrosome amplification, multipolar mitoses, and alterations of cytokinesis [36, 51, 70–74]. Cell cycle investigations have shown an accumulation of asbestos-treated cells in the G2/M phases of the cell cycle, consistent with a protracted mitosis [75–77]. It is known that mitosis impairment may promote chromosome missegregation, rearrangements, and aneuploidy, and delayed mitosis may promote DNA breakage, as shown with agents interacting with microtubule dynamics and other different conditions [66]. Therefore, the impact of asbestos on mitosis, which is due to the fiber internalization and the interaction with cell, is also an important effect to consider in the mechanisms of asbestos-induced carcinogenesis.

Repair processes are very important to resolve DNA damages. They include homologous and non-homologous recombination that may result in error-prone repair [78]. They may play a role in the genesis of MPM. On one hand, asbestos induces DNA breakage, as shown by the genotoxicity data in experimental assays. On the other hand, several publications reported pathogenic variants in DNA repair systems including recombination repair genes [39, 42].

3.2.2 Cell and Molecular Heterogeneity in MPM

A second MPM feature stands in its heterogeneity revealed at the cell and molecular levels. Pathological observations of MPM demonstrated a great morphological heterogeneity of the tumors [79]. This may reflect cell differentiation or different cell origin, as two main types of normal mesothelial cells, flattened and cuboidal, are distinguished and differentially distributed on the pleural sheets [80, 81]. In the same vein, recent data suggested that a tumor can be composed as a combination of epithelioid-like and sarcomatoid-like components, so-called histo-molecular gradients that encompass the tumor morphology and the molecular specificities [17]. This would be compatible with the in situ differences between normal mesothelial cells. Further analyses are needed to determine to what extent in situ normal mesothelial cell heterogeneity is pertinent to account for the origin of tumor heterogeneity.

Molecular heterogeneity of MPM is attested both by mutations and deregulation of signaling pathways. Molecular heterogeneity, in terms of mutations, is likely linked to the polyclonal and sub-clonal evolution of tumor cells, as shown by the intra-tumor heterogeneity [82–84]. Hippo pathway inactivation is a characteristic of some MPM. The role of the Hippo pathway is possibly linked to the structure of the pleura and to the mechanism of action of asbestos fibers. First, normal mesothelial cells form a monolayer at the serosal surface and are joined by junctions, which assure cell–cell and cell–basal membrane contacts [85, 86]. Hippo pathway activity is regulated by mechano-transduction and cell–cell adhesion and controls tight junctions [31, 87]. Its inactivation may abolish control of claudins, which are expressed in tight junctions, and differentially expressed in epithelioid compared to nonepithelioid MPM, and in MPM compared to healthy tissue [4, 17, 88–90]. Second, asbestos fibers provoke numerical chromosome changes and alteration of mitosis, especially the abolishment of cytokinesis, leading to in aneuploid cells including tetraploid cells. Interestingly, the Hippo pathway regulates the proliferation of tetraploid cells and blocks their proliferation. Asbestos fibers avoid cell abscission, and tetraploid and near-tetraploid cells are observed in asbestos-treated mesothelial cells and in MPM [91, 92]. Therefore, knockout of proliferation control may facilitate chromosome instability and the appearance of hypo-tetraploid or hyperdiploid cells, and lead to neoplastic evolution. It may be paradoxical that *NF2* seems more frequently mutated in nonexposed patients than in exposed patients, but *NF2* mutations in asbestos-exposed cells would lead to catastrophic mitosis [32].

Conversely, *BAP1*, the most frequently mutated gene in MPM, might prevent chromosome instability, by the regulation of γ-tubulin ubiquitination in *BAP1* wild-type cells [93, 94].

4 Conclusions

MPM remains thoroughly associated with asbestos fibers exposure in humans. For therapeutic purposes, numerous molecular studies have been carried out on human MPM to identify genomic alterations and activation state of signaling pathways. Experimental studies have been performed in knockout mice to assess the role of genes altered in human MPM. *BAP1* has been identified as a susceptibility gene in asbestos-exposed patients, and the Hippo pathway is the noteworthy pathway in MPM, among other frequently altered pathways in cancer.

Studies on human tumors have shown shared features between MPM tumors characterized by a high rate of chromosome rearrangements and recurrent mutations in a limited number of genes. Oppositely, a heterogeneity was evidenced between MPM at the morphological and molecular levels. Transcriptomic and proteomic studies have defined the MPM heterogeneity by the identification of individual MPM characteristics highlighting acknowledged neoplastic evolution like EMT, but so far without well-established steps of progression. Nonetheless, the original description of a histo-molecular continuum based on transcriptomic data linked to immunologic context and to patients' outcome was established [21].

Toxicology studies have documented the chromosome damage and the occurrence of potentially DNA-damaging inflammatory processes linked to asbestos exposure. The causal relationship between MPM and the mechanism of action of asbestos was consolidated by the occurrence of MPM in asbestos-exposed mice deficient in genes representative of human MPM.

Our present level of knowledge allows us to formulate hypotheses to link the identified MPM features to the mechanism of action of asbestos. In terms of genetics, the generation of abnormal mitoses in asbestos-interacting cells is likely preponderant. Improvement of our knowledge of the inflammatory microenvironment of the tumor cells should precise the role of inflammation in MPM evolution. Concerning heterogeneity, the pleural anatomy may account for the morphological heterogeneity, in addition to the neoplastic evolution. In terms of signal pathways alteration, an involvement of the Hippo pathway is likely related to its role in the regulation of membrane dynamics and growth [95, 96]. At least two elements should be considered. First, Hippo pathway components localize at cell junctions, which are important structures of the mesothelium that is formed by a monolayer of tightly joined mesothelial cell. Second, the Hippo pathway controls membrane junctions and cytoskeleton dynamics, and growth. The presence of solid material inside or near mesothelial cells impairs the chromosome and membrane dynamics during the

mitotic process. Further studies will likely clarify the relationships between mechanisms of action of asbestos and the molecular mechanism of mesothelial carcinogenesis.

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Chapter 3
Asbestos Fiber and Immunological Effects: Do Immunological Effects Play Any Role in Asbestos-Related Diseases?

Yasumitsu Nishimura, Naoko Kumagai-Takei, Suni Lee, Kei Yoshitome, Tatsuo Ito, and Takemi Otsuki

Abstract The immune system functions to eliminate abnormal cells that may arise for a variety of reasons. This process can help prevent tumor formation and is referred to as anti-tumor immunity. Inhaled asbestos fibers can accumulate in the non-lymphoid organ of the lungs as well as draining lymph nodes, exposing immune cells to the inhaled asbestos and thereby triggering potential immunological effects. On the basis of that idea, we have been investigating various kinds of asbestos exposure-mediated functional alterations in natural killer (NK), $CD4^+$ T helper (Th), $CD8^+$ cytotoxic T lymphocyte (CTL), and macrophage cells by in vitro experiments. The findings obtained indicate that exposure to asbestos causes decreased cytotoxicity of NK and CTL cells and decreased expression of cell surface activating receptors (NKp46, NKG2D), intracellular perforin levels, and IFN- γ production. Furthermore, an enhanced immune-suppressive role of Th (regulatory T (Treg)) cell function results, with increases in cell surface CTLA-4, and increased production of IL-10 and TGF-β cells as well as fibrogenic/immune-suppressive macrophages with high and lasting production of TGF-β. Interestingly, patients with malignant mesothelioma also show similar characteristics with respect to findings relating to peripheral blood mononuclear cells. Taken together, those data suggest that asbestos fibers elicit immune-suppressive potential, which might contribute to immune escape of abnormal mesothelial cells arising transiently, and promote the development of malignant mesothelioma in people exposed to asbestos. Continued investigations of these events may facilitate the development of early intervention strategies from an immunological perspective to mitigate the development of mesothelioma.

Keywords NK · CTL · Th1 · Treg · Macrophage

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1 Introduction

It is well known that asbestos fibers possess cytotoxic and pro-inflammatory effects that include mutagenicity and the production of reactive oxygen species [1–5], which are thought to account for the observed development of malignant mesothelioma. However, the body has an immune system that always checks for the presence of abnormal cells and can eliminate these cells in an effort to avoid tumor diseases. Therefore, if an abnormal mesothelial cell was transiently generated by exposure to asbestos, it should not survive the response of the immune system. Additionally, it is interesting that malignant mesothelioma is caused by low- or middle-dose exposure to asbestos rather than high doses, and can take a long period to develop following asbestos exposure [6]. This suggests that the development of mesothelioma might be more complex and is not caused only by toxic or pro-inflammatory effects of asbestos. These findings prompted the development of the hypothesis that functional alterations in the immune system might be related to the development of malignant mesothelioma following exposure to asbestos. Actually, it has been reported that asbestos fibers inhaled as a result of work-related activities or within a general environmental context accumulate in regional lymph nodes [7, 8], which suggests that even lymphocytes can potentially be affected by asbestos fibers. In this chapter, we introduce findings from our studies relating to asbestos-induced functional alterations in immune cells as determined by the investigation of peripheral blood obtained by patients with malignant mesothelioma. Finally, we explain the relationship between the observed immunological effects and mesothelioma.

2 Altered Functions of Immune Cells Caused by Exposure to Asbestos

2.1 Decreased Functions of Cytotoxic Lymphocytes

2.1.1 Natural Killer Cells

Natural killer (NK) cells play an important role as first defenders against abnormal cells in the context of anti-tumor immunity, as they promptly exert natural cytotoxicity against targets without any induction. In fact, the relationship between the cytotoxicity of NK cells and tumor disease has been reported clearly: Both men and women with low levels of natural cytotoxicity in peripheral blood show a high incidence ratio of tumor diseases after measurement of cytotoxicity compared with subjects that possess middle or high cytotoxicity [9]. In order to selectively target abnormal cells and leave healthy cells unscathed, cytotoxicity is regulated by the balance of signals derived from activating and inhibitory receptors expressed on the cell surface, which recognize certain types of ligands on the target cells [10–16].

The activating and inhibitory receptors transduce promoting and suppressive signals through extracellular signal-regulated kinase (ERK) and c-jun N-terminal kinase (JNK), respectively, leading to degranulation of cytotoxic granules and from which perforin and granzymes are released to injure targets [17, 18]. When the human NK cell line YT-A1 was continuously cultured with asbestos, it showed impaired cytotoxicity with decreased expression of cell surface NKG2D and 2B4 activating receptors and intracellular perforin and granzyme A after about 4 months of culture [19]. Degranulation was also reduced in YT-A1 exposed to asbestos, which showed lower phosphorylation of ERK following stimulation through NKG2D compared with the original cell line [20]. When human peripheral blood mononuclear cells (PBMCs) were cultured with asbestos, NK cells in the culture also showed altered expression of activating receptors, with cell surface expression of NKp46 (NCR1) activating receptor decreasing upon exposure to asbestos, although exposure to glass wool did not result in a decrease. Finally, when NK cells in peripheral blood of patients with malignant mesothelioma were analyzed for natural cytotoxicity and cell surface expression of activating receptors, lower levels of cytotoxicity per cell were observed compared with healthy people, in addition to lower levels of NKp46 expression, although NKG2D and 2B4 levels did not differ [19]. These findings indicate that exposure to asbestos causes impairment of natural cytotoxicity with altered expression of activating receptors, and might contribute to insufficient removal of abnormal cells arising in asbestos-exposed individuals, thereby leading to malignant mesothelioma.

2.1.2 $CD8^+$ T Cells

$CD8^+$ T cells, also referred to as cytotoxic T lymphocytes (CTLs), represent a population of cells that play a role in anti-tumor immunity by specifically recognizing and injuring target cell antigen, which differ from NK cells, although both cell populations utilize the same machinery of perforin and granzymes to effect cellular injury [21]. Naïve $CD8^+$ T cells require antigen stimulation to develop effector cells equipped with cytotoxicity and the production of cytokines such as IFN-γ, and the part of cells stimulated produces memory cells that function to maintain cytotoxicity induced against target cells [22, 23]. The mixed lymphocyte reaction (MLR) is an in vitro model that mimics antigen-specific responses of T lymphocytes by using two sets of whole immune cells obtained from different donors for responder and stimulator. When human PBMCs as responder were cultured with allogenic PBMCs as a stimulator for the MLR assay, the addition of asbestos into the culture resulted in a decrease in induced cytotoxicity of $CD8^+$ T cells against allogenic PBMCs and was accompanied with decreases in cell proliferation and production of TNF-α and IFN-γ [24]. Additionally, the $CD8^+$ T cells showed decreases in effector/activation cell surface markers (CD45RO and CD25) as well as an increase in naïve cells (CD45RA). To examine the effect of asbestos exposure on the maintenance of effective CTL function, the EBT-8 human $CD8^+$ T cell line, which expresses HLA-DR, a marker of T cell activation, was continuously cultured with asbestos for more than

one month in media supplemented with IL-2 to maintain cell proliferation. Asbestos exposure decreased intracellular expression of perforin, but not granzyme B, and stimulated the production of IFN-γ in EBT-8 [25]. Consistent with the aforementioned results, patients with malignant mesothelioma showed lower levels of intracellular perforin, but not of granzyme B, in peripheral blood $CD8^+$ T cells after stimulation compared with healthy or pleural plaque-positive individuals without tumors, although there was no difference in intracellular IFN-γ [26]. These findings indicate that asbestos exposure suppresses induction to effector CTLs during stimulation as well as maintenance of those effectors, and where $CD8^+$ T cells in mesothelioma patients have a similar character. Additionally, it has also been found that supplementation of media with IL-2 led to recovery of asbestos-induced low cytotoxicity of $CD8^+$ T cells in culture for the MLR assay, although altered expression of cell surface markers was not restored, suggesting possible restoration of impaired CTL function in mesothelioma patients with appropriate treatment [27].

2.2 Enhanced Immune-Suppressive Functions of Lymphocytes as well as Myeloid Cells

2.2.1 $CD4^+$ T Cells

From here, we introduce findings concerning the effects of asbestos exposure on suppressive functions of lymphocytes as well as myeloid cells. $CD4^+$ T lymphocytes function as the most important population of cells that decide the direction of the immune response following stimulation, thereby contributing to the promotion of different kinds of immune responses as well as immune suppression. In particular, the balance of T helper 1 (Th1) and regulatory T (Treg) cell functions is crucial for the success or failure of anti-tumor immunity following antigen stimulation [28, 29]. Th1 cells represent a major cell population that produces IFN-γ which stimulates dendritic cells as well as $CD8^+$ T cells directly and leads to the promotion of immune responses against tumors. CXCR3 is expressed on $CD4^+$ T cells as a chemokine receptor and representative marker for Th1 cell. Continuous exposure to asbestos decreased expression of CXCR3 and production of IFNγ in the MT-2 human $CD4^+$ T cell line as well as in vitro expanded peripheral blood $CD4^+$ T cells during culture [30, 31]. TGF-β and IL-10 are representative cytokines that are produced by Treg cells and function to suppress immune responses [32]. MT-2 cells also possess some character of Treg cells, since MT-2 is a T cell line immortalized by human T cell leukemia virus type 1 (HTLV-1) virus which causes adult T cell leukemia (ATL) and show immune-regulatory properties [33]. Exposure of the MT-2 cell line to asbestos resulted in high production of TGF-β and IL-10 as well as augmented performance in suppressing the proliferation of conventional $CD4^+$ T cells following stimulation, which is reduced by knockdown of TGF-β and IL-10 genes in asbestos-exposed MT-2 cells. Interestingly, when properties of peripheral blood $CD4^+$ T cells were compared among healthy (H), plaque-positive (P)

individuals and mesothelioma patients (M), the order of CXCR3 expression and production of IFN-γ in peripheral blood $CD4^+$ T cells was H > P > M and H = P > M, respectively. Additionally, exposure of the MT-2 cell line to asbestos resulted in increased cell surface expression of CTLA-4 [34], which functions in immune suppression by interfering with the interaction of CD28 with conventional T cells and CD80 and CD86 on antigen-presenting cells including dendritic cells and macrophages [35, 36]. Consistent with this finding is the fact that the expression of CTLA-4 on peripheral blood $CD4^+$ T cells is higher in patients with mesothelioma compared with patients with an asbestos-related benign disease such as diffuse pleural thickening (unpublished data). These results suggest that asbestos exposure might contribute to suppressed anti-tumor immunity related with the development of malignant mesothelioma.

2.2.2 Macrophages

Macrophages are responsible for phagocytizing exogenous as well as endogenous particles and fibers in lymphoid and non-lymphoid organs, where inflammatory cytokines including TNF-α, IL-6, and IL-1β are produced by macrophages [37, 38]. Inhaled asbestos fibers also chronically causes these responses, which subsequently contribute to the development of lung fibrosis as well as tumor diseases. Therefore, macrophage-induced inflammation has been a target of research in addition to investigations concerned with the treatment of asbestos-related diseases [1, 39]. However, one of our previous studies found that low-dose exposure to asbestos induced high and lasting production of TGF-β by alveolar macrophages in culture in the absence of any other types of cells and was not accompanied with apoptosis of macrophages, whereas high-dose exposure to asbestos resulted in apoptosis [40]. Additionally, those macrophages showed long survival with increased expression of anti-apoptotic factor Bcl-2. TGF-β is a key cytokine that promotes lung fibrosis, and also contributes to immune suppression as described above. In fact, M2-type macrophages that produce TGF-β function as a population of myeloid cells to suppress anti-tumor immunity [41]. Taken together, these findings suggest that functional alterations in macrophages with fibrogenic and immune suppressive activity following exposure to asbestos might play a direct role in asbestosis through the induction of fibrogenic responses as well as in the promotion of malignant mesothelioma through immune suppression.

3 Conclusion

The results obtained from our studies as mentioned above demonstrated that exposure to asbestos causes functional alterations in immune cells, manifesting as decreased cytotoxic activity of NK and $CD8^+$ T cells, and enhanced immune-suppressive activity of $CD4^+$ T cells and macrophages (Fig. 3.1). Additionally,

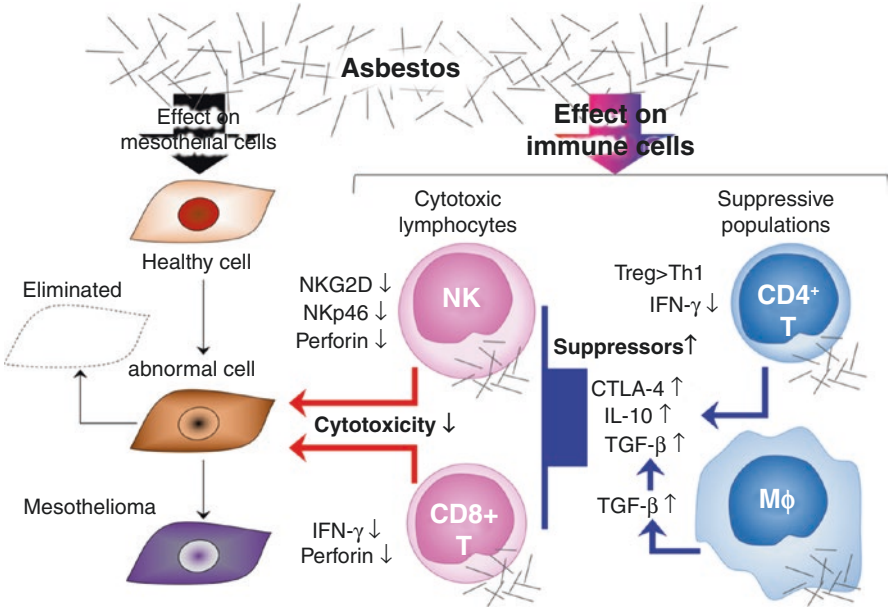

Fig. 3.1 Summarized illustration: Asbestos-induced functional alterations in immune cells. Exposure to asbestos causes decreased cytotoxicity of NK and $CD8^+$ T cells with decreased expression of activating receptors (NKp46, NKG2D), perforin, and IFN-γ, and induces enhanced suppressive functions of Treg cells and macrophages (Mϕ) with increases in CTLA-4, IL-10, and TGF-β. These alterations contribute to immune escape of abnormal mesothelial cells transiently resulting from exposure to asbestos, and promote the development of malignant mesothelioma

patients with malignant mesothelioma showed several kinds of altered gene expression similar to those found in asbestos-exposed immune cells. Taken together, these findings suggest that asbestos fibers possess immune-suppressive potential, which might contribute to immune escape of abnormal mesothelial cells arising transiently, and subsequent development of malignant mesothelioma in people exposed to asbestos. This knowledge will assist in the development of early intervention strategies from an immunological perspective to mitigate the development of mesothelioma. In fact, on the basis of data obtained from comprehensive analyses of patients with mesothelioma as well as individuals exposed to asbestos without tumors, we have proposed a screening tool with immunological scores for malignant mesothelioma as well as asbestos exposure (patent pending WO2016-167346). Additionally, the findings suggest the possible effectiveness of immunological treatment of mesothelioma, with particular focus on Treg cells and their factors including immune checkpoint molecules, and these are areas that our ongoing research is presently addressing.

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Chapter 4
Biomolecular Pathways in Mesothelioma: What Is New Perspective on Biomolecular Research for Mesothelioma?

Giovanni Gaudino, Michael Minaai, Michele Carbone, and Haining Yang

Abstract Exposure to asbestos and to other carcinogenic fibers causes mesothelioma, an aggressive tumor with poor prognosis. Tumorigenesis originates from a chronic inflammatory process driven by high mobility group box 1 (HMGB1) and the activation of the inflammatory factors, which induce the secretion of tumor necrosis factor-α (TNF-α) and other cytokines. Over time, the chronic inflammatory process induces cell survival, favoring the accumulation of DNA mutations that activate several activated pathways, promoting tumor growth. The discovery of germline heterozygous mutations of the BRCA-associated protein 1 (*BAP1*) gene, conferring higher susceptibility to mesothelioma, originated from studies of gene and environment interactions. Several pathways are relevant in mesothelioma, including NF2 and Hippo, receptor tyrosine kinases like EGFR and MET, intracellular kinases such as PI3K, ERK5, and others. However, HMGB1 and BAP1 represent the most frequent and key activators of oncogenic transformation and tumor progression in mesothelioma. Therefore, the pathways activated by these two proteins, both characterized by dual activity at nuclear and cytoplasmic levels, may offer the most promising perspectives for novel therapeutic approaches to antagonize a very aggressive and refractory cancer like mesothelioma.

Keywords Mesothelioma · BAP1 · HMGB1 · Chronic inflammation
Biomolecular pathways

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1 Introduction

Malignant mesothelioma is a rare but aggressive cancer, associated with exposure to asbestos or other carcinogenic mineral fibers, and characterized by poor prognosis and limited therapeutic response. The exposure of mesothelial cells of the pleural and peritoneal lining to asbestos fibers or to naturally occurring asbestos-like minerals, like erionite and others, leads to the development of chronic inflammation and production of mutagenic oxygen radicals. Exposed mesothelial cells, with the contribution of activated macrophages, survive and proliferate, accumulating genetic mutations, ultimately undergoing the process of malignant transformation [1, 2].

During a genomic analysis by high-density array comparative genomic hybridization (aCGH) focused on the 3p21 region, which is heavily rearranged in mesothelioma, we found the typical pattern of chromothripsis. This event occurs upon many chromosome rearrangements caused by a single catastrophic episode in different tumor cells, causing numerous gene alterations even after a few cell replications. Chromothripsis, which may lead to the dysregulation of proto-oncogenes or the loss of tumor suppressor functions, was subsequently postulated as a potential source of the expression of neoantigens that may increase immunogenicity of mesothelioma [2].

The different molecular pathways that may become activated during genome rearrangement and the acquisition of cell survival contribute to the susceptibility of mesothelial cells to environmental carcinogenic fibers and to drug resistance, a typical characteristic of mesothelioma cells [3]. We review here the molecular pathways and the role of gene and environment (GxE) interactions, which are relevant in the processes of onset and progression of mesothelioma, as well as the current perspectives for prophylactic and therapeutic approaches to mesothelioma.

2 HMGB1 and Asbestos Pathogenesis

The exposure of human mesothelial cells to asbestos or other carcinogenic asbestos-like mineral fibers causes their death that has been previously identified as apoptosis [4], until we clearly demonstrated that tumor necrosis factor-alpha (TNF-α) and NF-κB, two mediators of inflammation, were a key pathway of the cellular response to the insult provoked by the mineral fibers [5]. Afterward, we clarified that several fibers like crocidolite [6], erionite [7], and chrysotile [8] induce programmed necrosis in most exposed mesothelial cells. Programmed cell necrosis is a regulated process involving the passive release and the secretion of high mobility group box 1 (HMGB1), a damage-associated molecular protein (DAMP) that primes the formation of a pro-inflammatory microenvironment by binding mainly to the RAGE receptor of macrophages [6, 9]. In addition, HMGB1 cooperates with other pathways, such as those triggered by reactive oxygen species (ROS) also induced by fiber exposure, leading to the formation of the NLRP3 inflammasome that in turn

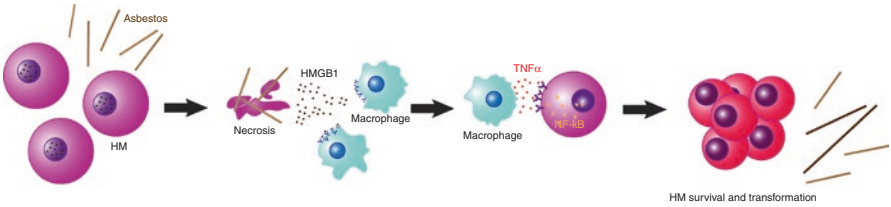

Fig. 4.1 Working hypothesis of asbestos carcinogenesis. Asbestos causes programmed necrosis in HM, leading to the release of HMGB1 into the extracellular space. HMGB1 triggers macrophages accumulation and the inflammatory response. Inflammatory factors, especially TNF-α, are secreted, bind to their receptors, and activate cell signaling pathways, like NF-κB, which increases the survival of asbestos-damaged HM. The continuous genetic damage, HM survival, and chronic inflammation, in the long run lead to the transformation of HM and the initiation of mesothelioma

provokes the secretion of several interleukins (IL-1β, IL-18, IL-1α), as well as of HMGB1 and TNF-α, establishing an autocrine chronic inflammation process. TNF-α binds to the receptors and activates the NF-kB pathway, which contributes to HM survival and malignant transformation (Fig. 4.1) [10]. The exposure to carcinogenic mineral fibers that are bio-persistent, like asbestos, erionite, and others, causes chronic inflammation in surviving mesothelial cells that further leads to transformation and mesothelioma development [1].

This oncogenic pathway can be hampered, preventing tumorigenesis or interfering with mesothelioma progression by different means: (1) antagonizing HMGB1 by using HMGB1 competitive inhibitors or monoclonal antibodies that significantly reduce the growth of mesothelioma xenografts [11]; (2) blocking the inflammatory process, as shown for aspirin that, by counteracting inflammation and HMGB1 activity, exerts antiproliferative activity in mesothelioma xenograft models [12]; and (3) interfering with HMGB1 signaling, as shown for ethyl pyruvate by inhibition of HMGB1 release and downregulation of the RAGE receptor with a significant reduction of mesothelioma aggressiveness [13].

3 Other Pathways Relevant to Mesothelioma Tumorigenesis

The neurofibromatosis type 2 (*NF2*) gene, when mutated, is responsible for inherited tumors of the nervous system. *NF2* is frequently mutated in mesothelioma and heterozygous deletion in mice results in enhanced tumor formation [14]. *NF2* encodes the Merlin protein, an initiator of the Hippo pathway, whose function is altered in approximately 40% of malignant mesotheliomas [15], leading to nuclear accumulation of other effectors of the Hippo pathway, the Yes-Associated Protein (YAP) and the WW Domain-Containing Transcription Regulator (WWTR1 or TAZ). The unbalanced localization of these proteins in the nucleus enhances the expression of multiple proto-oncogenes, which contribute to sustain survival of mesothelioma cancer cells [16]. The Hippo tumor suppressor pathway regulates

tissue growth, contact inhibition, stem cells, and tissue regeneration, offering a model for novel therapeutic strategies for mesothelioma.

Several cell signaling pathways involving tyrosine kinase receptors are frequently found activated in mesothelioma and even in mesothelial cells exposed to asbestos fibers. Following exposure of rat mesothelial cells to crocidolite asbestos fibers, epidermal growth factor receptor (EGFR) is found auto/trans-phosphorylated along with the activation of extracellular-regulated kinases 1 and 2 (Erk1/2). As a consequence, AP-1 is transcriptionally activated triggering signals related to tumor development and progression [17]. The hepatocyte growth factor (HGF) receptor, encoded by the *MET* gene, has been identified in activated (auto-phosphorylated) form in many thoracic tumors, including mesothelioma [18]. Moreover, overexpression and secretion of the MET ligand HGF has long been identified in mesothelioma cells [19]. The established autocrine circuit causes dysregulation of the signaling of one of the most powerful known oncogene, leading to uncontrolled proliferation, survival, migration, and invasiveness. The HGF/MET axis in mesothelioma involves the activation of phosphatidylinositol 3-kinase (PI3K), which in turn stimulates the mitogen extracellular signal-regulated kinase (ERK5)/fos-related antigen 1 (FRA-1), a pathway that enhances cell growth, motility, and invasiveness. Moreover, FRA-1 is able to positively regulate the expression of c-MET and of its co-receptor CD44 [20], further enhancing the oncogenic effect of the HGF/MET activation. Several elements of this complex pathway are targetable for therapeutic purposes. Small molecule inhibitors, like the MET/ALK kinase inhibitor crizotinib, the class I PI3K inhibitor BKM120, and the PI3R/mTOR dual inhibitor GDC-0980 were found effective, alone or in combination, in suppressing growth and migration of mesothelioma cells in tissue culture and in mouse models [21].

4 Gene and Environment Interaction and BAP1 in Mesothelioma

In the research field of carcinogens, the approach of combining genetics and environmental studies has become popular to study GxE interactions, and mesothelioma carcinogenesis represents a paradigmatic example [2].

The genetic susceptibility to mesothelioma has been first discovered in Cappadocia (Turkey) as dominantly transmitted in autosomal fashion in members of families environmentally exposed to erionite [22], leading to a real and devastating epidemic of mesothelioma [23]. Similar levels of exposure to erionite were found also in certain areas in the USA [7], highlighting the existence of a possible increased risk of similar epidemics in the US in the future [24]. The hypothesis that a gene and environment interaction could be the cause of mesothelioma development in some individuals was validated by the discovery of germline mutations in the *BAP1* gene, mapped at chromosome 3p21.3 and encoding the

BRCA1-associated Protein 1 (BAP1). Inherited mutations were identified in members of families where mesothelioma and uveal melanoma (UVM) were found with high incidence [25]. Afterward, additional different familial malignancies, such as clear cell renal carcinoma, cutaneous melanoma, basal cell carcinoma, meningioma, and cholangiocarcinoma were associated with germline mutated *BAP1*, postulating the existence of a BAP1 cancer syndrome [26]. The inheritance of the BAP1 cancer syndrome was confirmed by the finding of further families with members carrying *BAP1* germline mutations and predisposition to cancer development [27, 28] and of a large kindred dating back to the eighteenth Century with a high incidence of mesothelioma, UVM, and other cancers [29]. Patients with mesothelioma carrying germline *BAP1* mutations, compared to all sporadic mesotheliomas recorded in the United States Surveillance, Epidemiology, and End Results (SEER) database, resulted in a sevenfold improved survival [24]. Moreover, a later study conducted in selected patients with mesothelioma with a family history of *BAP1*-associated cancers and/or age under 50 years revealed that patients carrying pathogenic mutations of *BAP1* or of other cancer-associated genes were younger at diagnosis and had a significantly improved survival [30]. Further similar larger studies confirmed that mesothelioma is associated with germline mutations of *BAP1* or other genes with a frequency of approximately 10–12% and that prognosis and chemosensitivity is improved in these patients [31, 32].

BAP1 is particularly relevant in regulating GxE interactions also because it has different activities according to the intracellular localization. In the nucleus, BAP1 plays a key role in transcriptional regulation and DNA repair (reviewed in [33]). However, we discovered that BAP1 has a dual activity, both in the nucleus and in the cytoplasm. By studying primary fibroblasts derived from family members with heterozygous *BAP1* mutations, we demonstrated that reduced BAP1 levels destabilizes the type 3 inositol-1,4,5-trisphosphate receptor (IP3R3), with consequent lower mitochondria Ca^{2+} concentration, impaired apoptosis and cell death induced by environmental carcinogens including asbestos, and reduced sensitivity to pro-apoptotic drugs [34], which contributes to malignant transformation and tumor development. Moreover, primary fibroblasts from mutated *BAP1* display the "Warburg effect," with cellular metabolism unbalanced in favor of aerobic glycolysis, thus, a hallmark of cancer cells present in normal cells from *BAP1*-mutant carriers, helping to adjust to the metabolic stress typical of tumorigenesis [34] (Fig. 4.2).

Notably, it has been reported that loss of BAP1 function promotes the activity of enhancer of zeste homolog 2 (EZH2), a member of the polycomb repressive complex 2 (PRC2) that catalyzes histone H3 lysine 27 trimethylation (H3K27Me3) leading to the epigenetic silencing of different genes. Mesothelioma cells become dependent on EZH2 for maintaining the transformed status, making this molecule an attractive novel therapeutic target for all malignancies associated with *BAP1* mutations [35]. A phase II study of the EZH2 inhibitor tazemetostat (ClinicalTrials.gov identifier NCT02860286) yielded promising results with 51% of disease control rate after 12 weeks [36].

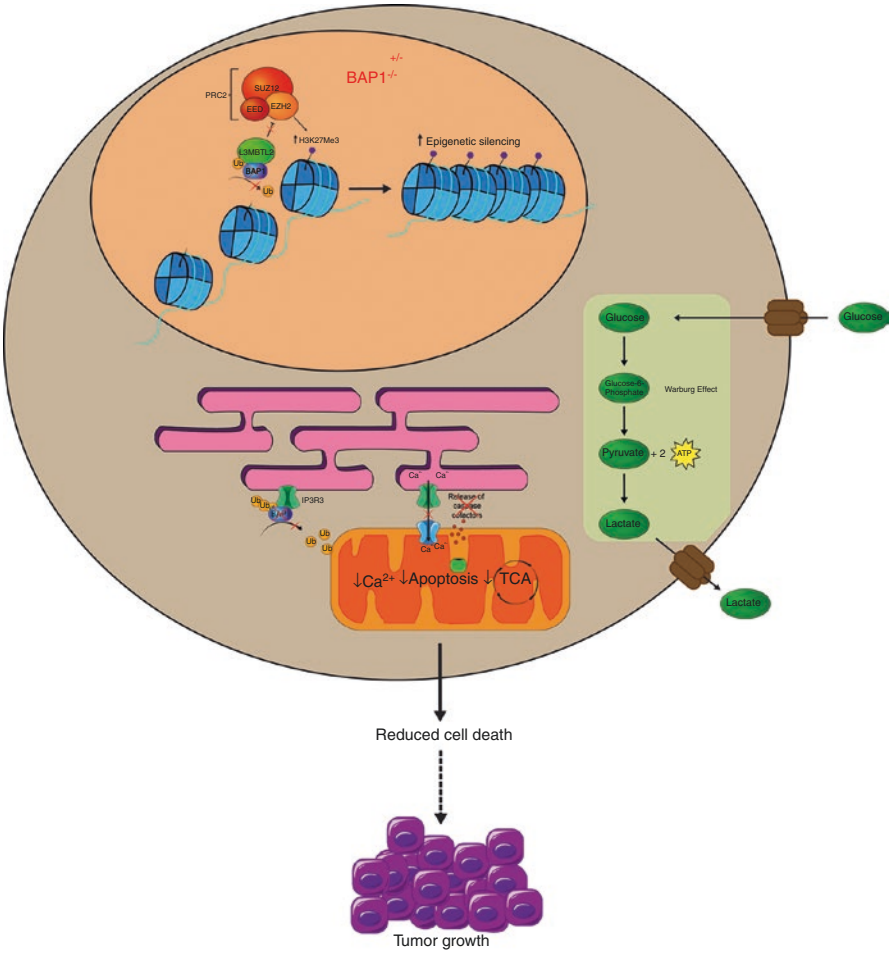

Fig. 4.2 Mechanisms of loss of BAP1 in causing cancer development. BAP1 has multiple functions and controls distinct cellular activities. In the nucleus, loss of BAP1 function promotes the activity of EZH2, a member of the PRC2 complex that catalyzes H3K27Me3, leading to the epigenetic silencing of different genes. In the cytoplasm, BAP1 deubiquitylates and thus stabilizes the IP3R3 receptor channel that regulates Ca^{2+} transfer from the ER to the cytoplasm. Loss of BAP1 causes reduced Ca^{2+} concentrations that impair mitochondrial respiration (TCA cycle), and the cells switch to aerobic glycolysis (Warburg effect) and release more lactate. Moreover, cells lacking BAP1 cannot release sufficient amounts of Ca^{2+} to start the apoptotic process. Thus, those cells carry DNA damage but have reduced cell death, and they keep dividing, which over time, may lead to malignant transformation and tumor development

Given the pleiotropic functions of BAP1 in mesothelioma tumorigenesis and that also somatic mutations of *BAP1* have been observed frequently (41–64%) in sporadic mesotheliomas [37–39], its related pathways are possible novel targets for therapeutic approaches.

5 Conclusions

As many other aggressive cancers, in mesothelioma many pathways are constitutively activated, as receptor and intracellular kinases (EGFR, MET, PI3K, etc.), developmental/morphogenic pathways (NF2, Hippo), and others. However, the pathways elicited by HMGB1 and BAP1 are of noteworthy relevance.

HMGB1 and inflammatory factors are the key triggers of carcinogenesis by mineral fibers, by inducing biomolecular pathways leading to chronic inflammation and cell survival. HMGB1 also contributes to mesothelioma progression and maintenance of the malignant condition. Therefore, targeting HMGB1 pathways, directly by using antagonist agents or indirectly interfering with the inflammatory process, may offer novel and effective strategies for mesothelioma prevention or therapy.

The identification of *BAP1* as a predisposition gene for the development of familial mesothelioma and other cancers is the product of combined genetics and environmental studies to elucidate gene and environment interactions. Mesothelioma is a heavily mutated cancer, albeit a relatively low number of genes are affected. *BAP1* is the most frequently mutated gene in mesothelioma, both as germline and somatic alterations. BAP1 has a pleiotropic effect, activating several different pathways, both at nuclear and cytoplasmic levels, making it an attractive therapeutic target.

Notably, HMGB1 and BAP1 share the property of having dual activities, according to the cell compartment where their activity is displayed (namely nucleus versus cytoplasm), proposing the suggestive hypothesis that a possible direct interaction between the two proteins may be involved in at least some, and possibly yet undiscovered dysregulated function in mesothelioma cells.

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Part III
Screening and Early Detection

Chapter 5
Biomarkers for Mesothelioma Screening: How Can We Identify Subjects Developing Mesothelioma in Asbestos-Exposed High-Risk Group?

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Keywords Hereditary cancer · Environmental cancer · Asbestos · Mesothelioma Cancer philosophy clinic

1 Introduction

Alfred G Knudson (1922–2016) was a man of great insight into the cancer genetics and a personal mentor of the author. Knudson developed the two-hit hypothesis [1, 2]. From this hypothesis, the concept of tumor suppressor gene was led. In 1954, a Norwegian pathologist, R. Eker found an animal model of inherited tumor [3]. The Eker rat developed bilateral, multiple, and dominantly inheritable renal tumors [4]. Utilizing syntenic homology between human and rat, a germline insertion was identified in the homologue of tuberous sclerosis complex (TSC) 2 gene (Tsc2) as the tumor predisposing mutation of the Eker rat. We, in researching hereditary kidney cancer in Eker rats, found that the product of the gene Erc, which appears with high frequency in simultaneous progressive process cancer, is secreted in the bloodstream, thereby providing a possible method for blood diagnosis. We reported that N-ERC/mesothelin could be a useful serum tumor marker for mesothelioma and

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have developed an ELISA kit (IBL. Co., Ltd. Gunma, Japan). “Environmental carcinogens” came to light in 1775 when the British surgeon Percival Pott reported scrotal cancer associated with chimney sweeps in “cancer stimulated by chimney soot” [5].

2 Mesothelioma as an Environmental Cancer

Mesothelioma is a highly aggressive tumor. It is estimated that as many as 43,000 people around the world die annually from this disease [6]. The suggestion that mesothelioma resulted from occupational exposure to asbestos was first made by Gloyne in Britain in 1935 [7]. Since that time, research on mesothelioma and its causal agents has progressed. IARC (International Agency for Research on Cancer) evaluated asbestos and pointed out its carcinogenic risk to humans in 1977. Subsequently, Hodgson and Darnton quantitatively presented the risks of mesothelioma (and lung cancer) in relation to asbestos exposure in 2000 [8]. While incidence has primarily been reported from developed countries, these reports are expected to increase significantly in developing countries where asbestos, the major causal agent of mesothelioma, is still broadly produced and used. In Japan alone, more than 10,900 people exposed to asbestos who developed mesothelioma, lung cancer, asbestosis, or diffuse pleural thickening (DPT), had been recognized and compensated through 2015 (Ministry of the Environment) [9]. Among these patients, the majority had experience working in factories producing asbestos-related goods. Because the latency period of mesothelioma is as long as 20–40 years after initial exposure to asbestos, and the cancer initially progresses mainly along the surfaces of pleura or peritoneum without forming masses, it has been challenging to diagnose this disease in its early stages and to perform complete surgical removal. The median survival time after diagnosis is 12 months [10].

3 Progress to Establish Early Diagnostic Systems of Mesothelioma

At present, most of the mesothelioma patients are diagnosed in advanced stages, although many efforts have been made to develop early diagnostic tools for mesothelioma. In this chapter, we reviewed the recent progress in diagnostic procedures that could lead to the early detection of mesothelioma in near future. We focused on the advances in (A) analysis of exhaled breath, (B) cytological studies in the pleural fluid, and (C) exploration of serum biomarker, of mesothelioma patients.

3.1 Analysis of Exhaled Breath of Mesothelioma Patients

Exhaled breath consists of liquid (water vapor) and gaseous fractions [11]. The latter includes volatile organic compounds (VOCs), part of which arise from endogenous sources. Endogenous VOCs are formed through biological reactions in tissues, and they are transported to the lung and released into breath. A single breath sample contains around 200 different VOCs [12]. Integrated patterns of multiple VOCs are informative as biomarker panels, and are reported to be associated with pathological conditions such as infectious diseases and cancer [13, 14]. Mesothelioma arises from the asbestos-related inflammatory changes that are characterized by reactive oxygen species (ROS) generated in "frustrated macrophages" or in iron-related chemical reactions. Their unique mechanisms of inflammation are likely to produce VOCs patterns unique to mesothelioma.

VOCs in exhaled breath have been analyzed by several techniques. Among them, gas chromatography-mass spectrometry (GC-MS) is considered to be the gold standard because it allows identification and quantification of individual compounds with high sensitivity [11]. VOCs in breath of asymptomatic asbestos-exposed (AEx) subjects, patients of benign asbestos-related disease (ARD), and those of mesothelioma were analyzed. As for discrimination between mesothelioma vs AEx, vs ARD, or vs (AEx + ARD), GC-MS showed the accuracy of 97%, 79%, or 94%, respectively [15]. These promising results will facilitate its practical use in early diagnosis of mesothelioma in near future, with advantages that sampling is noninvasive and possible from all breathing patients. Drawback of GC-MS is that this method is time consuming and requires expensive apparatus and well-trained technicians. Other than GC-MS, several techniques that can be performed in faster and easier ways are available to analyze breath VOCs, but sensitivities of those methods are not so high as that of GC-MS [11].

3.2 Cytological Studies in the Pleural Fluid of Mesothelioma Patients

In the guidelines for pathologic diagnosis of mesothelioma issued in 2009, cytological study was not considered to be reliable as histological ones [16]. However, based on many evidences, it has evolved into more refined one. In the guidelines in 2015, the cytological diagnosis of mesothelioma with ancillary techniques is mentioned to be as reliable as that based on histological study, although the sensitivity with cytology may be somewhat lower [17]. [The ancillary techniques include immunocytochemistry (ICC), immunohistochemistry (IHC), and fluorescent in situ hybridization (FISH).] Cytological study is much less invasive to the patients than histological study, and is recommended to be included as an accepted method for the diagnosis.

Cytological criteria of mesothelioma are described in literature [17, 18]. Briefly, the characteristic findings of mesothelioma cells in effusion include significantly larger mesothelial cells, spheres with a smooth surface or berry-like tissue fragments, extracellular matrix cores known as collagenous stroma, protrusions from cell membrane or blebbing, and cell-within-cell arrangements. Their representative figures are shown in Refs. [17, 18], and others.

As ancillary techniques, ICC on cytospins or IHC on cell blocks is required. At least two markers should be used to confirm the character of cells, because none of the single marker is specific enough. For the differential diagnosis between reactive mesothelial cells and mesothelioma, "desmin + calretinin" (positive in reactive cells) and "EMA + calretinin" (positive in mesothelioma) are recommended. For differential between adenocarcinoma and mesothelioma, "EMA + calretinin" (positive in mesothelioma and negative in adenocarcinoma) and "CEA + BerEp4" (negative in mesothelioma and positive in adenocarcinoma) or more markers are used [17]. By FISH, the homozygous deletion of p16INK is detected in 60–80% of epithelioid and 100% of sarcomatoid mesothelioma [19]. If p16INK is homozygously deleted, we can deny the possibility of benign reactive cells but we cannot exclude that of adenocarcinoma.

3.3 Exploration of Serum Biomarkers of Mesothelioma

At present, serum biomarkers are not clinically used for the early diagnosis of mesothelioma. Although the initial data of each marker showed promising results, none of them is rigorously validated in large-scale studies. Currently, many efforts are being made to explore new markers or new combination of already known markers.

3.3.1 High Mobility Group Box (HMGB) 1 Protein

HMGB1 is a 30-kDa transcription factor, and released from macrophages and monocytes by inflammatory stimulus. Activated macrophages/monocytes acetylates the HMGB1. Pathogenesis of mesothelioma is associated with inflammation caused by "frustrated macrophages," and therefore it is very likely that acetylated HMGB1 is increased in mesothelioma tissue. Actually, HMGB1 is extensively acetylated in serum of mesothelioma patients [20]. At the cutoff value of 2.00 ng/mL, the sensitivity and specificity of serum acetylated HMGB1 in differentiating mesothelioma patients from AEx and HC subjects was 100%, outperforming other previously proposed biomarkers [20]. More extensive studies will be required before it is used for clinical diagnosis.

3.3.2 Osteopontin and Fibulin-3

Osteopontin is a 32-kDa glycoprotein that binds to integrin. It mediates cell–cell interactions and is overexpressed in many tumors. A meta-analysis with 9 studies of serum and plasma osteopontin found a pooled sensitivity 0.57 and specificity 0.81 [21].

Fibulin-3 is a 57-kDa extracellular protein expressed on the basement membrane of blood vessels. A meta-analysis with 8 studies revealed pooled estimates of sensitivity 0.87 and specificity 0.89 [22].

3.3.3 Soluble Mesothelin-Related Protein

Soluble Mesothelin-Related Protein (SMRP) is almost identical to C-ERC (see below) shed into serum. The measurement of SMRP by MESOMARK assay kit is approved by the US Food and Drug Administration for the monitoring of patients with epithelioid and biphasic mesothelioma. A meta-analysis of 30 studies revealed a mean sensitivity 0.66 and specificity 0.97 for SMRP as a serum biomarker of mesothelioma when compared with HC subjects [23].

3.3.4 ERC/Mesothelin

A Brief History of Research on the ERC/MSLN Gene and Its Products

The ERC gene, originally discovered as Erc (expressed in renal carcinoma) gene in the study of the Eker (Tsc2 mutant) rat model [24], is the name given to its human homolog gene, which later was identified as the same as the MSLN gene [25]. (The Eker rat is a rat model that is predisposed to develop hereditary renal carcinomas due to two hit mutations of the tumor suppressor gene, Tsc2 [26]. The Eker rat strain was originally developed by R. Eker, a Norwegian pathologist. Dr. Knudson later introduced the Eker rat to the United States for hereditary cancer studies and maintained the mutation by breeding the rats on a normal Long-Evans strain background [27].

In the study of Eker rat renal carcinogenesis, Hino et al. found the following 4 genes were highly involved in renal carcinogenesis: the third component of complement (C3), the fos-related antigen 1 (fra-1) gene, the calpactine I heavy-chain (annexin II) gene, and an unknown gene, which was later named the Erc gene [26].

In 2000, Yamashita et al. determined the full sequence of the Erc gene cDNA and its exon–intron structure; also, the Erc locus (10q12-q21) and the locus of the putative human homologue (16p13.3) were mapped in the respective chromosomes by fluorescence in situ hybridization (FISH) [28]. At the nucleotide sequence level, the

rat Erc gene showed 67.6% identity with human ERC cDNA [28]. Discovered through mesothelin (MSLN) protein research, the MSLN gene was found to be located in the same region [25]. The ERC/MSLN gene encodes several proteins and its primary product is a full-length 71-kDa precursor protein, which is cleaved physiologically by a furin-like protease into a 31-kDa N-terminal fragment (N-ERC) that is secreted into the blood and a 40-kDa C-terminal fragment (C-ERC) that remains membrane-bound. N-ERC, also known as megakaryocyte potentiating factor (MPF), is a soluble protein released into the extracellular space [29]. C-ERC, also known as mesothelin—first recognized by the monoclonal antibody K1 in human mesotheliomas and ovarian cancers [30]—is a glycoprotein tethered to the cell surface by a glycosyl phosphatidyl inositol anchor.

4 Conclusion: Development of a Series of N-ERC ELISA Systems as Diagnostic Biomarkers of Mesothelioma

ERC-based ELISA development targeted the 31-kDa N-terminal (N-ERC). Shiomi et al. used a mouse monoclonal anti-ERC antibody MoAb 7E7 and a rabbit anti-ERC antibody PoAb-282 to develop an ELISA system for detecting N-ERC in sera of mesothelioma patients [31]. Shiomi et al. continued searching for other antibody clones to improve ERC-based ELISA sensitivity and established a novel sandwich ELISA system by using MoAb 7E7 (previously reported) and MoAb 16 K16 (7–16 ELISA) in 2008 [32]. In 2014, Sato et al. further established a new ELISA system by using MoAb 7E7 and a novel MoAb 20A2 (7–20 ELISA) to improve the reproducibility of the previous 7–16 ELISA system. In a study of 53 referred patients to Juntendo University Hospital from June 2005 to March 2013, the 7–20 ELISA system displayed improved sensitivity and specificity compared with the previous 7–16 ELISA system. Regarding the epithelioid type, in particular, the AUC (area under the curve) was 0.91, the sensitivity was 0.95, and the specificity was 0.76 in plasma [33]. Although the number of patients enrolled was small, the 7–20 ELISA system was clinically proven useful for precise diagnosis of the epithelioid type of pleural mesothelioma. In addition, “Human N-ERC/Mesothelin Assay Kit—IBL” was commercialized by Immuno-Biological Laboratories Co. Ltd. (IBL) in 2013. The Assay Kit has been used as a tool, combined with PET/CT scans and biopsy, to diagnose mesothelioma at clinical practices in Japan. N-ERC as diagnostic marker—a large-scale screening of construction workers for early diagnosis of asbestos-related mesothelioma by N-ERC ELISA in Japan.

A 5-year large-scale screening of Japanese construction workers who were or had been at risk of asbestos exposure was initiated in Feb. 2007. As of Mar. 2012,

approximately 40,000 participants were enrolled in this research study and a total of 179,201 blood samples from 85 research sites across Japan were collected and analyzed for N-ERC levels by 7–16 ELISA. Samples with N-ERC levels above 8 ng/ml were sent to Juntendo Medical School for a second 7–16 ELISA test, along with the HAMA (human anti-mouse immunoglobulin antibody) test. Approximately 900 subjects (~2000 blood samples) were recommended for examinations, including CT scans, at hospitals. One-hundred ninety subjects did follow the advice and had further examinations for diagnosis of mesothelioma and other asbestos-related diseases. Hirohashi et al. reported that overall, 62 participants were ultimately identified as the "high-risk" population and referred to have further assessment [34]. "High-Risk" was defined as the following: (1) human anti-mouse antibody (HAMA) not detected, (2) absence of any evidence of renal dysfunction based on medical history and a laboratory test, (3) age ≥ 35 years, and (4) detection of abnormal values (> 8.0 ng/ml) of N-ERC on more than two occasions during the annual assessments.

Serum N-ERC can also be used as the sensitive monitoring marker of mesothelioma. Figure 5.1 shows the clinical course of a mesothelioma patient, followed by the serum N-ERC value. After extrapleural pneumonectomy (EPP) and additional radiation therapy, the N-ERC level decreased to almost normal ranges. By recurrence, it increased but CDDP + PEM treatment returned it to normal range again. However, during withdrawal period, it increased again and this increase was not suppressed by GEM+VNR therapy.

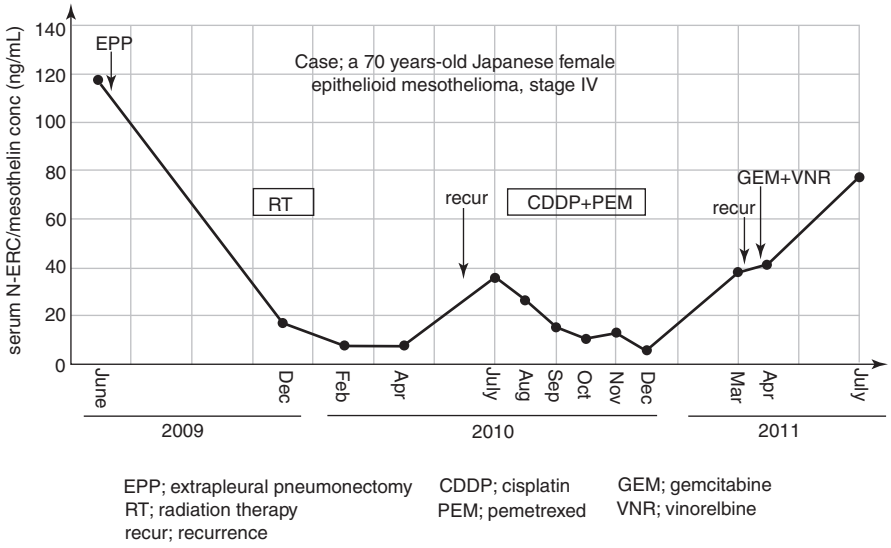

Fig. 5.1 Clinical course of a mesothelioma patient followed by serum N-ERC/mesothelin

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Chapter 6
Pleural Plaques as a Predictive Imaging Marker for Cancer Screening in Asbestos-Exposed Subjects: Can Pleural Plaques Be a Tool beyond Estimating Past Asbestos Inhalation?

Yasuo Morimoto, Chinatsu Nishida, Taisuke Tomonaga, and Hiroto Izumi

Abstract We reviewed relationship between the radiographic features of the pleural plaque and asbestos lung cancer. Because there is not a consistent opinion about pleural plaques and lung cancer considering some cohort and case-control studies, there is still controversy about whether or not pleural plaques in chest X-rays are a predictor of asbestos lung cancer. Although the usefulness of pleural plaques for screening of lung cancer is controversial, there are many reports that chest computed tomography (CT) imaging is more useful than X-rays in detecting pleural plaques. The presence of pleural plaques in chest CT images does have a tendency to be able to predict asbestos lung cancer.

As other than pleural plaques in chest CT images, some inflammatory and fibrotic abnormalities such as most fibrosis signs (subpleural nodules, septal lines, parenchymal bands, and honeycombing), ground-glass opacities, thickened bronchial walls, pleural plaque extent, and adherences may be able to be predictors of asbestos-related lung cancer.

Keywords Asbestos · Pleural plaque · Lung cancer · Screening

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1 Introduction

It has been reported that asbestos-related lung cancer is induced by high doses of cumulative asbestos. According to the Helsinki Criteria for occupational diseases associated with asbestos exposure, a cumulative exposure of more than 25 fiber-years is associated with lung cancer, and corresponds to a twofold risk of lung cancer [1, 2]. A twofold risk of lung cancer is related to retained fiber levels of two million amphibole fibers (> 5 μm) per gram of dry lung tissue or five million amphibole fibers (> 1 μm) per gram of dry lung. This lung fiber concentration is approximately equal to 5000 to 15,000 asbestos bodies per gram of dry tissue, or 5 to 15 asbestos bodies per milliliter of bronchoalveolar lavage fluid. Electron microscopic fiber analyses are recommended when the asbestos body concentration is less than 10,000 asbestos bodies per gram of dry tissue. Pleural plaques in the absence of asbestosis have 1.4 times the risk of lung cancer, not 2 times [3]. Although pleural plaques are important evidence of asbestos exposure, there is not enough evidence of the relationship between pleural plaques and lung cancer. That relationship is reviewed here.

2 Pleural Plaque

Pleural plaques are reported to be the most common manifestations of asbestos exposure as an incidental finding in radiological reports. They are localized, board-shaped pleural thickening, and most occur in the parietal pleura, rarely occurring in the visceral pleura, including the interlobular pleura. Macroscopically, pleural plaques appear as glossy, gray-white regions of pleural thickening. Pathologically, they are hyaline pleural fibrosis covered by normal mesothelial cells, with asbestos bodies or fibers found within them occasionally. They frequently undergo dystrophic calcification over time, which makes them much more readily visible radiographically. Pleural plaques occur more commonly in subjects with the increase in time since first exposure to asbestos and with greater cumulative exposure, especially with exposure to amphibole varieties of asbestos [4]. The frequency of the calcification is considered to be around 10–15% and appears from a lag time of >20 years since first exposure to asbestos, and the frequency with the calcification increases with the progress of time.

Most incidental findings of pleural plaques in chest x-rays (Fig. 6.1) are easily identified on plain films by their sharp, often foliate borders and by a raised straight surface with clear, cut off edges when seen face on and with irregular margins when seen in profile on the chest wall or diaphragm [5]. They appear in a variety of shapes, such as nodular, linear, reticular, rhombic, and map-like shapes. Pleural plaques are asymmetric, and calcified pleural plaques are often observed. The range of thickening in pleural plaques is between 1 and 10 mm, with the range between 1 and 5 mm, especially being often observed [6]. The dorsal lateral area of the chest wall 7–10

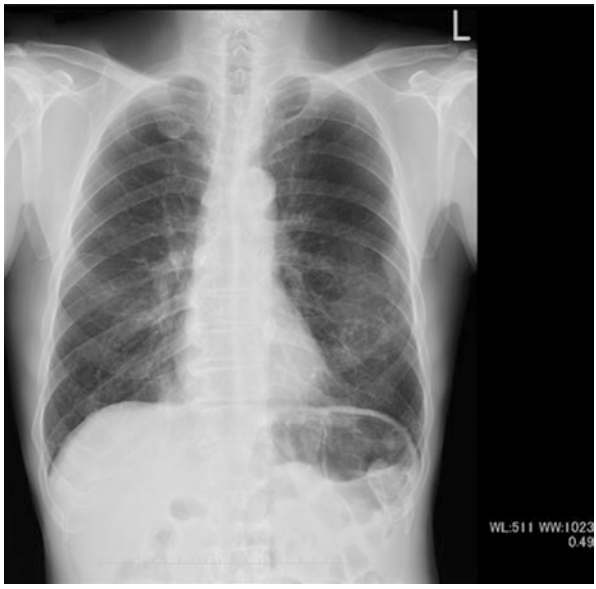

Fig. 6.1 Findings of pleural plaques in chest X-rays

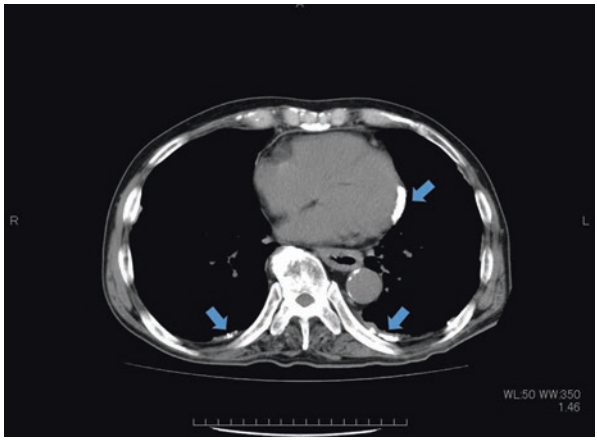

Fig. 6.2 Findings of pleural plaques in chest CT images. Plural pleural plaques were observed in bilateral pleural and pericardial pleura

rib level, the lateral area 6–9 rib level, the area of the dome of the diaphragm, and the paravertebral area are known as a common site of pleura plaques, and they are usually not seen in the apices of the lung or in the costophrenic angles.

In CT scans, pleural plaques appear as circumscribed quadrangular pleural elevations with sharp borders and tissue density, sometimes calcified, with usual topography in at least some of the images (Fig. 6.2), and are commonly located in the posterolateral and para-spiral regions of the thorax [6]. Lesions with a thickness of around 2–3 mm can be detected clearly.

There is a greater rate of detection of pleural plaques in chest CT images than in chest X-rays [7]. Although chest X rays of pleural plaques are reported to be effective for screening, with a sensitivity of 0.94 and a specificity 0.73, the rate of detection is reported to be between 8 and 40%. The rate of detection by CT is considered to be more than 95%, and pleural plaques can be detected clearly.

3 Relationship between Pleural Plaque and Lung Cancer in Chest Radiographs

There are many reported references to the relationship between the radiographic features of pleural plaques and lung cancer (Fig. 6.3), and in chest radiographs, there are both positive and negative findings that pleural plaques are useful for screening of asbestos-exposed lung cancer.

A review of the literature by Weiss [8] included six cohort studies, four lung cancer case-control studies, and three autopsies studies. Of the 13 investigations, only 3 supported the hypothesis that there is a higher risk of lung cancer in subjects with pleural plaques: 2 cohort studies from the same city in England with much the same data, and 1 case-control study. The authors concluded that pleural plaques were not associated with an increased risk of lung cancer in the absence of asbestosis. Some later reports showed that pleural plaques indicated an increased risk of asbestos-related lung cancer, while other reports showed that they did not. Ameille et al. [9] reviewed asbestos-related cancer risk with pleural plaques, and showed cohort studies of lung cancer associated with pleural plaques in the absence of asbestosis. Cullen et al. [10] conducted a subgroup analysis of 7965 male heavy smokers and 4060 asbestos-exposed men in the USA, and, after adjusting for age, smoking, history, duration of asbestos exposure, found radiographic evidence that

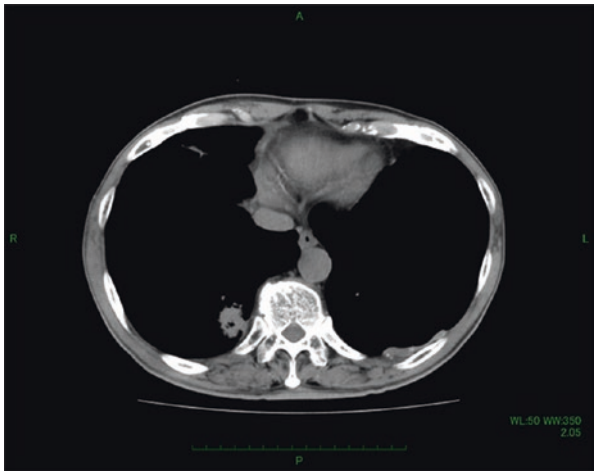

Fig. 6.3 Findings of lung tumors and pleural plaques in chest CT images. Lung tumor in right lower lobes and typical pleural plaques in left side were observed

pleural abnormalities (bilateral thickening or plaques) indicated an increased risk of lung cancer (RR, 1.91; 95% CI, 1.12–3.27). Karijalinen et al. [11] used the Finnish Cancer Registry from 1967–1995 to follow up Finish patients with asbestos-related benign pleural disease (n = 4887), and found that men with benign pleural plaques had a slightly raised risk of lung cancer (SIR = 1.3, CI = 1.0–1.8).

Brims et al. [4] used plain chest radiography to conduct two cohort studies over more than 25 years of crocidolite mine and mill workers and predominantly construction and manufacturing industry workers with mixed asbestos fiber exposure. Among the 3486 male subjects, the prevalence of pleural plaques in plain chest radiographs was 16.3% and 40% in Cohort 1 and 2, respectively. The hazard ratio for the presence of pleural plaques and lung cancer was 0.85 (0.49–1.48) and 0.85 (0.47–1.54), respectively. Taken together, the authors concluded that pleural plaques did not present a lung cancer risk in either cohort. The usefulness of pleural plaques in assessing the risk of lung cancer is controversial under present conditions.

One reason why there is not a consistent opinion about pleural plaques and lung cancer is that there are so many abnormalities in chest X-rays that it is difficult to clearly identify pleural plaques. Posteroanterior projection cannot readily reveal noncalcified plaques located anteriorly or posteriorly [9]. It is also difficult to distinguish between pleural plaques and subpleural fat in opacities on the lateral chest wall that are seen tangentially. We consider that the conflicting results of the studies referred to above reflect the difficulty in identifying pleural plaques.

4 Relationship between Pleural Plaque and Lung Cancer in Chest Computed Tomography

Although the usefulness of pleural plaques for screening of lung cancer is controversial, there are many reports that chest computed tomography (CT) imaging is more useful than X-rays in detecting pleural plaques. Pairon et al. [12] conducted a study with a 6-year follow-up of lung cancer mortality in 5402 male asbestos-related workers in four regions of France, and analyzed the relationship between benign asbestos-related abnormalities in chest CT scans and lung cancer mortality. The Cox proportional hazard model demonstrated a statistically significant association between pleural plaques and lung cancer mortality (HR 2.41 (1.21–4.85)) after adjusting for smoking and asbestos cumulative exposure index. Pleural plaques were an independent risk factor for lung cancer death in asbestos-exposed workers, and the researchers suspected that pleural plaques could be used as an additional criterion in the definition of high-risk populations eligible for CT screening.

In a study in Italy using low-dose computed tomography (LDCT), Silva et al. [13] reported a relationship between the prevalence of pleural plaques and the incidence of lung cancer and mortality in 2303 participants (1570 men, 733 women) aged 50–75 years. Among the men, asbestos exposure was consistently self-reported

by 128/1570 (8.2%), and 1374/1570 (87.5%) consistently denied asbestos exposure. The number of men with pleural plaques was 31, and the prevalence of pleural plaques in the asbestos-exposed and unexposed men was 6.3% (8/128) and 1.7% (23/1374), respectively. There was a trend of higher frequency of lung cancer among the male subjects with pleural plaques (9.7% with vs. 4.2% without pleural plaques). The mortality of lung cancer for all men with pleural plaques was HR 5.48 (95% CI 1.61–18.70). The authors showed through screening by LDCT that pleural plaques could be a risk factor of lung cancer mortality for all their subjects, including those who were not aware of occupational exposure.

Other research has found negative findings about the relationship between pleural plaques and lung cancer. Brims et al. [4] conducted two cohort studies for more than 25 years in crocidolite mine and mill workers and predominantly construction and manufacturing industry workers with mixed asbestos fiber exposure, using LDCT. Among 3486 male subjects, the prevalence of pleural plaques in chest CT images was 48.8% and 72.5% in Cohorts 1 and 2, respectively. However, the hazard ratio for the presence of pleural plaques and lung cancer by LDCT (1.54 (0.41–5.87) in Cohort 1 and 0.85 (0.21–2.48) in Cohort 2) was not significant. Taken together, the authors concluded that pleural plaques did not confer a risk of lung cancer in either cohort.

There are some reports on a relationship between the characteristics of pleural plaques and lung cancer. Yusa et al. [14] reported a relationship between the extent of pleural plaques and the body concentration of pulmonary asbestos in lung tissue in 207 lung cancer patients with occupational asbestos exposure. Seventy-five percent (51/70) of the patients were determined by chest CT images to have extensive plaques, with one-fourth or more of the inner chest wall having a pulmonary asbestos body concentration of more than 5000 asbestos bodies per gram of dry lung tissue. Chest X-ray showed that forty-four percent (27/61) of the patients had plaques in less than one-fourth of the inner chest wall, with a pulmonary asbestos body concentration of more than 5000 asbestos bodies per gram of dry lung tissue. Therefore, it is thought that extensive plaques could be a predictor in screening of asbestos-related lung cancer because the widespread pleural plaques corresponded to a cumulative exposure of 25 fiber-years, which can lead to asbestos-related lung cancer.

Vehmas et al. [15] followed up 584 construction workers (574 males and 10 females), and used HRCT to analyze the relationship between pleural plaques and death from lung cancer. Pleural plaque calcification and maximal thickness were significant predictors of respiratory cancer deaths in a backward regression model. This characteristic of pleural plaques is expected to be useful in the future for determining the risk factor in asbestos-related lung cancer.

Although a relationship between the presence of pleural plaques and mesothelioma has not been proven, one report showed a positive relationship between them. Pairon et al. [16] studied 5287 retired or unemployed workers who had previously been occupationally exposed to asbestos for 7 years, and examined the relationship between the incidence of mesothelioma and the presence of pleural plaques in chest CT images. When typical parietal or diaphragmatic pleural plaques were seen in CT

scans, a statistically significant hazard ratio (6.8, 95%CI 2.2–21.4) was observed after adjustment for time since first exposure to asbestos and cumulative exposure index.

5 Relationship between Pleural Plaque and Pulmonary Function

Although it has been said that pleural plaques do not reflect pulmonary function, a recent study by Kopylev et al. [17] showed contrary findings. In a meta-analyses of 20 studies that reviewed the relationship between the presence of pleural plaques and pulmonary function in asbestos-exposed populations, the presence of pleural plaques was associated with statistically significant decreases in FVC (4.09%pred, 95%CI 2.31–5.86) and FEV1 (1.99%pred, 95% CI 0.22 to 3.77) in asbestos-exposed workers. The presence of pleural plaques was associated with a small but statistically significant mean difference in FVC and FEV1 in comparison to asbestos-exposed individuals without plaques or other abnormalities.

6 Relationship between Other Factors and Lung Cancer

It has been reported that, in radiographic features other than pleural plaques in chest CT images, some inflammatory and fibrotic abnormalities can be predictors of asbestos-related lung cancer. It is known that asbestosis (Fig. 6.4) is a precursor of

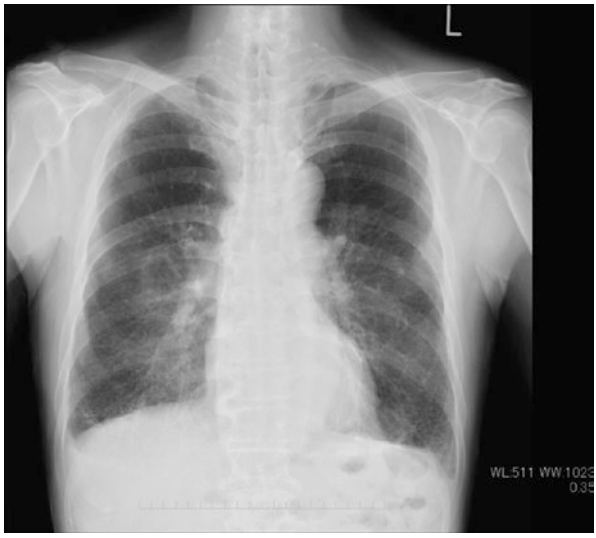

Fig. 6.4 Appearance of asbestosis in chest X-ray

asbestos-related lung cancer [18], and it is anticipated that future studies will examine the relationship between components of radiographic features of asbestosis and asbestos lung cancer.

In the above-mentioned study of 584 construction workers by Vehmas et al. [15], they analyzed not only pleural plaques but also inflammatory and fibrotic findings related to asbestosis in high-resolution computed tomography (HRCT) and their relationship to death from lung cancer. All of the emphysema signs that they studied were significant predictors of all-cause deaths, as were most fibrosis signs (subpleural nodules, septal lines, parenchymal bands, and honeycombing), ground-glass opacities, thickened bronchial walls, pleural plaque extent, and adherences (Fig. 6.5). Significant predictors of respiratory cancer death were subpleural septal lines, parenchymal bands, subpleural nodules, honeycombing, centrilobular emphysema, ground-glass opacities, thickened bronchial walls, and bronchiectasis, based on a Cox regression model adjusted for sex, age, body mass index, pack-years of smoking, and years of asbestos exposure. Other variables significant in a backward regression model were subpleural curvilinear opacities. Significant predictors of non-malignancy respiratory deaths were subpleural septal lines, parenchymal bands, subpleural nodules, honeycombing, centrilobular emphysema, paraceptal emphysema, panlobular emphysema, ground-glass opacities, thickened bronchial walls, and extent of pleural plaques.

Pulmonary function, as a factor other than radiographic features, might be a predictor of asbestos lung cancer. Swiatkowska et al. [19] examined 6882 subjects registered in a health surveillance program of asbestos-related diseases, and analyzed lung function and the incidence of lung cancer in asbestos-exposed workers. Cox's proportional hazards model, after adjustment for age, gender, number of cigarettes, duration of smoking, and cumulative asbestos exposure, revealed that the hazard ratio of lung cancer was 1.4 (95% CI: 0.94–2.08) for the subjects with FEV1 less than 90%, and 1.95 (HR = 1.86; 95%CI: 1.12–3.08) for those with FEV1 less

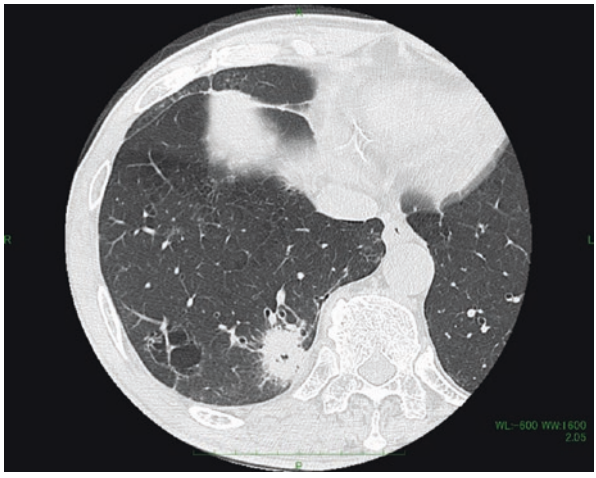

Fig. 6.5 Emphysema sign in cheat HRCT image. Signs of both emphysema and lung tumor were observed in right lung

than 70%, compared with the subjects with FEV1 greater than or equal to 90%. As for late stages of lung cancer, such as stages III and IV, the HR of lung cancer was 2.54 (95% CI: 1.32–4.08), even for the subjects with FEV1 less than 90%.

Swiatkowska et al. [20] also performed case-control studies within a cohort that included 7374 former workers of asbestos processing plants over the years 2000–2013, in which they analyzed the relationship between lung cancer incidence and its risk factors. The relative risk of lung cancer was twice as high in the subjects who smoked more than 20 pack-years (OR = 2.23, 95% CI 1.45–3.46) than it was in the case of the nonsmokers. The risk of lung cancer continued for 30 years after cessation of asbestos exposure.

Although it is unknown whether or not fine crackles detected in auscultation can predict asbestos lung cancer, it is a relatively easy screening method for asbestos-related diseases. In a review by Piirila [21], the presence of fine crackles in asbestos workers correlated with roentgenological and histological honeycombing, and also correlated with the duration of exposure to dust containing asbestos. Jarad [22] reported that repetitive mid or late inspiratory crackles were detected by auscultation in seven of 32 subjects (22%) with an ILO score of less than or equal to 1/0 among all subjects (32) with an ILO score of 0/0, 0/1, and 1/0. The frequency of detecting crackles by auscultation was 50% and 45% in subjects with an ILO score of 0/1 and 1/0, respectively. Considering that asbestosis is a precursor of asbestos lung cancer, we recommend the use of auscultation in medical examinations for asbestos workers, especially in high-risk groups such as smokers.

7 Conclusion

In summary, although there is still controversy about whether or not pleural plaques in chest X-rays are a predictor of asbestos lung cancer, the presence of pleural plaques in chest CT images does have a tendency to be able to predict asbestos lung cancer. There is also a possibility that a decrease in pulmonary function is related to asbestos lung cancer.

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Part IV
Pathology and Diagnosis

Chapter 7
Anatomical Structure of the Pleura and Mesothelial Cells: What Are the Characteristic Features?

Kenzo Hiroshima

Abstract It is important to understand the anatomy of the visceral and parietal pleura for an accurate pathological diagnosis of malignant mesothelioma. Elastic stains are helpful in understanding the microscopic anatomy of the pleura. Although there is controversy regarding the anatomy of the visceral pleura, it is believed to comprise five layers: mesothelial, submesothelial, external elastic, interstitial, and internal elastic. The submesothelial layer contains capillaries and lymphatic vessels in disease conditions, and the mesothelioma cells proliferate in this layer at an early stage. The interstitial layer is rich in capillaries and lymph vessels and is the plane of cleavage for pleurectomy/decortication. Some of the elastic fibers in the internal elastic layer are continuous with those in the alveolar wall. The anatomy of the parietal pleura is not fully clear. It is believed to comprise five layers: mesothelial, submesothelial, internal elastic, fibroadipose, and external elastic. The distance between the mesothelial and external elastic layers is variable and increases when fibrosis of the fibroadipose layer occurs. When extrapulmonary pneumonectomy is performed, the plane of the external elastic layer is dissected. Fat tissue, endothoracic fascia, striated muscles, and ribs are present outside the external elastic layer. Bundles of elastic fibers connect the parietal pleura and periosteum. The endothoracic fascia and external elastic layer of the parietal pleura are continuous from the thoracic wall to the peritoneal wall. The internal elastic layer in the thoracic wall runs in the direction of the diaphragm at the costophrenic angle.

Keywords Mesothelial cell · Visceral pleura · Parietal pleura · Internal elastic layer · External elastic layer · Endothoracic fascia

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1 Introduction

The use of video-assisted thoracic surgery for biopsy of the parietal pleura is recommended for the definite pathological diagnosis of malignant mesothelioma, particularly at its early stage [1]. It is important to understand the anatomy of the visceral and parietal pleura for an accurate pathological diagnosis of malignant mesothelioma. It is also important to consider the microscopic anatomy of the visceral and parietal pleura to evaluate pleural invasion in lung cancer according to the Union for International Cancer Control pathological staging of lung cancer [2]. Although some descriptions of the visceral pleura anatomy have been described in textbooks and journal articles, some confusion still exists among authors regarding the understanding of the anatomy of the visceral pleura. A few books have described the anatomy of the parietal pleura. In this chapter, the normal structure of the pleura and mesothelial cells will be described based on published books, manuscripts, and pathological studies on surgical and autopsy samples of the pleura by the author of this chapter.

2 Macroscopic Structure of the Pleura

The surface of the lung is covered by a continuous, serous membrane called the visceral pleura. The visceral pleura extends into the fissures between the lobes (the interlobar fissures). The parietal pleura covers the internal surface of the thoracic cavity and is subdivided according to the part of the body that is in contact with the pleura: mediastinal pleura, cervical pleura, costal pleura, and diaphragmatic pleura. The visceral pleura reflects at the hilum and continues as the parietal pleura. The pulmonary ligament is a fused triangular-shaped pleural fold that is formed by merging the visceral and parietal pleura, which extends from the hilum to the mediastinal surface of the lower lobe.

The pleural cavity is a space between the visceral and parietal pleura that contains a small volume of serous fluid. The amount of pleural fluid is small, and a volume of <1 mL can be detected from the pleural cavity of a healthy individual. This fluid prevents contact between the visceral and parietal surfaces and helps the lung move smoothly and efficiently during respiration. This serous fluid also generates surface tension so that the lungs fill with air and expand when the thorax expands.

3 Mesothelial Cells

Mesothelial cells are stretchable and range in size from 16.4 ± 6.8 to 41.9 ± 9.5 μm in diameter [3]. The cytoplasm rises over a central oval nucleus. They may appear flat, cuboidal, or columnar. The flat cells contain microfilaments, few mitochondria, poorly developed rough endoplasmic reticulum (ER), and Golgi apparatus. Flat

cells usually represent stretched quiescent cells on the visceral surface or cells that cover a very rigid substructure such as a rib. The cuboidal or columnar cells contain abundant microfilaments, mitochondria, rough ER, well-developed Golgi bodies, and microtubules. A cuboidal or columnar mesothelial cell shape indicates that the cells are metabolically active or that they are associated with a substructure that is loose or fatty.

A tight junction joins the cell at the apical portion of the mesothelial layer. The mesothelial cells may overlap without being attached to each other at other junctions [4]. This overlap disappears during deep inspiration.

The mesothelial cell surface is covered with microvilli, which are approximately 0.1 μm in diameter and 0.5–3 μm in length [3]. More microvilli are found on the visceral pleura than on corresponding regions of the parietal pleura. The exact function of mesothelial microvilli has not been defined. However, the main function of microvilli is to enmesh glycoproteins rich in hyaluronic acid to decrease the friction between the lung and thorax. Hyaluronic acid is secreted by mesothelial cells and mesenchymal cells in the subpleural space.

Mesothelial cells are frequently dislodged from their surfaces and freely float in the pleural fluid where they become round or oval in shape. When the mesothelial cells float freely in the pleural fluid, they can transform into macrophages capable of phagocytosis and erythrophagocytosis [5].

Mesothelial cells are immunohistochemically positive for mesothelial markers, such as calretinin, D2-40, and WT1, whereas in normal conditions, the staining intensity of these markers is weak or negative.

The mesothelial layer is extremely fragile; when disrupted, the defect is repaired via mitosis and migration of mesothelial cells [6]. The nucleus of mesothelial cells increases in size, and the nucleoli become prominent under reactive conditions such as pneumothorax or pleuritis. In some cases, under reactive conditions, the proliferation of mesothelial cells becomes prominent, and stratification and papillary proliferation of mesothelial cells occur; moreover, the staining intensity of mesothelial markers increases. The differentiation between atypical mesothelial proliferation and early-stage mesothelioma is difficult. Homozygous deletion of the p16/CDKN2A gene is detected in up to 80% of pleural mesotheliomas with fluorescence in situ hybridization but not in reactive mesothelial hyperplasia [1]. Analysis of the p16/CDKN2A gene is useful for the differentiation of atypical mesothelial proliferation and mesothelioma. Positivity for breast cancer 1-associated protein 1 (BAP1) and methylthioadenosine phosphorylase (MTAP) by immunostaining has also been reported to be an excellent biomarker for mesothelioma with 100% specificity for malignant mesothelioma in the context of mesothelial proliferation [7, 8].

4 Anatomy of the Visceral Pleura

Nagaishi has classified the pulmonary pleura into five layers, i.e., (1) mesothelial, (2) submesothelial, (3) external elastic, (4) interstitial, and (5) internal elastic [9]. A textbook by Dail and Hammar also divides the visceral pleura into five layers: (1) a

mesothelial layer, (2) a thin submesothelial connective tissue layer, (3) a superficial elastic layer, (4) a loose subpleural connective tissue layer, and (5) a deep fibroelastic layer [10]. A textbook by Corrin also divides the visceral pleura into five layers: (1) a surface mesothelium and its basement membrane, (2) a thin layer of connective tissue, (3) a prominent outer elastin layer, (4) a band of collagen, and (5) an inner elastin layer (which is continuous with the alveolar elastin) [11]. However, there are different opinions regarding the terminology of the parts of pleura. Some authors have termed the layer situated immediately under the submesothelial layer as the "internal elastic layer" [12, 13].

The International Association for the Study of Lung Cancer (IASLC) reported only four layers in the visceral pleura: (1) a single layer of mesothelial cells resting on a basement membrane, (2) a submesothelial connective tissue layer, (3) an elastic fiber layer, and (4) a connective tissue layer [14].

Elastic stains are helpful in understanding the microscopic anatomy of the pleura and in assessing the invasion of the visceral pleura by primary lung cancer cells and the invasion of the lung by pleural mesothelioma. IASLC recommends that a tumor that invades beyond the thick elastic layer should be regarded as having penetrated the pleura [14]. However, Corrin describes that penetration of the outer elastic layer should be regarded as the criterion of pleural penetration [11], and the author of this chapter agrees with Corrin.

The idea that visceral pleura is composed of five different layers is widely accepted and the author of this chapter agrees with it. The terminology of each layer of the visceral pleura proposed by Nagaishi [9] is used and discussed in this chapter (Fig. 7.1).

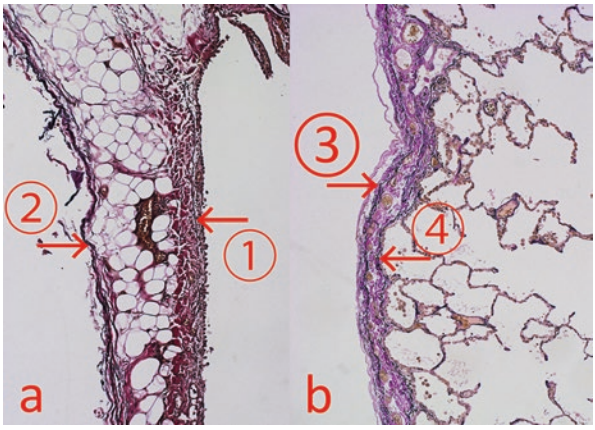

Fig. 7.1 Anatomy of the pleura. (a) Parietal pleura and the lung. The parietal pleura is divided into five layers: the mesothelial, submesothelial, internal elastic (①), fibroadipose, and external elastic layers (②)
(b) Visceral pleura. The visceral pleura is divided into five layers: the mesothelial, submesothelial, external elastic (③), interstitial, and internal elastic layers (④).(Byori to Rinsho 35:Supplement, p174, 2017 (in Japanese), with permission)

4.1 Mesothelial Layer

The mesothelial layer consists of a monolayer of flat single cells. The size of the mesothelial cells is uniform, but the shape of each cell is not uniform. The nucleus is round or ovoid and situated at the center of the cell.

4.2 Submesothelial Layer

The submesothelial layer is a thin layer comprising connective tissue immediately under the mesothelial layers. This layer does not contain capillaries or lymphatic vessels under normal conditions. However, this layer contains capillaries and lymphatic vessels in the presence of an abnormal lesion in the pleura. Mesothelioma cells may proliferate in this layer at its early stage, although no nodules or any abnormalities are macroscopically detected on the visceral pleura.

4.3 External Elastic Layer

This layer is situated immediately under the submesothelial layer. It is composed of several elastic fibers. They run parallel with the pleural surface and form anastomoses and complex networks.

4.4 Interstitial Layer

This layer is situated between the external and internal elastic layers. These layers form connective tissue, and the collagen fibers of the interstitial layer are continuous with the interlobular connective tissue. The interstitial layer is rich in capillaries and lymph vessels.

Collagen fibers are observed in the interstitial layer and run parallel with the pleural surface. Some elastic fibers from the external and internal elastic layers are interwoven with the collagen fibers.

This layer is the plane of cleavage for pleurectomy/decortication [10] because the connective tissue is relatively loose.

4.5 Internal Elastic Layer

This is the layer that is close to the pulmonary parenchyma. The internal elastic layer comprises a few elastic fibers, and the internal elastic layer is not as prominent as the external elastic layer. However, when fibrosis occurs in the internal elastic

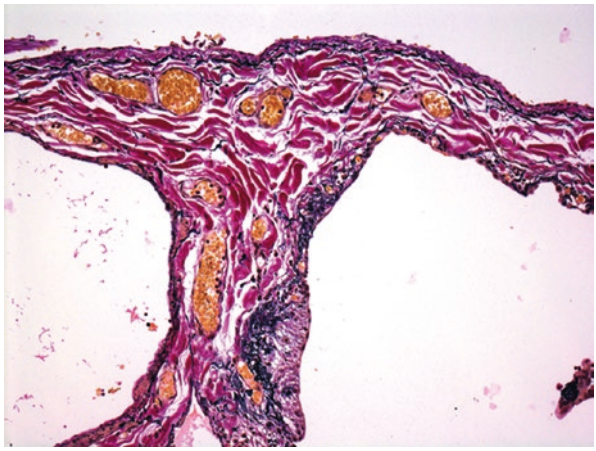

Fig. 7.2 Visceral pleura. Some of the elastic fibers in the internal elastic layer are continuous with those of the alveolar wall. (Byori to Rinsho 35:Supplement, p. 176, 2017 (in Japanese), with permission)

layer, the number of elastic fibers in the internal elastic layer increases, and the internal elastic layer becomes prominent.

Some of the elastic fibers present in the internal elastic layer are continuous with the elastic fibers of the alveolar wall (Fig. 7.2). This bundle of elastic fibers may fix the visceral pleura to the pulmonary parenchyma. The elastic fibers in this region contain elastic fibers belonging to the subpleural connective tissue and those belonging to the alveolar wall. Based on these findings, the internal elastic layer is regarded as part of the pulmonary parenchyma. Considering that the internal elastic layer is part of the pulmonary parenchymal wall, it is reasonable to evaluate the lung cancer that invades the internal elastic layer as pl0 and the lung cancer that invades the external elastic layer as pl1 [11, 15].

5 Anatomy of the Parietal Pleura

In the textbook by Dail and Hammar, microscopic anatomy of the parietal pleura is described [10]. The surface of the parietal pleura is covered by a layer of mesothelial cells under which lies a thicker layer of fibroelastic tissue. Beneath this layer, there exists the subpleural fibroadipose tissue and skeletal muscle of the chest wall. The textbook by Corrin claims that the amount and distribution of elastic fibers in the parietal pleura are irregular, but there is no description on the microscopic anatomy of the parietal pleura [11]. The report from IASLC describes that the anatomy of the parietal pleura is more variable than that of the visceral pleura [14]. Generally, it is covered by a mesothelial layer that rests upon a basement membrane and a thin layer of loose connective tissue. This is followed by a discontinuous elastic layer and another layer of loose connective tissue. Below this lies a dense collagenous layer or endothoracic fascia that may contain varying amounts of fat on both sides, and after this layer, are the skeletal muscle fibers of the chest wall. Masaoka reported

that the parietal pleura is composed of six layers: mesothelial, submesothelial, internal elastic, connective tissue, fat tissue, and external elastic [16]. The anatomy of the parietal pleura is described in this chapter based on the modified scheme of Masaoka (Fig. 7.1).

5.1 Mesothelial Layer

The mesothelial layer comprises a monolayer of flat single cells.

5.2 Submesothelial Layer

The submesothelial layer is a thin layer comprising connective tissue that lies immediately under the mesothelial layer.

5.3 Internal Elastic Layer

This layer is situated immediately under the submesothelial layer. The internal elastic layer consists of one or a few thin elastic fibers. Elastic fibers in the internal elastic layer are continuous with those of the external elastic layer in some cases, and consequently, the fibroadipose tissue layer cannot be discerned. The parietal pleura on the rib is thin compared with that on other regions, and elastic fibers in the internal elastic layer and external elastic layer cannot be differentiated from each other.

5.4 Fibroadipose Tissue Layer

This layer is situated immediately under the internal elastic layer. It is composed of connective tissue and fat tissue.

5.5 External Elastic Layer

This layer is situated immediately under the fibroadipose tissue layer. The distance between the mesothelial and external elastic layers is variable. This distance is approximately 0.2 mm in a healthy individual and increases to $\geq$1 mm when the

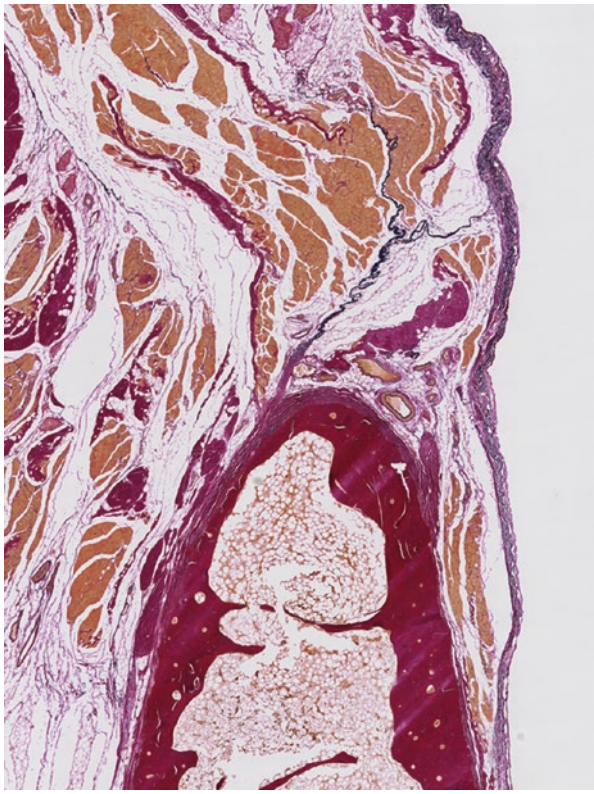

Fig. 7.3 Parietal pleura. The parietal pleura along the ribs is thin compared with that in other regions. Outside the external elastic layer are adipose tissue, endothoracic fascia, striated muscle, and rib. Bundles of elastic fibers connect the external elastic layer and the periosteum, which is a dense fibrous membrane covering the surfaces of bones. (Byori to Rinsho 35:Supplement, p178, 2017 (in Japanese), with permission)

parietal pleura thickens owing to fibrosis of the fibroadipose tissue layer. When extrapulmonary pneumonectomy is performed, the plane of the external elastic layer is dissected.

Adipose tissue, endothoracic fascia, skeletal muscle fibers of the thoracic wall, and ribs are located outside the external elastic layer. Bundles of elastic fibers connect the external elastic layer of the parietal pleura and periosteum, which is a dense fibrous membrane covering the surfaces of bones (Fig. 7.3). This bundle of elastic fibers may fix the parietal pleura to the thoracic wall and may prevent the parietal pleura from sliding against the thoracic wall.

6 Anatomy of the Endothoracic Fascia at the Costophrenic Angle

The endothoracic fascia runs outside the parietal pleura. The manner in which the endothoracic fascia runs at the costophrenic angle is controversial. One opinion is that the endothoracic fascia in the thorax turns at right angles toward the direction

of the diaphragm at the costophrenic angle [17]. Another opinion is that the endothoracic fascia is continuous from the neck to the pubis or pelvis and does not run in the direction of the diaphragm [18].

The author of this chapter analyzed the anatomy of the endothoracic fascia at the costophrenic angle in autopsy samples obtained from seven autopsy cases. Tissue at costophrenic angles, including the parietal pleura, endothoracic fascia, ribs, and skeletal muscle fibers of the thoracic wall, were sampled from cadavers and were formalin-fixed, decalcified, and paraffin-embedded. Thin sections were cut from the blocks and stained with hematoxylin and eosin and elastic van Gieson stain (Fig. 7.4). All cases showed the same anatomy at the costophrenic angle. The endothoracic fascia is continuous from the thoracic wall to peritoneal wall, and the external elastic layer in the thoracic wall runs alongside the endothoracic fascia and is continuous with elastic fibers in the abdominal wall. Conversely, the internal elastic layer in the thoracic wall runs in the direction of the diaphragm and is continuous with elastic fibers in the diaphragm.

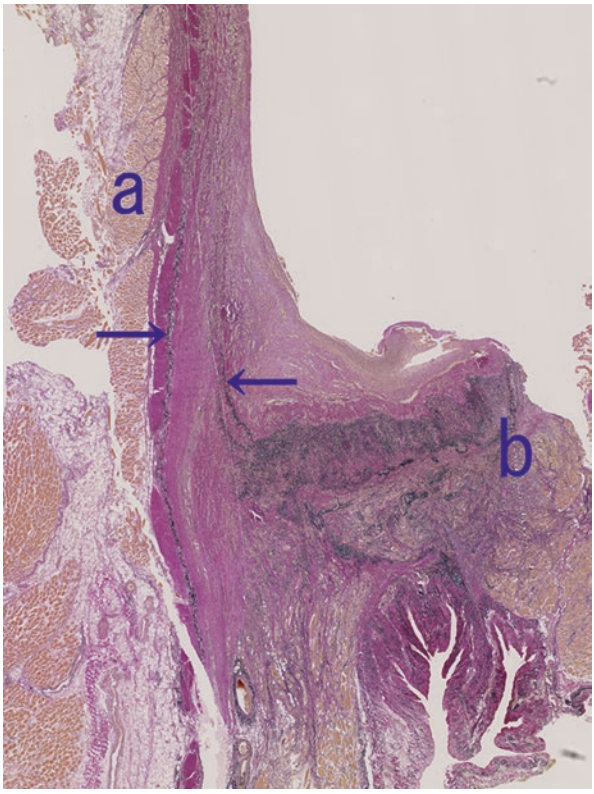

Fig. 7.4 Parietal pleura. The external elastic layer (→) in the thoracic wall runs alongside the endothoracic fascia and is continuous with elastic fibers in the abdominal wall. The endothoracic fascia is attached to the external elastic layer. Skeletal muscles in the thoracic wall (a) are located outside the endothoracic fascia. The internal elastic layer (←) in the thoracic wall runs in the direction of the diaphragm and is continuous with elastic fibers in the diaphragm (b). (Byori to Rinsho 35:Supplement, p178, 2017 (in Japanese), with permission)

7 Conclusion

Elastic stains are helpful in understanding the microscopic anatomy of the pleura and in assessing the invasion of the visceral pleura by primary lung cancer cells or pleural mesothelioma cells. The visceral pleura is divided into five layers: the mesothelial, submesothelial, external elastic, interstitial, and internal elastic layers. The parietal pleura is also divided into five layers: the mesothelial, submesothelial, internal elastic, fibroadipose tissue, and external elastic layers. Outside the external elastic layer resides adipose tissue, endothoracic fascia, skeletal muscle fibers of the thoracic wall, and the ribs. Bundles of elastic fibers connect the external elastic layer of the parietal pleura and the periosteum, which maintains the alignment of the parietal pleura against the thoracic wall.

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Chapter 8
Histologic Classification of Tumors of the Pleura: How Has the WHO Classification Progressed After 2015?

Yuichi Ishikawa

Abstract Malignant mesothelioma is a representative mesothelial malignant tumor. The current World Health Organization classification includes four subtypes of mesothelial tumors; diffuse malignant mesothelioma, localized malignant mesothelioma, well-differentiated papillary mesothelioma, and adenomatoid tumor. Diffuse and localized mesotheliomas are further divided into three histological subtypes: epithelioid mesothelioma, sarcomatoid mesothelioma, and biphasic mesothelioma. Under the sarcomatoid mesothelioma of diffuse type, desmoplastic mesothelioma is defined.

By introducing a panel of immunohistochemical markers including calretinin, precision of pathological diagnosis of mesothelioma has been markedly improved. Also, WT-1 and podoplanin (D2–40) are utilized as sensitive and specific mesothelial markers as well as CEA and TTF-1 are used as negative markers for epithelioid mesothelioma. On the other hand, there are only a few markers for sarcomatoid mesothelioma.

In this review, the progress after 2015 WHO classification will be described in terms of more detailed subtyping of mesothelioma, various markers for grading and classification, and newly emerging concepts, based on histology, immunohistochemistry, and other molecular methodology including fluorescent in situ hybridization. Additionally, characterization of calretinin-expressing lung adenocarcinoma is described to help differential diagnosis of mesothelioma from its mimickers.

Keywords Histological classification · WHO classification
Immunohistochemistry · Epithelioid mesothelioma · Sarcomatoid mesothelioma

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1 Introduction

Malignant mesothelioma is a representative mesothelial malignant tumor and the causal relevance with past exposure to asbestos is recognized well. The current World Health Organization classification [1] includes four subtypes of mesothelial tumors: diffuse malignant mesothelioma, localized malignant mesothelioma, well-differentiated papillary mesothelioma, and adenomatoid tumor as shown in Table 8.1. Diffuse and localized mesotheliomas are further divided into three histological subtypes: epithelioid mesothelioma, sarcomatoid mesothelioma, and biphasic mesothelioma. Under the sarcomatoid mesothelioma of diffuse type, desmoplastic mesothelioma is defined. There have been no reports with localized desmoplastic mesothelioma.

Mesothelioma is so rare that its pathological diagnosis has been a challenge to most pathologists. However, after introducing a panel of immunohistochemical markers including calretinin, precision of pathological diagnosis of mesothelioma has been markedly improved. Currently, a number of mesothelial markers are routinely used and hence pathologists can make quite precise diagnosis even at biopsy. Among them, calretinin expression is a hallmark of mesothelioma, particularly epithelioid mesothelioma. Besides calretinin, numerous useful markers for mesothelioma diagnosis, epithelioid mesothelioma, in particular, have been emerging.

Usefulness of calretinin, WT-1, and podoplanin (D2–40) for epithelioid mesothelioma has been established as positive markers for mesothelium. Also, negative markers have been recognized widely; CEA, TTF-1 for pleural lesion as well as MOC-31 or BerEP4. These markers are usually utilized as a panel of antibodies. However, sarcomatoid mesothelioma is often negative for some of those antibodies and therefore the diagnosis must be made occasionally on the basis of keratin expression only. It is noted that these antibodies are markers of mesothelium, not mesothelioma. So, the distinction of regenerative mesothelial cells with cellular atypia from actual mesothelioma has been at issue.

In this review, the progress after 2015 WHO classification will be described in terms of more detailed subtyping of mesothelioma, various markers for grading

Table 8.1 Mesothelial tumors described in the WHO classification of tumors of the pleura (2015)

Diffuse malignant mesothelioma
Epithelioid mesothelioma
Sarcomatoid mesothelioma
Desmoplastic mesothelioma
Biphasic mesothelioma
Localized malignant mesothelioma
Epithelioid mesothelioma
Sarcomatoid mesothelioma
Biphasic mesothelioma
Well-differentiated papillary mesothelioma
Adenomatoid tumor

and classification and newly emerging concepts, based on histology, immunohistochemistry (IHC), and other molecular methodology including fluorescence in situ hybridization (FISH).

2 Grading Systems

Based on a previous study by Kadota et al. [2], which proposed a grading system of pleural epithelioid mesothelioma using nuclear atypia and mitotic count, Rosen et al. [3] performed a multi-institutional study on the grading system updated by adding another feature, necrosis, and by employing international series of cases (17 institutions and 776 cases), which demonstrated that the nuclear grade, defined as a sum of nuclear atypia level (Fig. 8.1) and mitotic count, was clearly associated with survival. Also, they proposed an alternative scoring system, the mitosis-necrosis score, which was a strong association with the nuclear grade and the overall survival.

3 Architectural Patterns of Pleural Mesothelioma

Nicholson et al. [4] proposed a revision of mesothelioma classification by including architectural (16 histologic and 3 stromal) patterns as shown in Table 8.2. Some of them have only histological implications, but others have clinical, diagnostic, and prognostic implications as well. For example, adenomatoid mesothelioma resembles adenomatoid tumor, but its nature is malignant. Transitional mesothelioma is between epithelioid and sarcomatoid in terms of histology, but its clinical behavior may be like sarcomatoid. Further research should be warranted.

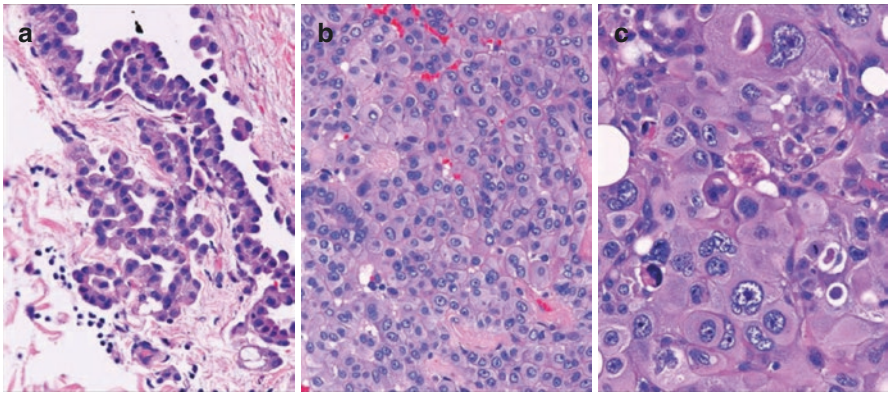

Fig. 8.1 Typical nuclear atypia of epithelioid mesothelioma. (**a**) Mild nuclear atypia, (**b**) moderate atypia, and (**c**) severe atypia (from Rosen et al. Mod Pathol, 2018; 31:598–606, with permission) [3]

Table 8.2 Proposed architectural patterns for pleural mesothelioma (Nicholson et al. 2019)

Histologic patterns		Comments	Differentiation grades
a.	Tubular	Common in epithelioid mesothelioma.	Well
b.	Papillary	Common in epithelioid mesothelioma.	Well
c.	Tubulopapillary	Common in epithelioid mesothelioma.	Well
d.	Trabecular		Moderately–poorly
e.	Solid		Poorly
f.	Micropapillary	Similar to lung micropapillary. Single-cell pattern is also included.	Moderately
g.	Adenomatoid	Malignant epithelioid mesothelioma, resembling adenomatoid tumor.	Moderately
h.	Microcystic		Moderately
Cytologic features			
i.	Pleomorphic		(poorly)
j.	Transitional	Intermediate between epithelioid and sarcomatoid morphologies.	(poorly)
k.	Rhabdoid		(poorly)
l.	Deciduoid		(poorly)
m.	Small cell	Epithelioid mesothelioma, morphologically resembling small cell lung cancer. No prognostic significance.	(poorly)
n.	Clear cell		Not determined
o.	Signet ring	Epithelioid mesothelioma, morphologically resembling signet ring cell adenocarcinoma. No prognostic significance.	(poorly)
p.	Lymphohistiocytic		(poorly)
Stromal features			
q.	Myxoid		
r.	Desmoplastic		
s.	Heterologous elements	Sarcomatous elements such as osteosarcoma, chondrosarcoma, and rhabdomyosarcoma.	

Three-tier classification of adenocarcinoma according to differentiation grades: well-, moderately-, and poorly-differentiated adenocarcinomas are commonly used in pathology practice for adenocarcinomas in general. Similarly, epithelioid mesothelioma may be classified as well-, moderately-, and poorly differentiated. As shown in Table 8.2, well-differentiated mesotheliomas may include papillary and tubulopapillary; moderately-differentiated mesotheliomas may include trabecular, adenomatoid, microcystic, and micropapillary; and poorly-differentiated mesothelioma may include mesotheliomas composed mainly of pleomorphic cells or showing solid, transitional patterns. We expect to confirm whether the classification of

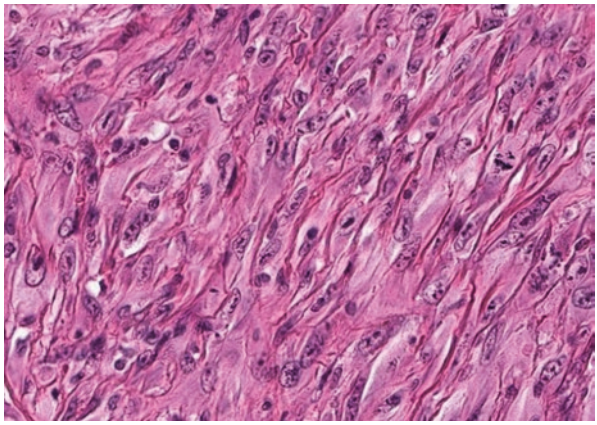

Fig. 8.2 Transitional pattern of mesothelioma. Sheet of cohesive large plump epithelioid cells losing their epithelioid appearance but not overtly spindle shaped, intermediate pattern between epithelioid and sarcomatoid (from Churg et al. Lung Cancer, 2018; 124:95–10, with permission) [4]

epithelioid mesothelioma according to this three-tier grading is useful or not to estimate prognosis (Fig. 8.2).

4 Mesothelioma In Situ

In many organs including esophagus, uterine cervix, and lung, carcinomas without stromal invasion are frequently observed and the concept of "carcinoma in situ" has been used in practical medicine. Also in the field of lung medicine, bronchial squamous cell carcinoma in situ and peripheral adenocarcinoma in situ have been established and appropriately treated. By contrast, although the concept of mesothelioma in situ was described in 1992 [5], there was difficulty to make a diagnosis of the condition accurately. In fact, there are almost no clinical findings, only the patients occasionally showing pleural effusion. Histologically, it was reported that mesothelioma in situ shows monolayered mesothelia with cytological atypia, but diagnosis of mesothelioma in situ required overt invasive lesions besides the in situ lesions to make sure they were true mesotheliomas.

The situations have been drastically changed after BRCA1-associated-protein 1 (BAP1) antibody has become available. BAP1 is a tumor suppressor gene, located in the short arm of Chromosome 3 and, although the gene harbors the name of BRCA1, its functional association with BRCA1 is not well understood. Notably, immunohistochemical loss of BAP1 expression is observed in 66% of malignant mesothelioma [6], and therefore BAP1 immunostains are very useful to diagnose mesothelioma in situ as well. As it is known that p16/CDKN2A loss by FISH analysis is a useful marker for mesothelioma even for effusion cytology [7], mesothelial proliferating lesions with both immunohistochemical BAP1 loss and histological characteristics will become a formal definition of mesothelioma in situ [8] (Fig. 8.3).

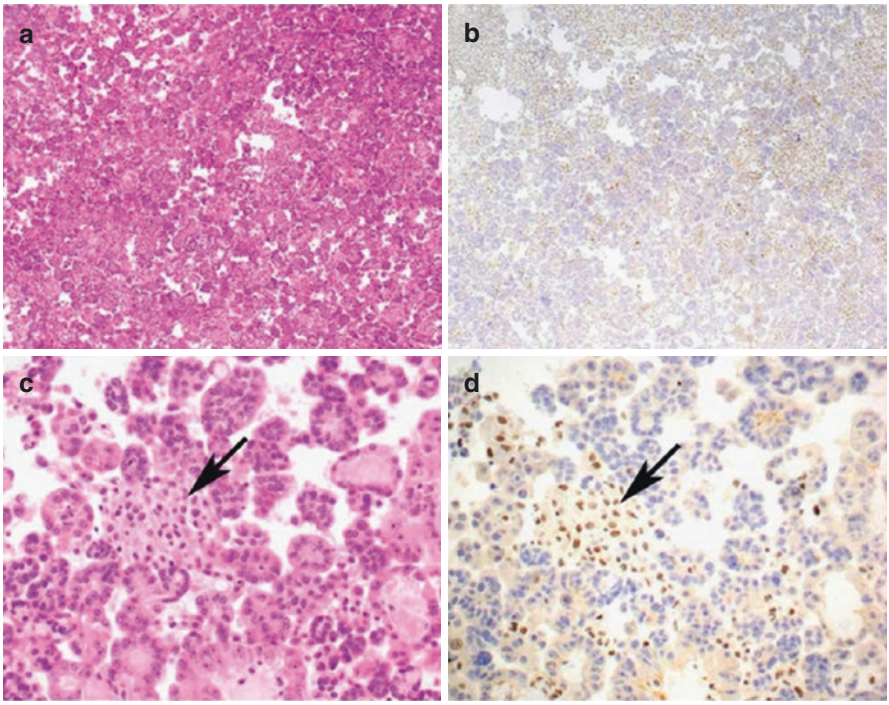

Fig. 8.3 BAP1 stains in sections made from an effusion cell block of epithelioid mesothelioma (**a**, **c**). All the neoplastic cells show completely negative staining for BAP1 (**b**). Positive nuclear staining in nonneoplastic stromal and inflammatory cells (arrows) is noted and acts as an internal positive control (**d**) (from Andrici et al. Mod Pathol, 2015; 28:1360–1368, with permission) [9]

5 Markers to Distinguish Mesothelioma from Other Tumors and Reactive Mesothelial Proliferation

It is critically important to distinguish malignant mesothelioma from other tumors such as lung cancer and pleural sarcoma. Currently, a panel of antibodies for mesothelium are available, including calretinin, WT-1, podoplanin (D2–40), and so forth, sometimes called "mesothelioma markers." Negative markers for pleural mesothelium are also used, e.g., CEA, TTF-1, and napsin A. However, all of the "mesothelioma makers" are for mesothelial cells, not actually mesothelioma cells. Therefore, the panel of antibodies are useless to distinguish mesothelioma from reactive mesothelial proliferation. If specific markers for mesothelioma are available, that would be extremely useful. So far, several markers including Glut-1, IMP3, CD146, and desmin were expected to be useful to discriminate mesothelioma from reactive mesothelial cells [10], but subsequent analyses revealed these were not sufficiently helpful.

Tsuji et al. reported that a mucin-like membrane protein, sialylated protein HEG homolog 1 (HEG1), is a specific marker for malignant mesothelioma [11]. They

produced a monoclonal antibody against sialylated HEG1, SKM9–2, which can detect even sarcomatoid and desmoplastic mesothelioma as shown in Fig. 8.4 [12]. Also, the antibody is reported to be quite specific to discriminate mesothelioma from lung cancers [13]. Further analysis in different populations will be encouraged.

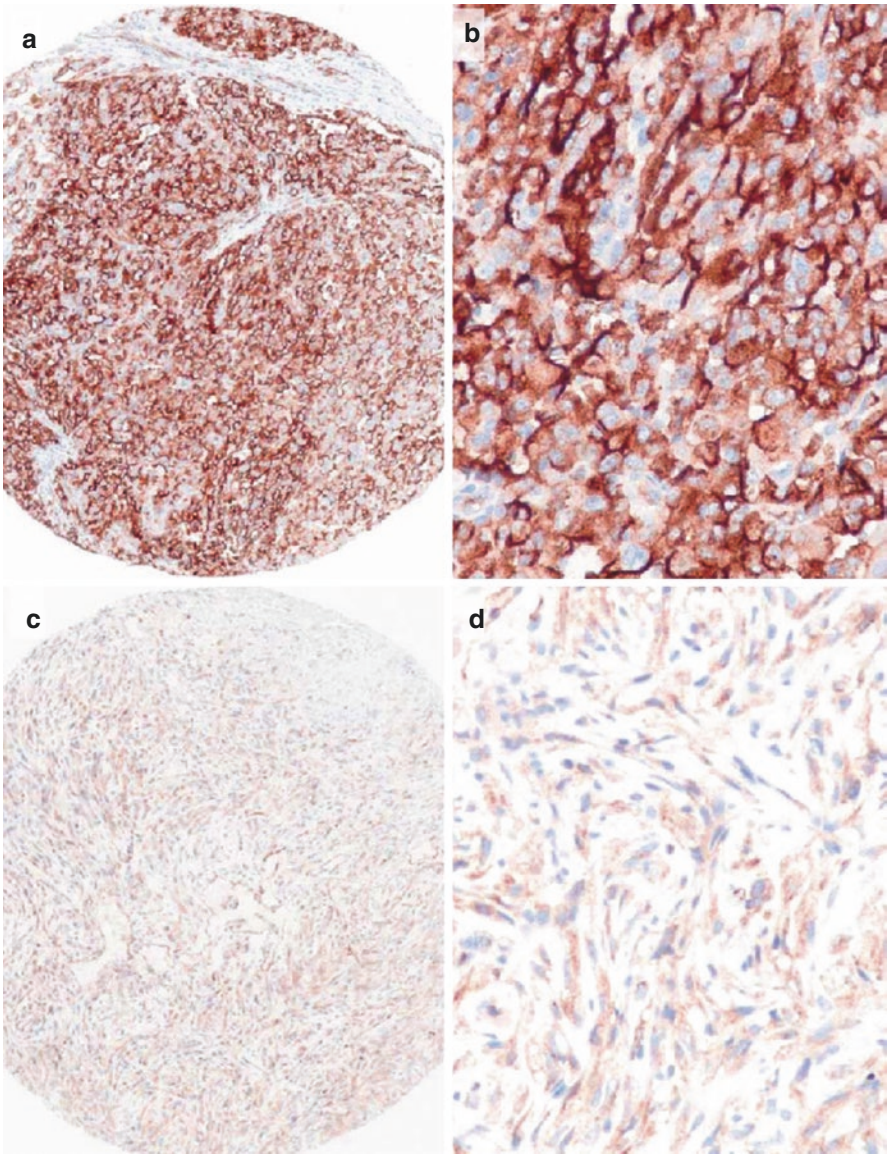

Fig. 8.4 HEG1 immunostaining of mesotheliomas. A and B, Strong diffuse membrane staining in an epithelioid mesothelioma. C and D, Diffuse membrane staining in sarcomatoid mesothelioma. (from Naso et al. Am J Surg Pathol. 2020 Mar 19. https://doi.org/10.1097/PAS.0000000000001469. Online ahead of print, with permission) [12]

Dr. K. Nabeshima's group in Fukuoka has developed a useful method to detect CDKN2A (*p16*) loss, using not FISH analyses but immunohistochemistry [14]. A gene coding methylthioadenosine phosphorylase (MTAP) is closely located to CDKN2A at 9p21 locus and was reported to be deleted together with CDKN2A in almost all cases of mesothelioma determined by FISH. Moreover, it was confirmed that a combination of MTAP and BAP1 immunohistochemistry was able to distinguish sarcomatoid MPM from fibrous pleuritis [15, 16]. Since FISH analysis is not necessarily available in every hospital, combinatorial immunohistochemistry of MTAP and BAP1 will replace FISH analysis in the future.

6 Novel Mesothelioma Markers

To diagnose sarcomatoid mesothelioma, differential diagnoses include sarcomatoid carcinoma of the lung, which has a sarcomatoid component such as a spindle cell area. Amatya et al. discovered that MUC4 was expressed in pulmonary sarcomatoid carcinoma but not in sarcomatoid mesothelioma, using gene expression and clustering analyses [17]. Previously MUC4 was reported to be useful to distinguish epithelioid mesothelioma from lung adenocarcinoma because no epithelioid mesothelioma expressed MUC4 and most of the lung adenocarcinoma expressed MUC4 [18]. Although there are some arguments on usefulness of MUC4 immunohistochemistry [19, 20], the applicability of MUC4 to distinguish sarcomatoid mesothelioma from pulmonary sarcomatoid carcinoma is being proved.

Immunohistochemistry of GATA binding protein 3 (GATA3) has been suggested to be useful to separate sarcomatoid/desmoplastic mesothelioma from pulmonary sarcomatoid carcinoma [21]. This report is interesting because it is quite difficult to discriminate sarcomatoid and desmoplastic mesothelioma from sarcomatoid carcinoma of the lung. Further studies in different populations should be warranted.

7 Calretinin-Expressing Adenocarcinoma and Its Distinction from Pleural Mesothelioma

There are pulmonary adenocarcinomas that express calretinin, a mesothelioma marker, which may complicate differential diagnosis of mesothelioma. Matsuda et al. performed a study on characterization of calretinin-expressing lung cancer, which may be useful for separation of lung cancer from mesothelioma [22], since calretinin is one of the most sensitive and specific mesothelioma markers.

Calretinin expression in 250 consecutive cases of lung adenocarcinomas was evaluated using immunohistochemistry. Among them, 15% (37/250) of adenocarcinomas expressed calretinin, including those with partial and weak expression. In the calretinin-positive 37 adenocarcinomas, expression percentages of Wilms' tumor-1

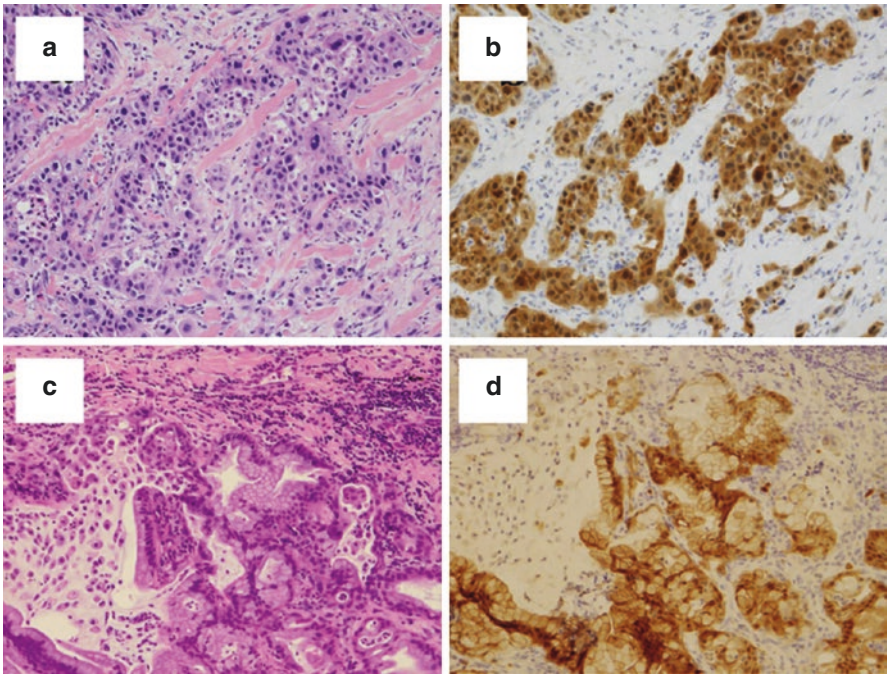

Fig. 8.5 Photomicrographs of calretinin-positive adenocarcinoma; histology and immunohistochemical expression of calretinin. (**a**) Solid adenocarcinoma and (**b**) calretinin staining. (**c**) Invasive mucinous adenocarcinoma and (**d**) calretinin staining. (from Matsuda et al. Pathology—Research and Practice 2020; 216; 152,817, with permission) [22]

was 6%, that of podoplanin (D2–40) was 3% and those of claudin-4 and TTF-1 were 82 and 52%, respectively, indicating that other mesothelial markers were only rarely expressed and epithelial markers were highly expressed. There were more smokers and less EGFR mutations in calretinin-positive tumors than in negative tumors. Further, calretinin expression was associated with a poor prognosis for stage I tumors of adenocarcinoma ($p < 0.001$). In conclusion, calretinin-positive lung adenocarcinomas share characteristics with adenocarcinomas arising in smokers and can be differentiated from mesothelioma with the use of other mesothelial and epithelial markers (Fig. 8.5).

8 Conclusion

The current histological classification of pleural mesothelioma is the WHO classification published in 2015 [1]. After that, quite a few advances were made including histological grading, new markers for separation of resembling tumors and non-tumorous conditions and new mesothelioma markers. Also, there are some

progresses to characterize lung tumors mimicking mesothelioma. Based on these progresses, the next classification of mesothelioma histology will be expected to improve from the present one [3, 4].

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Chapter 9
Pathology of Mesothelioma, Subtypes, and Rare Variants: What Is the Role of Immunohistochemical Markers in Differential Diagnosis?

Tohru Tsujimura, Michiko Yuki, Yoshiyasu Shinohara, and Ayuko Sato

Abstract Malignant pleural mesothelioma (MPM) is an asbestos-related aggressive tumor arising from mesothelial cells on the pleural surface. The definitive diagnosis of MPM is made histopathologically, supported by clinical and radiological findings, but morphological discrimination between MPM and other malignant tumors and that between MPM and non-neoplastic reactive mesothelial hyperplasia (RMH) are often difficult. In such cases, ancillary diagnostic techniques, fluorescence *in situ* hybridization (FISH) and immunohistochemistry (IHC), are effective to diagnose MPM. Immunohistochemical markers, mesothelial-related positive and negative markers, are especially essential for discrimination between MPM and other malignant tumors. Immunohistochemical detection of BRCA1-associated protein 1 (BAP1) protein loss and that of methylthioadenosine phosphorylase (MTAP) protein loss are also useful for differentiating MPM from RMH similar to detection of *CDKN2A* (*p16*) homozygous deletion by FISH. Moreover, BAP1 IHC and MTAP IHC, as well as *CDKN2A* (*p16*) FISH, are effective for diagnosing early-stage MPM and assessing malignancy of mesothelial cells in pleural effusion. In this chapter, we focus on the pathological approach including auxiliary diagnostic techniques, particularly IHC, in the differential diagnosis of MPM.

Keywords Immunohistochemistry · BAP1 · MTAP

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1 Introduction

Malignant mesothelioma (MM) is an asbestos-related aggressive tumor arising from mesothelial cells on the serosal surfaces of the pleural, peritoneal, and pericardial cavities and tunica vaginalis testis [1, 2]. Malignant pleural mesothelioma (MPM) is most common, and its incidence is dramatically increasing until 2030, as a result of widespread use of asbestos.

The definitive diagnosis of MPM is made histopathologically, supported by clinical and radiological findings, but morphological discrimination between MPM and other malignant tumors including secondary tumors involving the pleura and that between MPM and non-neoplastic reactive mesothelial hyperplasia (RMH) are challenging for pathologists. Since the most reliable pathological criterion for malignancy has been mesothelial proliferation invading deeply into subpleural adipose tissues, the diagnosis of early-stage MPM, in which mesothelial proliferation is localized on the pleural surface or limited within the submesothelial fibrous tissues of the pleura, was so hard. Moreover, since invasion cannot be assessed with specimens used in effusion cytology, the definitive diagnosis of MPM by effusion cytology has not been accepted [3].

The pathological diagnosis of MPM is basically performed by two steps, confirmation of mesothelial origin and evaluation of malignancy. Immunohistochemistry (IHC) is essential for advancing the two steps. Various immunohistochemical markers for investigating the origin of tumors have been reported [1, 4, 5]. In addition, immunohistochemical markers, BRCA1-associated protein 1 (BAP1) and methylthioadenosine phosphorylase (MTAP), have been recently developed based on genetic alternations of MPM. By using BAP1 IHC and MTAP IHC, it is now possible to differentiate between MPM, particularly early-stage MPM, and RMH [6–9] and to identify MM cells in pleural effusion [10]. In this chapter, we focus on histopathology of MPM and immunohistochemical approach to the differential diagnosis of MPM, such as MPM versus other malignant tumors and MPM versus RMH.

2 Histopathology

MPM is classified into diffuse malignant mesothelioma (DMM) and localized malignant mesothelioma (LMM) in the growth pattern, and most cases are DMM. DMM is histopathologically subtyped to epithelioid mesothelioma (EM), sarcomatoid mesothelioma (SM) including desmoplastic mesothelioma (DM), and biphasic mesothelioma (BM) (Table 9.1, Fig. 9.1), and EM is most common. Since the prognosis is the worst in SM, followed by BM and EM, the classification of histological subtypes is very important [1, 5]. The histopathology of LMM is similar to that of DMM, but LMM has a better prognosis than DMM [11]. Well-differentiated papillary mesothelioma of the pleura is classified as a borderline behavior tumor, but that of the peritoneum as a benign tumor (Table 9.1).

Table 9.1 WHO classification of mesothelial tumors of the pleura and peritoneum

Mesothelial tumors	Morphology codes
Pleura	
Diffuse malignant mesothelioma	
Epithelioid mesothelioma	9052/3
Sarcomatoid mesothelioma	9051/3
Desmoplastic mesothelioma	9051/3
Biphasic mesothelioma	9053/3
Localized malignant mesothelioma	
Epithelioid mesothelioma	9052/3
Sarcomatoid mesothelioma	9051/3
Biphasic mesothelioma	9053/3
Well-differentiated papillary mesothelioma	9052/1
Adenomatoid tumor	9054/0
Peritoneum	
Malignant mesothelioma	9050/3
Well-differentiated papillary mesothelioma	9052/0
Adenomatoid tumor	9054/0

The morphology codes are from the International Classification of Diseases for Oncology (ICD-O). Behavior is coded /0 for benign tumors; /1 for unspecified, borderline, or uncertain behavior; /2 for carcinoma in situ and grade III intraepithelial neoplasia; and /3 for malignant tumors. This table has been modified from Refs. [1, 2]

Adenomatoid tumor is regarded as a benign tumor in both pleura and peritoneum (Table 9.1). Tumors and diseases to be distinguished from MPM are listed in Table 9.2.

2.1 Epithelioid Mesothelioma

EM is defined as a malignant tumor originating from mesothelial cells and showing epithelioid morphology (Figs. 9.1a and 9.2a–d). Tissue structure pattern and cell morphology of EM are extremely diverse. Tissue structure pattern consists of tubulopapillary, micropapillary, acinar, trabecular, adenomatoid tumor like, solid, and adenoid cystic, and the commonly encountered patterns are solid, tubulopapillary, and trabecular. Characteristics of cell morphology include clear cell, deciduoid, lymphohistiocytoid, small cell, rhabdoid, pleomorphic, and transitional, and they are involved in the formation of rare variants, such as lymphohistiocytoid mesothelioma mimicking malignant lymphoma or lymphoepithelioma-like carcinoma, small cell mesothelioma mimicking small cell carcinoma, and transitional mesothelioma (TM). TM was first described as a tumor with a sheet-like growth pattern in

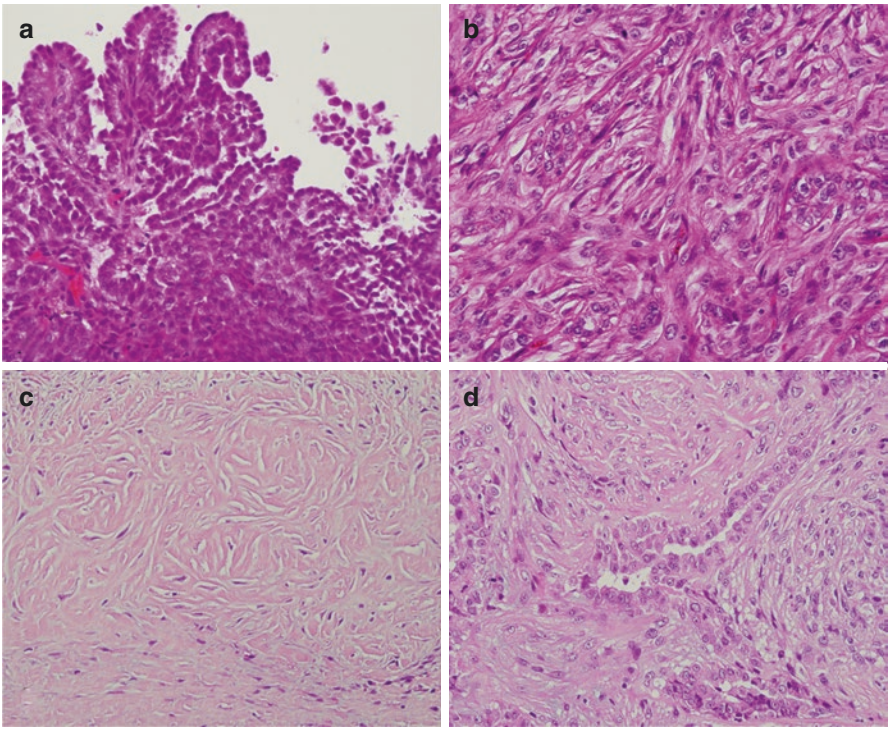

Fig. 9.1 Histopathological classification. Hematoxylin and eosin stains. Epithelioid mesothelioma (**a**), sarcomatoid mesothelioma (**b**), desmoplastic mesothelioma (**c**), and biphasic mesothelioma (**d**)

Table 9.2 Tumors and diseases to be distinguished from malignant pleural mesothelioma

Histological subtype	Tumors and diseases to be differentiated
Epithelioid mesothelioma	Primary lung adenocarcinoma
	Metastatic/invasive tumors to the pleura
	Reactive mesothelial hyperplasia
Sarcomatoid mesothelioma	Sarcoma of the chest wall, pleura, and lung
	Sarcomatoid carcinoma
Desmoplastic mesothelioma	Fibrous pleuritis
Biphasic mesothelioma	Biphasic synovial sarcoma
	Carcinosarcoma
	Pulmonary blastoma

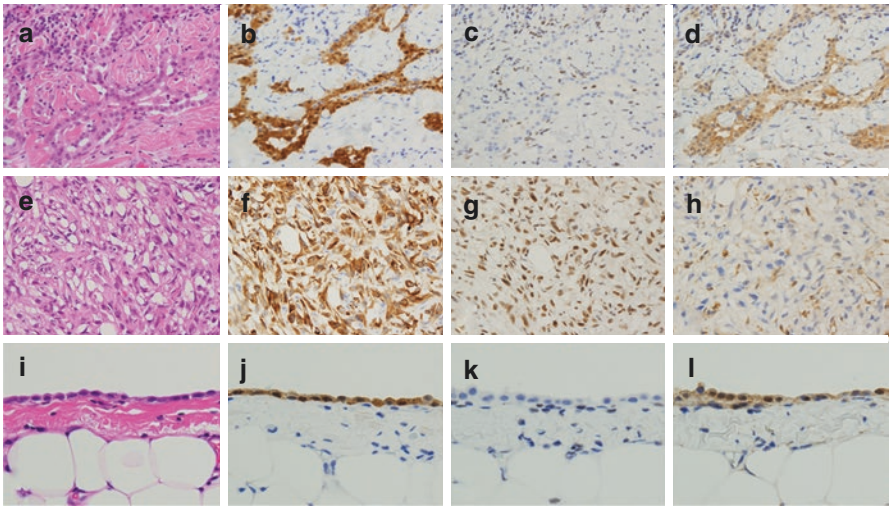

Fig. 9.2 Immunohistochemistry. Hematoxylin and eosin stains (**a**, **e**, and **i**) and immunohistochemical staining for calretinin (**b** and **j**), BAP1 (**c**, **g**, and **k**), MTAP (**d**, **h**, and **l**), and AE1/AE3 (**f**). A case of calretinin-positive epithelioid mesothelioma with BAP1 loss and MTAP retained is shown in (**a**)–(**d**). A case of AE1/AE3-positive sarcomatoid mesothelioma with BAP1 retained and MTAP loss is shown in (**e**)–(**h**). A case of calretinin-positive atypical mesothelial cells with BAP1 loss and MTAP retained on the pleural surface is shown in (**i**)–(**l**)

which cells are cohesive but have elongated morphology [1]. Recently, it has been proposed that TM should be considered as an aggressive subtype of MM, characterized by distinct structural criteria, reticulin pattern, and transcriptomic profile, and should be classified as a non-EM, at minimum as a subgroup of SM, and not as an EM variant of MM [12].

2.2 Sarcomatoid Mesothelioma

SM is defined as a malignant tumor arising from mesothelial cells and showing spindle cell morphology (Figs. 9.1b and 9.2e–h). Spindle cells grow in complex or arrayed in bundles, with various graded of nuclear atypia and mitotic activity. Necrosis is often observed in tumors. SM sometimes includes heterologous elements, such as rhabdomyosarcomatous, osteosarcomatous, or chondrosarcomatous elements. These elements should be differentiated from osteoid and chondroid metaplasia.

DM is defined as a malignant tumor arising from mesothelial cells and being characterized by atypical spindle cells arranged in a patternless pattern within the dense, hyalinized, collagenous stroma that constitutes at least 50% of the tumor (Fig. 9.1c). DM is classified as a subtype of SM. Since the atypia of spindle cells is not high in many cases of DM, the differentiation of DM from fibrous pleuritis is particularly required (Table 9.2). Invasion into adipose tissues is the most supportive finding for DM. The presence of bland necrosis, cellular stromal nodules, and other areas of EM or SM are also effective for diagnosing DM. On the other hand, zonation, which is characterized by a dense infiltration of cells on the pleural surface and a decrease of the cell density with more fibrosis towards the chest wall, is useful to diagnose fibrous pleuritis.

2.3 Biphasic Mesothelioma

BM is defined as a malignant tumor containing both cell types of EM and SM, in which each subtype should constitute at least 10% of the tumor (Fig. 9.1d). Tumors with biphasic patterns, such as biphasic synovial sarcoma, carcinosarcoma, and pulmonary blastoma, should be differentiated from BM (Table 9.2). It should be careful that the spindle cell component of BM might be a non-neoplastic desmoplastic stroma growing due to invasion of EM cells [13, 14].

3 Immunohistochemistry

The pathological diagnosis of MPM is basically performed by two steps, confirmation of mesothelial origin and evaluation of malignancy. IHC is essential for advancing the two steps.

3.1 Immunohistochemical Markers for Confirming Mesothelial Origin

Immunohistochemical markers that are useful for distinguishing MPM, particularly EM, from lung adenocarcinoma/squamous cell carcinoma are summarized in Table 9.3. Since none of the immunohistochemical markers with 100% sensitivity and 100% specificity are present in the differential diagnosis of MPM, an immunohistochemical approach to confirm mesothelial origin should be performed by using an immunohistochemical panel including two or more mesothelial-related positive markers and two or more negative markers. When EM of the pleura is suspected, calretinin (Fig. 9.2b), podoplanin (D2-40), and Wilms tumor-1 (WT1) are usually

Table 9.3 Immunohistochemical markers useful for distinguishing malignant pleural mesothelioma from lung adenocarcinoma/squamous cell carcinoma

	Markers	Localization	Mesothelioma	Lung ADC	Lung SCC
Mesothelial markers	Calretinin	Nucleus and cytoplasm	100%	5–10%	40%
	Podoplanin (D2-40)	Cell membrane	90–100%	≤15%	50%
	WT1	Nucleus	70–95%	0%	0%
	Cytokeratin5/6	Cytoplasm	75–100%	2–20% focal	100%
Lung ADC/SCC markers	CEA	Cytoplasm	< 5% focal	80–100%	NA
	TTF-1	Nucleus	0%	75–85%	NA
	Napsin A	Cytoplasm	0%	80–90%	NA
	Claudin 4	Cell membrane	0%	100%	95%
	MOC31	Cell membrane	2–10% focal	95–100%	97–100%
	BerEP4	Cell membrane	≤20% focal	95–100%	85–90%
	BG8	Cytoplasm	3–7% focal	90–100%	80%
	p40	Nucleus	2.5% focal	NA	100%

This table has been modified from Refs. [1, 5]
ADC adenocarcinoma, *SCC* squamous cell carcinoma, *WT1* Wilms tumor-1, *CEA* carcinoembryonic antigen, *TTF-1* thyroid transcription factor-1, *BG8* blood group 8, *NA* not available

used as positive markers and carcinoembryonic antigen (CEA), thyroid transcription factor-1 (TTF-1), and Napsin A as negative markers. Claudin 4 is a good marker for carcinoma because most carcinomas are positive and MPM is negative [4, 5, 15]. On the other hand, it should be careful that calretinin and podoplanin (D2-40) are relatively positive in lung squamous cell carcinoma. p40 is useful to distinguish MPM from lung squamous cell carcinoma [5, 16].

Not only general carcinoma markers, such as CEA, Claudin 4, MOC31, BerEP4, and blood group 8 (BG8), but also other organ-specific markers are helpful in the differential diagnosis between EM and secondary carcinomas involving the pleura [1, 5]. For example, estrogen receptor (ER), progesterone receptor (PgR), gross cystic disease fluid protein 15 (GCDFP-15), and mammaglobin are markers for breast carcinoma. PAX8, CDX2, and prostate-specific antigen are markers for renal cell carcinoma, gastrointestinal adenocarcinoma, and prostatic adenocarcinoma, respectively.

When SM is suspected, pancytokeratin, AE1/AE3 and CAM5.2, is recommended as a positive marker (Fig. 9.2f) and other sarcoma markers, such as S100, CD34, and smooth muscle actin, are used as negative markers [17]. However, it should be careful that about 10% of SM are negative for pancytokeratin and some sarcoma, such as angiosarcoma, synovial sarcoma, leiomyosarcoma, and SMARCA4-deficient thoracic sarcoma [18] are positive. The identification of the chromosomal translocation *t(X;18)* is helpful in the diagnosis of synovial sarcoma. The positive rate of calretinin, podoplanin (D2-40), and WT1 is not high in SM.

In peritoneal cases, calretinin is useful to differentiate between peritoneal MM and serous adenocarcinoma. However, WT1 is not effective to distinguish these tumors, because most serous adenocarcinomas as well as peritoneal MM are positive for WT1. Claudin 4 and MOC31 are good negative markers for peritoneal MM. ER and PgR are useful negative markers for peritoneal MM in female cases [5].

3.2 Immunohistochemical Markers for Evaluating Malignancy

It has been believed that the most reliable pathological criterion for malignancy is mesothelial proliferation invading deeply into subpleural adipose tissues. While this criterion is still useful, genetic alternations detected by FISH and IHC is now included in the criterion for malignancy. FISH detecting the *CDKN2A* (*p16*) homozygous deletion is effective to evaluate malignancy of proliferating mesothelial cells, leading to the discrimination of MPM from RMH. Genetic alternations in the *BAP1* gene generally result in loss of BAP1 expression in cell nucleus at the protein level. IHC detecting loss of BAP1 expression is useful to separate benign from malignant mesothelial proliferations [6–8]. The *MTAP* gene is located on the 9p21 chromosomal region where the *CDKN2A* (*p16*) gene exists. Loss of MTAP expression detected by IHC is correlated with the deletion status of *CDKN2A* (*p16*) FISH in MPM [8, 9]. MTAP can be also a useful immunohistochemical marker for distinguishing between benign and malignant proliferation of mesothelial cells [8–10].

4 Differential Diagnosis of Malignant Mesothelioma and Non-neoplastic Reactive Mesothelial Hyperplasia

The submesothelial basal lamina is immature, leading to allow non-neoplastic mesothelial cells to infiltrate into the submesothelial fibrous tissues. Non-neoplastic mesothelial cells can be also entrapped in organizing tissues formed during serosal inflammatory processes. So, it is difficult to distinguish between genuine invasion of MPM cells and inflammation-induced infiltration and entrapment of non-neoplastic mesothelial cells. Moreover, since non-neoplastic mesothelial cells growing on the serosal surface are often strongly atypical, benign and malignant mesothelial proliferations on the serosal surface are hardly distinguished morphologically [3].

CDKN2A (*p16*) homozygous deletion is found only in MM cells and not in all non-neoplastic mesothelial cells by FISH, indicating that the specificity of *CDKN2A* (*p16*) homozygous deletion is 100% [19]. On the other hand, the sensitivity of *CDKN2A* (*p16*) homozygous deletion is approximately 45% to 85% for EM and the sensitivity is much higher for SM than EM and is up to 100%. The specificity and the sensitivity of MTAP IHC for detection of *CDKN2A* (*p16*) homozygous deletion by FISH have been reported to be 98% and 78%, respectively, indicating that MTAP IHC is a reliable surrogate for *CDKN2A* (*p16*) FISH in evaluating malignancy of mesothelial cells [8, 9].

Loss of BAP1 expression is detected only in MM, but not in non-neoplastic mesothelial cells, by IHC. The specificity of BAP1 loss is considered to be 100% [19], even if germline mutations of the *BAP1* gene have been reported [20]. Surprisingly, BAP1 loss is also found in mesothelial cells growing as a monolayer on the pleural surface, showing that these mesothelial cells are neoplastic (Fig. 9.2i–l). Based on these findings, a new concept of mesothelioma *in situ* has been proposed [21]. Its criterion consists of the following: (1) mesothelial cells with BAP1 loss on the pleural surface are proliferating as a monolayer, (2) no invasion is observed by images and direct observation of the pleural and the peritoneal cavities at the time of biopsy, and (3) no invasive mesothelioma has occurred for at least 1 year after biopsy, supporting that invasive tumors are unlikely to be present in areas other than the biopsy site at the time of biopsy. Thus, BAP1 is a useful immunohistochemical marker to evaluate malignancy of proliferating mesothelial cells, but it should be noted that the sensitivity of BAP1 loss is approximately 60–70% in EM and 15% in SM.

Since invasion cannot be assessed with specimens used in effusion cytology, the definitive diagnosis of MPM by effusion cytology has not been accepted so far. However, the application of BAP1 IHC and MTAP IHC to cellblock sections has made it possible to evaluate malignancy of mesothelial cells in pleural effusion, leading to the confident diagnosis of MPM by pleural effusion cytology [10].

5 Differential Diagnosis of Desmoplastic Mesothelioma and Fibrous Pleuritis

The diagnosis of DM is very important due to its poor prognosis, but it is difficult to distinguish morphologically DM from benign fibrous pleuritis. On the other hand, *CDKN2A* (*p16*) homozygous deletion is detected in all SM including DM and no deletion in fibrous pleuritis, indicating the specificity of *CDKN2A* (*p16*) homozygous deletion is 100% and the sensitivity is 100% [22]. IHC detecting MTAP loss has been reported to be effective in distinguishing SM from fibrous pleuritis (Fig. 9.2h) [23], but the utility of MTAP IHC in diagnosis limited to DM is unclear. IHC detecting BAP1 loss does not appear to help separate DM from fibrous pleuritis since the frequency of BAP1 mutations is low in SM including DM. Therefore, FISH detecting *CDKN2A* (*p16*) homozygous deletion is a particularly important diagnostic technique to discriminate between DM and fibrous pleuritis.

6 Conclusion

It is important to identify the histological subtype, EM, SM, and BM, of MPM, since the histological subtype is a good predictor of prognosis and since tumors and diseases to be distinguished from MPM vary by the histological subtype. The pathological diagnosis of MPM is basically performed by confirmation of mesothelial

origin and evaluation of malignancy. Immunohistochemical markers, such as mesothelial-related markers (positive markers) and other organ-specific markers (negative markers), play an important role to determine tumor origin. IHC using immunohistochemical markers such as BAP1 and MTAP and FISH detecting *CDKN2A* (*p16*) homozygous deletion are indispensable to evaluate malignancy of proliferating mesothelial cells. BAP1 IHC and MTAP IHC have made it possible to find early-stage MPM and to determine malignancy of mesothelial cells in pleural effusion even if the invasion cannot be confirmed histopathologically. In summary, the development of immunohistochemical markers has greatly advanced the differential diagnosis of MPM.

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Chapter 10
Cytopathologic Diagnosis of Mesothelioma: Can We Diagnose Mesothelioma Based on Fluid Cytological Materials Without Biopsy?

Kazuki Nabeshima, Makoto Hamasaki, Yoshiaki Kinoshita, Masayo Yoshimura, and Shinji Matsumoto

Abstract Early diagnosis and initiation of treatment lead to longer survival in malignant pleural mesothelioma (MPM). Since more than 80% of MPM cases start with pleural effusions, cytologic diagnosis with effusion smears is critical for improved clinical outcomes. A three-step approach is usually undertaken for the diagnosis of MPM. The first step is to detect atypical mesothelial cells; the second step is to verify its mesothelial origin using immunohistochemistry (IHC); the third step is differentiating MPM cells from reactive mesothelial hyperplasia (RMH) or reactive mesothelial cells (RMC). Genomic-based ancillary assays that can effectively distinguish MPM from RMH/RMC, including BRCA-1 associated protein-1 (BAP1) and methylthioadenosine phosphorylase (MTAP) IHC and 9p21 and neurofibromin 2 (*NF2*) fluorescence in situ hybridization (FISH), have recently been developed. These ancillary assays enable the confirmation of the neoplastic and malignant nature of atypical mesothelial cells detected in the cytologic preparations or that of a single layer of surface mesothelial cells found in the in situ phase of mesothelioma. However, cautious interpretation and familiarity with potential challenges of data interpretation while assessing BAP1 and MTAP IHC results in cell blocks are warranted.

Keywords BAP1 · MTAP · CDKN2A (p16) · NF2 · Cytology · Mesothelioma

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1 Introduction

The incidence of pleural mesothelioma shows a strong correlation with asbestos exposure. Cumulative asbestos consumption is a significant predictor of death from malignant mesothelioma [1]. Mesothelioma typically occurs 30–40 years after asbestos exposure. In Japan, asbestos import increased during the period between the 1960s and 1990s; thus, deaths from pleural mesothelioma have rapidly increased since 2000, with more than 1500 deaths reported in 2017. Based on historical asbestos usage trends, a peak in pleural mesothelioma incidence is expected in the fourth decade of the twenty-first century, and approximately 100,000 cumulative deaths are estimated [2].

The prognosis of malignant pleural mesothelioma (MPM) is still poor; the median survival is approximately 14 months even after surgery and the 5-year survival is just 12% [3]. However, starting treatment at stage I, in which the disease is restricted to the unilateral pleura, leads to longer survival (30 months of median survival) [4]. Accurate pathological diagnosis in pleural biopsies or pleural effusion smears is essential for early initiation of treatment. Since more than 80% of MPM cases start with pleural effusions, effective cytologic diagnosis with effusion smears is important for improving clinical outcomes in MPM.

2 Diagnosis of MPM Using Effusion Cytology

The WHO classification of tumors (2015) categorized malignant mesothelioma into epithelioid, biphasic, and sarcomatoid mesothelioma for prognostic relevance and to guide treatment decisions [5]. Sarcomatoid mesothelioma shows the shortest survival, with 4 months of median survival, while epithelioid mesothelioma is characterized by 14 months of median survival. The use of effusion smear cytology is limited to epithelioid mesothelioma because only epithelioid mesothelioma cells derived from either epithelioid mesothelioma or the epithelioid portions of biphasic mesothelioma can be desquamated into pleural effusions. This chapter will provide a comprehensive overview of the current state of application of effusion smear cytology in the diagnosis of epithelioid mesothelioma.

The diagnosis of MPM using effusion cytology specimens has been controversial, and the International Mesothelioma Interest Group (IMIG) guidelines for histopathology published in 2009 and 2013 did not recommend this approach to diagnosis [6, 7]. However, a recent survey of 55 laboratories revealed that approximately two-thirds use an effusion specimen to provide a definitive diagnosis of mesothelioma [8]. Furthermore, as per the 2015 IMIG guidelines for the cytopathologic diagnosis of epithelioid or mixed-type malignant mesothelioma, while cytomorphological detection of MPM by an experienced cytopathologist is feasible, a definitive diagnosis should be supported by ancillary techniques [9], such as those described in the subsequent sections.

Diagnosing MPM is usually a three-step process. The first step is to detect atypical mesothelial cells; the second step is to verify their mesothelial origin using immunohistochemistry (IHC); the third step is differentiating MPM from reactive mesothelial hyperplasia (RMH) or reactive mesothelial cells (RMC). The atypical cells are morphologically characterized by a greater number and larger size of cells and cell clusters with lobulated or flower-like contours [10], in addition to multinucleation, hump-like cellular processes, cell-in-cell engulfment, thick basophilic cytoplasm, and blurring of cell contours [11]. Furthermore, the cytomorphological characteristics of MPM cells that harbor the genetic alterations frequently associated with mesothelioma have also been elucidated. These features include the presence of larger clusters consisting of >10 cells, more cell-in-cell pattern imparting the appearance of a hump-like cytoplasmic protrusion on one edge of a small cluster, and greater multinucleation (i.e., > 2 nuclei), which is statistically more commonly seen in cases with *p16* homozygous deletion or BRCA-1 associated protein-1 (BAP1) loss [12]. In routine clinical practice, it is also recommended that attempts to establish a malignant diagnosis should be undertaken when the sample contains exceptionally large numbers of mesothelial cells even when the nuclear atypia is less apparent [9]. In such cases, genomic-based ancillary assays described below will often confirm the presence of malignant cells.

The verification of the mesothelial origin of the atypical MPM-like cells identified in cytologic preparations entails using IHC for their differentiation from carcinoma cells that could have metastasized to the pleura. The guidelines suggest that positivity for at least two mesothelial markers such as calretinin, WT1, podoplanin (D2–40), and HEG-1, and negativity for at least two mesothelial-exclusion markers (carcinoma-favoring markers) such as claudin-4, CEA, TTF-1, Ber-EP4, MOC31, and MUC4, provide confirmatory evidence for the mesothelial origin [13]. Claudin-4 and MUC4 are reported to have 100% specificity for differentiating carcinoma cells from mesothelioma cells [14, 15], although claudin-4 has higher sensitivity than MUC4. On the other hand, HEG-1 is reported to have 100% specificity in distinguishing mesothelioma cells from adenocarcinoma cells, while some squamous carcinoma cells and most sarcoma cells also show HEG-1 reactivity [16].

Deep tumor cell invasion into the adipose tissue is a good indicator of malignancy and is a clear distinguisher of MPM from RMH or RMC. However, in small biopsies with shallow stromal invasion or in cytologic preparations, determining malignancy and its differentiation from reactive hyperplasia or cells is sometimes challenging. Genomic-based ancillary assays, including loss of BAP1 expression (BAP1 loss) detected by IHC and 9p21 homozygous deletion detected by fluorescence in situ hybridization (FISH), have been shown to be very useful for this differentiation with 100% specificity [13, 17]. In addition, we have recently reported that IHC detection of loss of methylthioadenosine phosphorylase (MTAP) expression (MTAP loss) is a reliable surrogate assay for FISH detection of 9p21 homozygous deletion [18]. The utility of MTAP IHC in a combination with BAP1 IHC was also confirmed in a multi-institutional study that revealed excellent interobserver agreement and interlaboratory reproducibility: loss of MTAP showed approximately 80% sensitivity and nearly 100% specificity for detecting 9p21 homozygous

Table 10.1 A combination of MTAP IHC, BAP1 IHC, and 9p21 FISH for distinguishing MPM from RMH in tissue sections

Assay	Total	MPM		RMH		Sensitivity (%)	Specificity (%)
		HD/ loss	No HD/ loss	HD/ loss	No HD/ loss		
BAP1 IHC	96	54	42	0	37	56.3	100
MTAP IHC	86	49	37	0	37	57.0	100
9p21 FISH	184	129	55	0	37	70.1	100
Combination							
BAP1 IHC/9p21 FISH	96	81	15	0	37	84.4	100
BAP1 IHC/MTAP IHC	86	71	15	0	37	82.6	100

MPM, malignant pleural mesothelioma; RMH, reactive mesothelial hyperplasia; HD, homozygous deletion; BAP1, BRCA-1 associated protein-1; MTAP, methylthioadenosine phosphorylase; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization

deletion [19]. Although the sensitivity of either of these assays on their own is insufficient, the combined use of 9p21 FISH (or MTAP IHC) and BAP1 IHC enhances the sensitivity (Table 10.1). More recently, we reported that neurofibromin 2 (*NF2*) deletion as detected by FISH is characterized by hemizygous loss in MPM, as has been shown in peritoneal mesothelioma [20]. Hemizygous *NF2* loss showed 53.2% sensitivity and 100% specificity in differentiating MPM from RMH. In that cohort, a triple combination of *NF2* FISH, 9p21 FISH, and BAP1 IHC yielded greater sensitivity (100%) than that detected for either diagnostic assay alone or either combination of two of these assays [21]. Thus, *NF2* FISH is also effective for distinguishing MPM from RMH in a combination with other diagnostic assays.

3 Genomic-Based Assays and Malignant Mesothelioma In Situ

Asbestos interacts with mitotic spindles to cause chromosome missegregation during mitosis, which leads to aneuploidy [22]. Cytogenetic and comparative genomic hybridization (CGH) analyses have identified frequent losses in chromosome 3p, 9p, and 22q regions upon asbestos exposure. The genes linked with the development of mesothelioma in such regions were determined to be *BAP1* in 3p region, *CDKN2A* (*p16*) in 9p region, and *NF2* in 22q region [23–26]. Recently, next-generation sequencing (NGS) analysis also confirmed these genetic alterations [27].

The aforementioned ancillary assays were developed based on these genetic alterations and applied for the differentiation of MPM from RMH or RMC. *BAP1* gene is a tumor suppressor gene located in the 3p21 region and has a role in transcription factor regulation, chromatin modification, and double-strand DNA repair [28]. Somatic mutations in *BAP1* have been observed in approximately 60% of

MPM cases, and a biallelic loss of *BAP1* or inactivating mutations cause loss of normal nuclear staining detected by IHC [29]. This phenomenon is illustrated in Fig. 10.1a which shows the loss of BAP1 protein expression in nuclei of MPM cells, while inflammatory cells, which serve as internal positive controls, express BAP1 in their nuclei. According to a recent meta-analysis of over 1800 published mesothelial biopsy and cytology cases, IHC detection of nuclear BAP1 loss is 100% specific for malignant mesothelioma vs. RMH [30].

CDKN2A is also a tumor suppressor gene located in the 9p21 region and is involved in the negative regulation of the cell cycle. Homozygous deletion of *CDKN2A* in malignant mesothelioma was first reported in the early 1990s [24], and its detection by 9p21 FISH was revealed to be useful in the 2000s for diagnosing MPM [31, 32]. For differentiating MPM from RMH, 9p21 FISH shows a sensitivity of 45%–85% in epithelioid MPM and 67%–100% in sarcomatoid MPM [17]. None of the reported RMH cases have shown 9p21 homozygous deletion [17, 31–33], and therefore the detection of this deletion has 100% specificity for MPM vs. RMH differentiation. The 9p21 region is labeled with red fluorescence in FISH. Thus, 9p21 homozygous deletion is detected by the loss of two red signals, as shown in Fig. 10.1b. However, longer turnaround times, greater expense, and limited access to FISH technology and expertise compared to IHC have prompted interest in a reliable IHC-based surrogate assay for 9p21 FISH. The *MTAP* gene is located adjacent

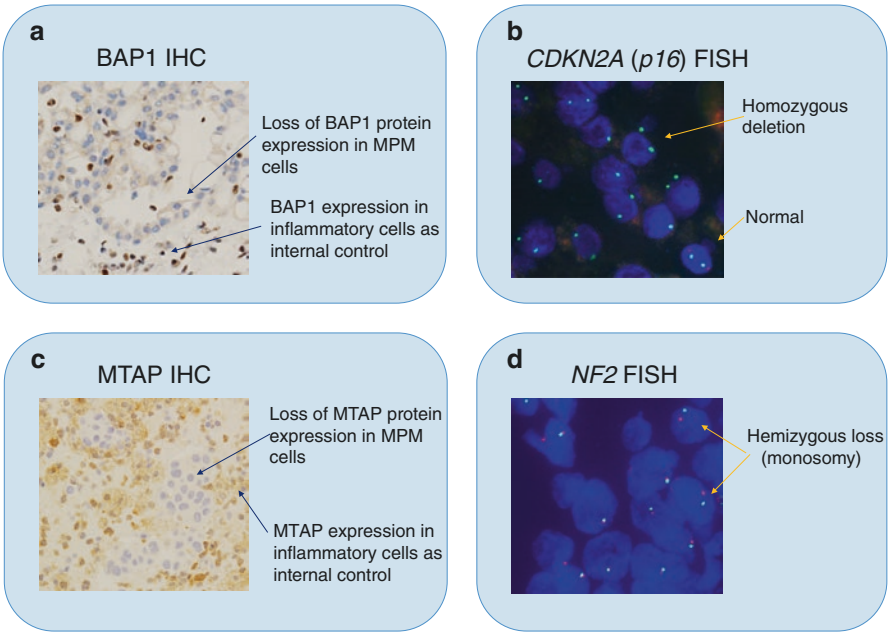

Fig. 10.1 Genomic-based ancillary assays. (**a**), BAP1 immunohistochemistry (IHC) showing loss of nuclear staining; (**b**), *CDKN2A* (*p16*) FISH showing homozygous deletion; (**c**), MTAP IHC showing loss of cytoplasmic staining; (**d**), *NF2* FISH showing hemizygous loss (monosomy)

to *CDKN2A* on chromosome 9p21, and early FISH studies showed *MTAP* co-deletion in up to 90% of pleural and peritoneal malignant mesothelioma cases with *CDKN2A* homozygous deletion [34]. However, *MTAP* FISH has not been adopted for routine clinical diagnostic use. Conversely, MTAP IHC using a monoclonal anti-MTAP primary antibody yielded excellent specificity and acceptable sensitivity for detecting *CDKN2A* homozygous deletion [18, 19]. Subsequent studies also showed that MTAP IHC could be reliably applied to cellblock preparations (Fig. 10.1c) [35] and in the differential diagnosis of sarcomatoid mesothelial lesions [36]. In reactive mesothelium, MTAP expression is retained and 9p21 FISH also shows a normal signal, while MTAP expression is lost and 9p21 FISH reveals homozygous deletion in an MPM lesion. Table 10.1 shows the data obtained for the application of MTAP IHC in the differentiation of MPM from RMH in tissue sections. In epithelioid MPM, MTAP IHC, BAP1 IHC, and 9p21 FISH showed 57, 56, and 70% sensitivities, respectively, which increased upon combining these assays—84.4% for a combination of BAP1 IHC and p16 FISH, and 82.6% for a combination of BAP1 IHC and MTAP IHC.

NF2 is a tumor suppressor gene located on chromosome 22q12.2 encoding for moesin-ezrin-radixin-like protein (merlin) [25, 26]. NF2 modulates the Hippo and mammalian target of rapamycin (mTOR) signal transduction pathways which regulate cell proliferation, growth, and apoptosis. Genetic alterations of *NF2* are the third most frequently observed in mesothelioma [25, 26]. Hemizygous *NF2* loss as detected by FISH was identified in 35% of peritoneal mesotheliomas [20]. Using *NF2* FISH, we reported that hemizygous loss was the dominant form of *NF2* deletion in MPM (Fig. 10.1d) and that *NF2* FISH in a combination with other diagnostic assays was effective for distinguishing MPM from RMH [21].

These genomic-based ancillary assays that can be applied to histological sections have greatly improved the ability to distinguish MPM from RMH, and new criteria for mesothelioma in situ (MIS) have recently been proposed using the assays [37, 38]. It has been postulated that malignant mesothelioma also has an in situ phase as observed for other types of epithelial malignancies. In the early 1990s, Whitaker et al. [39] first suggested that MIS can possibly be diagnosed histologically. They presented cases lacking a grossly visible tumor in which atypical mesothelial cells proliferated as a single layer or small papillary lesions on the pleural surface, however, accompanied by underlying invasive mesothelioma at a microscopical level. It was unclear whether the surface mesothelial cells are indeed MIS or represent the spread of the underlying invasive tumor along the pleural surface. Furthermore, because reactive mesothelial cells themselves can be atypical cytologically, it is quite difficult to differentiate mesothelioma cells of the in situ phase from reactive mesothelial cells based on routine cytomorphology. In such circumstances, Churg et al. [38] proposed to define MIS by the presence of a single layer of surface mesothelial cells exhibiting a loss of BAP1 nuclear immunostaining, absence of evidence of tumor via either imaging and/or direct examination of pleura/peritoneum, and absence of invasive mesothelioma development for at least 1 year.

Thus, it is now widely accepted that a single layer of surface mesothelial cells, which cannot be morphologically differentiated from mesothelioma, are considered

mesothelioma cells when they clearly show BAP1 loss immunohistochemically. Similarly, atypical mesothelioma-like cells in smears or cell blocks can also be diagnosed as mesothelioma cells when they show a clear loss of BAP1 nuclear immunostaining. Furthermore, *CDKN2A/p16* homozygous deletion and *NF2* hemizygous loss can also be used for this diagnosis because they also show 100% specificity in distinguishing MPM from RMH or RMC.

4 Application of Genomic-Based Assays to the Cytologic Diagnosis of MPM

We have applied a combination of BAP1 and MTAP IHC and 9p21 FISH to cell blocks of pleural effusions [35]. BAP1 loss was determined by loss of nuclear staining, while MTAP loss was determined by loss of cytoplasmic staining (Fig. 10.2). Generally, loss of MTAP occurs in both the nucleus and the cytoplasm. However, in certain cases, only nuclear or cytoplasmic MTAP expression is lost. In such cases, the cytoplasmic expression has been found to correlate with 9p21 homozygous deletion. Thus, a loss of cytoplasmic MTAP expression should be interpreted as a

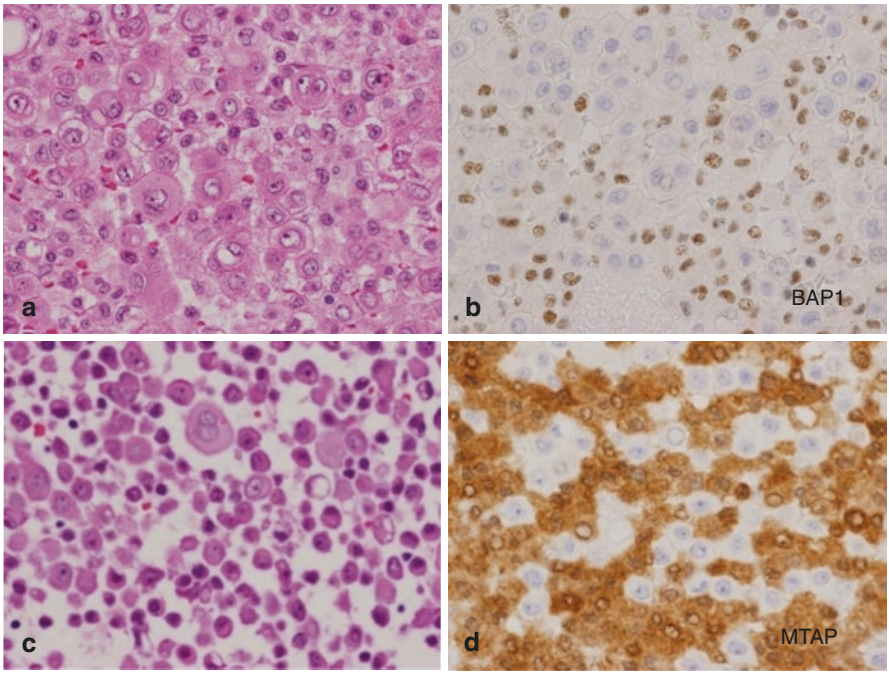

Fig. 10.2 BAP1 loss and MTAP loss in cell blocks. BAP1 loss was determined by loss of nuclear staining, while MTAP loss was determined by loss of cytoplasmic staining. (**a**) and (**c**), Hematoxylin and eosin staining; (**b**), BAP1 immunostaining; (**d**), MTAP immunostaining

Table 10.2 Application of a combination of MTAP IHC, BAP1 IHC, and 9p21 FISH for differentiating MPM cells from RMC in cell blocks

	MPM (n = 90)		RMC (n = 31)			
Assay	Loss	Normal	Loss	Normal	Sensitivity (%)	Specificity (%)
BAP1 IHC	52	28	0	31	65.0	100
MTAP IHC	37	36	0	31	50.1	100
9p21 FISH	51	39	0	31	56.7	100
Combination						
BAP1 IHC/9p21 FISH	73	7	0	31	91.3	100
BAP1 IHC/MTAP IHC	65	8	0	31	89.0	100

MPM, malignant pleural mesothelioma; RMC, reactive mesothelial cells; HD, homozygous deletion; BAP1, BRCA-1 associated protein-1; MTAP, methylthioadenosine phosphorylase; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization

loss of MTAP expression regardless of the nuclear MTAP expression status. The cytoplasmic MTAP staining loss yielded 84.1% sensitivity and 100% specificity in correlating with FISH findings of 9p21 homozygous deletion, with an excellent kappa coefficient (k = 0.80) [40].

The results for the application of genomic-based assays in the differentiation between MPM cells and RMC in cell blocks are shown in Table 10.2. All assays had 100% specificity for the differentiation of MPM from RMH, as seen in tissue sections. Compared with individual assays, the sensitivity increased to 91.3% in a combination of BAP1 IHC and 9p21 FISH, and to 89% in a combination of BAP1 and MTAP IHC. Thus, a combination of either BAP1 and MTAP IHC or BAP1 IHC and 9p21 FISH effectively distinguishes MPM cells from RMC in cell blocks. Additionally, NF2 FISH can also be applied to cytologic preparations and is useful in cases wherein both BAP1 and MTAP are retained along with a normal pattern of 9p21 FISH, and the hemizygous loss of *NF2* is the only genetic aberration (data not shown).

However, the interpretation of MTAP and BAP1 IHC in cell blocks presents some unique challenges. While the interpretation of IHC is straightforward when MPM cells form clusters in cell blocks, it becomes complicated when MPM cells are single and scattered. In this latter case, it is difficult to determine whether stained cells are reactive or neoplastic in immunostained sections. In such cases, double immunostaining for EMA and MTAP or BAP1 with the assessment of MTAP or BAP1 staining only in probably neoplastic EMA positive cells is recommended. When the double immunostaining fails or is technically infeasible, a conclusive diagnosis cannot be made. Similarly, no diagnostic conclusions can be drawn from analysis when internal positive control cells such as histiocytes and lymphocytes do not stain positive for BAP1 or MTAP.

Finally, based on our findings and the reported literature in this field, we would like to propose the diagnostic workflow for MPM (Fig. 10.3). The overtly malignant morphology such as that seen with fat invasion by tumor cells results in the diagnosis of MPM. However, in cases with ambiguous histo- or cytomorphology, IHC

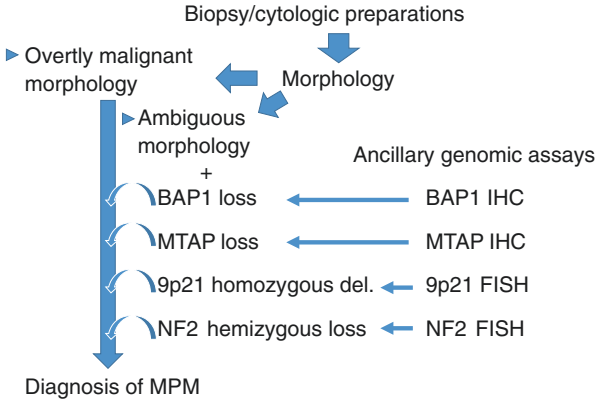

Fig. 10.3 Diagnostic workflow for malignant pleural mesothelioma (MPM). In the case of ambiguous morphology, ancillary genomic assays effectively help differentiate MPM from reactive mesothelial hyperplasia or cells

detection of BAP1 or MTAP loss or FISH detection of 9p21 homozygous deletion or *NF2* hemizygous loss, support the diagnosis of MPM.

5 Conclusions

Even in cytologic preparations, the defining cytomorphology of MPM cells in conjunction with immunostaining enables the detection of potential MPM cells, which are then definitively differentiated from RMC using genomic-based ancillary assays. However, it is prudent to exercise caution while interpreting IHC results for BAP1 or MTAP loss in cell blocks and be familiar with potential challenges associated with such interpretation.

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Chapter 11
Circulating Tumor Cells in Mesothelioma: What Is the Role of Liquid Biopsy in Clinical Practice of Pleural Mesothelioma?

Kazue Yoneda and Fumihiro Tanaka

Abstract Liquid biopsies may overcome the limitations of traditional tissue biopsies. Circulating tumor cells (CTCs), tumor cells that are shed from the primary tumor and circulate in peripheral blood, can be useful as a surrogate of micrometastasis. Cell-based liquid biopsies to detect CTCs have a variety of advantages over cell-free liquid biopsies. However, isolation and detection of rare CTCs contaminated among a large number of normal hematologic cells remain a technical challenge. In fact, the "CellSearch" system is the only approved system for clinical use, but our previous studies have indicated that it provided insufficient sensitivity in the detection of CTCs in patients with thoracic malignant tumors including malignant pleural mesothelioma (MPM). Accordingly, we have developed a novel microfluidic CTC-capture and detection system named "universal" CTC-chip with a unique advantage that any antibody to capture CTCs can be easily bound to the chip, and have shown that a variety of tumor cells spiked are captured with the "universal" CTC-chip system. The novel CTC-chip system provides new insight into not only the detection of CTCs but also further molecular analysis of CTCs, which may lead to realizing "precision medicine" in MPM.

Keywords Liquid biopsy · CTCs: circulating tumor cells · Microfluidic device Podoplanin

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1 Introduction

Pathological diagnosis and molecular analysis, which are essential for treatment decision-making of a cancer patient, have been generally performed on a biopsied tumor tissue. However, tissue biopsies, either of a primary site or of a metastatic site, are usually associated with some risks. In addition, a tissue sample may not represent true pathological characteristics or molecular profiles, due to tumor heterogeneity both within a tumor and between different tumor sites in a patient. More importantly, a molecular profile may be altered after the initiation of a treatment, which may cause acquired resistance to the treatment [1, 2]. In clinical practice, however, longitudinal tissue sampling to monitor the emergence of acquired resistance, as well as the alteration in molecular profile, can be performed only in selected patients because tumor tissues are not easily accessible, and repeated biopsies are associated with higher risks. In contrast to tissue sampling, peripheral blood sampling can be easily and repeatedly performed [3–5]. Accordingly, a variety of blood-based biopsies, referred to as "liquid biopsies," have been studied to overcome limitations of tissue biopsies, as entire tumor heterogeneity can be captured in real time by "cell-free" or "cell-based" liquid biopsies [5, 6].

Fragmented DNA, originated from tumor cells undergoing necrosis or apoptosis, can be found in the serum or plasma, and tumor-specific genes contained in circulating cell-free DNA were detected after amplification by polymerase chain reaction (PCR). "Cell-free" biopsies have an advantage of higher sensitivity, especially achieved with the recent advance of technologies such as digital PCR or next-generation sequencing (NGS), and many studies have revealed that "cell-free" liquid biopsies provide high sensitivity in the detection of tumor-specific gene mutations in advanced stages of a variety of malignant tumors (Fig. 11.1) [5–9].

"Cell-based" liquid biopsies, detection of circulating tumor cells (CTCs) that are shed from the primary tumor and circulate in the peripheral blood, are more challenging, because of technical difficulty in the capture of rare tumor cells contaminated in a large number of normal hematologic cells. However, as compared to cell-free liquid biopsies, cell-based methods potentially have various advantages as follows: (1) morphological confirmation of CTCs, (2) quantitative analysis of CTCs, and (3) isolation of CTCs for further analysis methods genetic analysis or immunostaining to evaluate the expression of specific antigens (i.e., programmed death-ligand 1 [PD-L1], a potential biomarker to predict the efficacy of anti-PD-1/PD-L1 antibodies) [5].

Therefore, many systems to detect CTCs have been developed, but CTCs had not been established as a clinical biomarker mainly due to lack of reproducibility and accuracy [10, 11]. The CellSeach (Veridex LLC., Raritan, NJ), an automated immuno-magnetic isolation system of CTCs, is the only approved CTC-detection system for clinical use. In the "CellSearch" system, an antibody against the epithelial cell adhesion molecule (EpCAM) is employed to capture CTCs, because the EpCAM, a pan—epithelial marker, is abundantly expressed on the surface of tumor cells of epithelial cell origin. The most important advantage is its higher reproducibility [12, 13]. We have previously investigated the clinical significance of CTC in

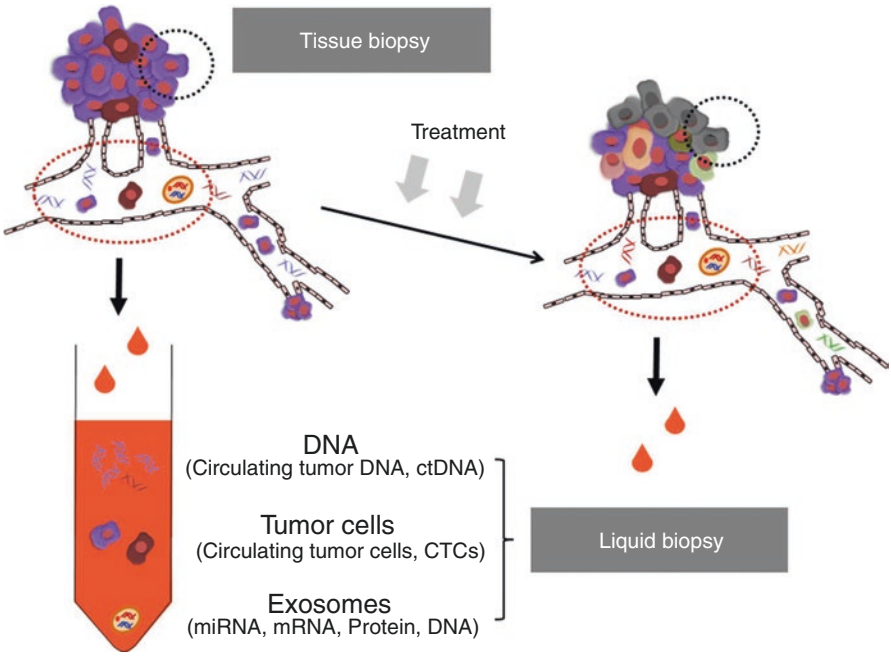


Fig. 11.1 ([5] Surg Today Fig. 2) Tissue biopsies versus liquid biopsies. Pathological and molecular information at the biopsy site of a tumor can be obtained with tissue biopsies. Peripheral blood may contain tumor cells as well as tumor-derived DNA and extracellular vesicles (exosomes) that come from all tumors in the whole body, so the heterogeneous tumor characteristics can be non-invasively monitored with liquid biopsies. *DNA* deoxyribonucleic acid, *RNA* ribonucleic acid

thoracic malignancies such as primary lung cancer or malignant pleural mesothelioma (MPM). Especially in MPM, as an invasive pleural biopsy is necessary for diagnosis, a less invasive marker such as CTC is expected to not only mass screening in high-risk populations with asbestos exposure but also materials for the "precision medicine." Accordingly, we examined the clinical implication of CTCs in MPM, and we have tried to develop a novel CTC detection system with higher sensitivity.

2 Clinical Significance of CTCs Detected with the CellSearch in Thoracic Malignancies

The CellSearch system may be useful for predicting the tumor progression and prognosis as well as the therapeutic efficacy in primary lung cancer [14, 15]. In a clinical study of 125 patients with primary lung cancer, the CellSearch provided a significant diagnostic performance in the prediction of distant metastasis, but failed to detect CTCs in 31.0% (9/29) of patients with clinically apparent distant metastasis [14].

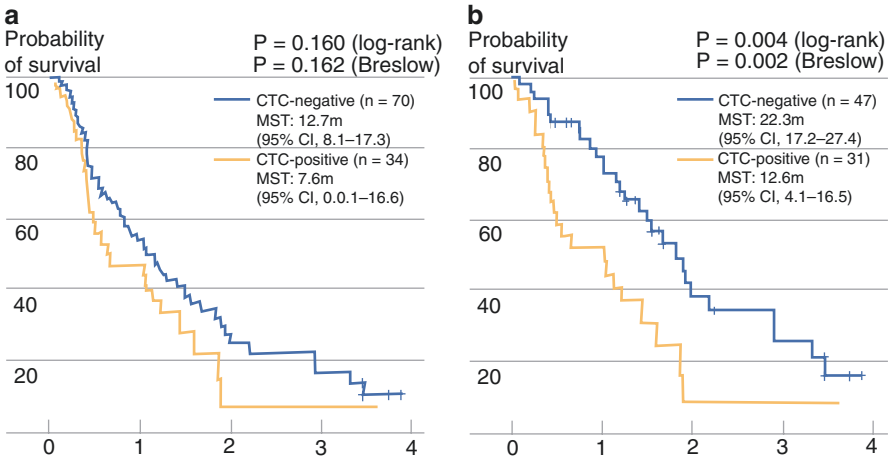


Fig. 11.2 ([16] Ann Surg Oncol Fig. 4) Survival curves according to CTC count in MPM. (**a**) Survival curves according to CTC count in all MPM patients, (**b**) survival curves according to CTC count in epithelioid-type MPM patients. *MST* mean survival time

In a clinical study of MPM, CellSearch-CTC was useful for the discrimination of MPM and nonmalignant diseases such as pleuritis. In addition, CTC-positive patients were significantly poor prognosis, especially in epithelioid MPM (Fig. 11.2). Although these results indicate the clinical usefulness of CTCs in MPM, the CTC-positive (more than 1 CTC per 7.5 mL of peripheral blood) rate was low at 32.7% (34/104), and it was suggested the sensitivity of detecting CTCs by "CellSearch" is not sufficient [16].

The most important reason for the low sensitivity is that the CellSearch can principally capture tumor cells with EpCAM expression [12]. MPM, originating from the mesothelium, not from the epithelium, rarely or weakly express EpCAM, and may not be captured with an EpCAM-dependent system such as the CellSeach. These results clearly indicate that more sensitive systems to detect CTCs, regardless of EpCAM expression status, are needed for clinical use of "cell-based" liquid biopsies.

3 Detection of CTCs with "CTC-Chip"

3.1 Development of CTC-Chip

CTC-chip is a novel microfluidic platform for detecting CTCs. Nagrath and coworkers have developed a CTC-chip, consisting of an array of 78,000 micro-posts coated with anti-EpCAM antibodies, and CTCs are captured by the interaction of these

cells with the EpCAM-coated micro-posts under laminar flow conditions [17, 18]. According to the initial report, the CTC-chip achieved a higher sensitivity in identification of CTCs; higher numbers of CTCs (5–1281 CTCs per mL) with approximately 50% purity were isolated in 115 of 116 (99%) blood samples taken from patients with a variety of malignant tumor including lung, prostate, pancreatic, breast, and colon cancer. In addition, even in early-stage disease, CTCs were detected in 7 of 7 prostate cancer patients [17]. The high sensitivity and specificity suggest that CTC-chip is a promising tool for the detection of CTCs, but no additional study to confirm or validate its high performance has been reported.

A novel polymeric CTC-chip is composed of UV light-curable resins was developed by Ohnaga and coworkers [19]. The novel CTC-chip provides a variety of advantages over the "original" CTC-chip as follows: lower cost, higher durability, and improved transparency. In addition, the chip surface is made reactive by incorporation of resins having an epoxy group, any antibody easily can conjugate to the chip, so we named "universal" CTC-chip. The "universal" CTC-chip potentially captures not only EpCAM-positive CTCs but also EpCAM-negative CTCs by conjugating an antibody against a specific antigen expressing on tumor cells; MPM cells expressing podoplanin can be captured by an anti-podoplanin antibody conjugated to the CTC-chip (Fig. 11.3).

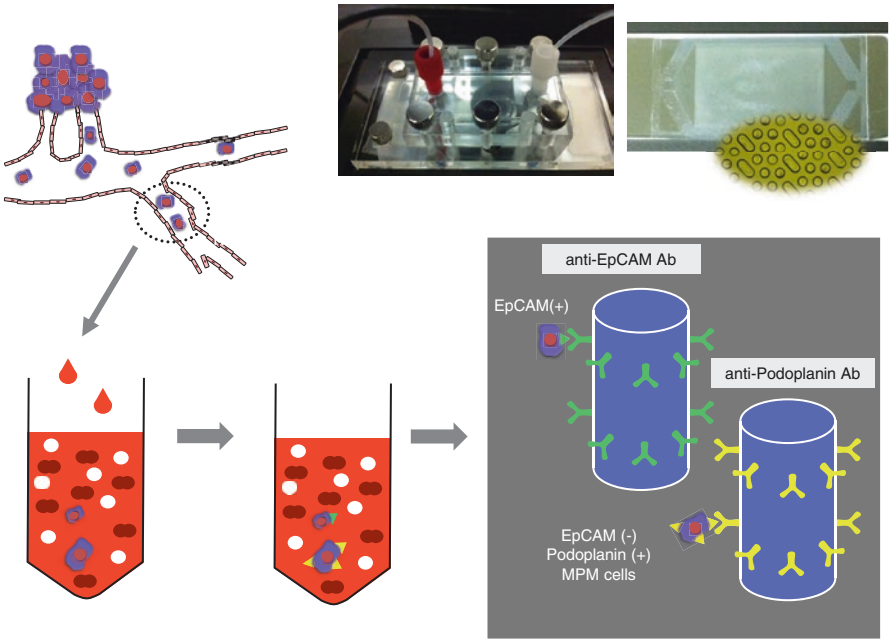


Fig. 11.3 "Universal" CTC-chip system. The "universal" CTC-chip potentially captures not only EpCAM-positive CTCs but also EpCAM-negative CTCs by conjugating an antibody against a specific antigen-expressing on tumor cells

3.2 Isolation and Detection of Tumor Cell Lines with CTC-Chip

We employed the novel "universal" CTC-chip, and conducted a series of experiments to examine the efficacy in capturing CTCs using tumor cell lines with or without EpCAM expression as follows [20–23].

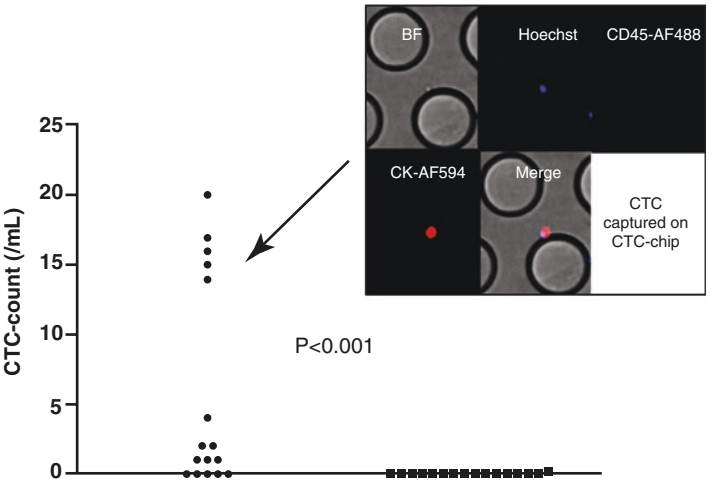
First, we examined the EpCAM expression status of cell lines with immunochemical staining and with flow cytometry. PC-9, a human lung adenocarcinoma cell line, strongly expressed EpCAM. In contrast, ACC-MESO-1, ACC-MESO-4, NCI-H226, MSTO-211H, and NCI-H28 a human MPM cell line, did not express EpCAM. ACC-MESO-4 and NCI-H226 strongly expressed podoplanin, ACC-MESO-1 moderately expressed podoplanin, and MSTO-211H and NCI-H28 did not express podoplanin.

Next, we examined the capture efficacy of CTC-models (tumor cells spiked in the blood sampled from a healthy volunteer). The CTC-chip was first incubated with a goat anti-mouse IgG antibody and then incubated with an antibody to capture tumor cells, either an anti-EpCAM antibody (clone HEA125) or an anti-podoplanin antibody (clone E1 or NZ-1.2). A 1-mL of cell suspension sample containing 100 or 500 tumor cells, labeled with CFSE, was applied to the CTC-chip coated with the anti-EpCAM antibody (EpCAM-chip) or the anti-podoplanin antibody (podoplanin-E1 or podoplanin-NZ-1.2-chip). PC-9 cells expressing strong EpCAM cells were effectively captured by the EpCAM-chip with approximately 90% capture efficiency, and not by the podoplanin-E1-chip. Mesothelioma cells were not captured by the EpCAM-chip [20]. ACC-MESO-4 and NCI-H226 cells, showing strong podoplanin expression, were effectively captured by the podoplanin-E1-chip with capture efficiency of 84.1% and 76.3%, respectively. MSTO-211H and NCI-H28 did not express podoplanin were not captured by the podoplanin-chip (approximately less than 10%) [22]. The podoplanin-NZ-1.2-chip achieved more effective capture with 97.9% and 97.6% capture efficiency with ACC-MESO-4 and NCI-H226 cells [23].

Finally, CTCs were Immuno-fluorescently stained on the CTC-chip. Each cell with a round to oval morphology, a Hoechst33342-positive nucleus, positive staining for CK in the cytoplasm, and negative staining for CD45 was judged as a CTC [22, 23].

4 Clinical Implications of CTCs Detected with the CTC-Chip in MPM

CTC-chip was showed superior cell detection efficiency over CellSearch. A total of 16 peripheral blood samples drawn from 15 patients with MPM (11 samples from 11 patients with epithelioid type, 4 samples from 3 patients with biphasic type, and



		CTC-chip	CellSearch
CTC-count	median	1.5/ml	0/7.5ml
	range	0-20/ml	0-1/7.5ml
CTC-positive (CTC-count≥1)		11/16 (68.8%)	1/16 (6.3%)

Fig. 11.4 ([22] Cancer Sci Fig. 4) Circulating tumor cell (CTC) count in patients with malignant pleural mesothelioma (MPM) with the CTC-chip and CellSearch. Among cells captured on the CTC-chip, only cells with positive nuclear staining by Hoechst33342 (blue), positive cytoplasmic staining with cytokeratin (CK) by Alexa594 (red), and negative CD45 staining by Alexa488 (green) were judged as tumor cells. Blood samples (1 and 7.5 mL) from each patient with MPM were applied to the podoplanin-chip and CellSearch, respectively. The CTC count is represented as the number of CTCs in 1 mL blood for the podoplanin-chip and that in 7.5 mL blood for CellSearch. *BF* bright field

1 sample from 1 patient with sarcomatoid type) were subjected to quantitative analyses for CTCs by the podoplanin-chip or by CellSearch. the CTC-positivity was significantly higher with the CTC-chip than with CellSearch (68.5% vs 6.3%, $P < 0.001$) (Fig. 11.4) [22].

We further analyzed additional blood samples drawn from a total of 25 patients with MPM. Overall, CTCs were detected in 16 patients (sensitivity, 64.0%), and there was no significant difference in the sensitivity to detect CTCs according to pathological subtypes of MPM (CTC-positivity, 58.8% [10/17] for epithelioid type, 75.0% [3/4] for biphasic type, and 75.0% [3/4] for sarcomatoid type; $P = 0.734$). As expected, the sensitivity was significantly higher in advanced stages of diseases (CTC-positivity, 0.0% [0/4] in stage IA, 42.9% [3/7] in stage IB, 56.3% [9/10] in stage IIIB, and 100.0% [4/4] in stage IV disease; $P = 0.003$) (Fig. 11.5). As stage IIIB and IV diseases are generally recognized as "unresectable" disease, a ROC curve analysis was carried out to determine the optimal cut-off value of CTC count in discrimination between "unresectable" disease (stage IIIB or IV disease) and

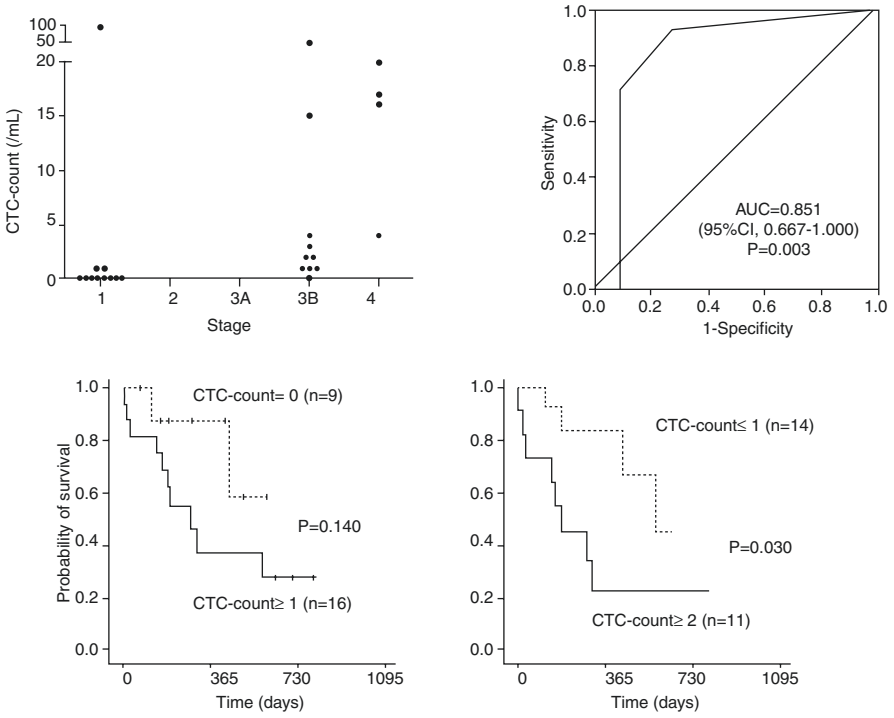


Fig. 11.5 ([22] Cancer Sci Fig. 5) Clinical implications of circulating tumor cells (CTCs) detected with the CTC-chip in malignant pleural mesothelioma (MPM). Top left panel, distribution of CTC count according to disease stage. CTCs were detected in peripheral blood sampled from 25 patients with MPM with the podoplanin-chip. The number of CTCs (CTC count) was significantly higher in advanced stages of disease (stages IIIB and IV). Top right panel, receiver operating curve to predict "unresectable" disease (stage IIIB or IV). CTC count provided a significant diagnostic performance in discrimination of "unresectable" (stage IIIB or IV) disease from potentially "resectable" disease. Bottom panels, overall survival curves according to CTC count. Patients with high-CTC tumor (CTC count ≥2) showed a significantly poorer prognosis than those with low-CTC tumor (CTC count, 0 or 1)

"resectable" disease (stage IIIA or earlier). The area under the ROC curve was 0.851 (95% confidence interval, 0.667–1.000), indicating a significant diagnostic performance of the CTC-test to predict "unresectable" disease in MPM (P = 0.003; Fig. 11.5). The optimal cut-off value of the CTC-count was estimated as "2" which provided a very high specificity (90.9%) with moderate sensitivity (64.3%).

When the cut-off value of "1" was used, the sensitivity was higher (92.3%) but the specificity was modest (72.7%). When patients were classified into high- and low-CTC patients according to the cut-off value of 2, high CTC (CTC-count ≥2) was significantly associated with a poor prognosis (Fig. 11.5) [22].

These results could indicate that the CTC-count evaluated with the novel CTC-chip system is potentially useful as a biomarker in the diagnosis and treatment of MPM patients, which should be validated in future prospective studies.

5 Conclusion

Although CTC-test as a "cell-based" liquid biopsy may be potentially useful for diagnosis or prognosis of MPM, insufficient sensitivity for early diagnosis, monitoring of clinical course, or predicting therapeutic effect.

Considering the clinical use of the CTC-chip system, the capture efficiency should be further increased, for which a variety of conditions including selection and concentration of each antibody attached to the chip, incubation time, and temperature for antibody coating, and flow rate in applying sample should be optimized. Another anti-podoplanin antibody, clone NZ-1.2 has a higher affinity than clone E1, achieved almost 100% of capture efficiency to podoplanin positive MPM cell lines, and more CTC were detected in clinical samples of MPM [23].

Another strategy to achieve higher performance in capturing CTCs is the use of multiple antibodies to capture tumor cells. Mesothelin or epidermal growth factor receptor (EGFR) were often expressed MPM cells, can be a target for capturing mesothelioma cells. In a preliminary study, podoplanin-negative HCI-H28 and MSTO-211H cells with EGFR expression can be effectively captured by the chip coated with an anti-EGFR antibody (unpublished data). The CTC-chip, when coated with an anti-EpCAM antibody, an anti-podoplanin antibody, and an anti-EGFR antibody, may effectively capture all tumor cells.

These results suggest that the "universal" CTC-chip system is a promising modality to capture a variety of EpCAM-negative tumor cells including those undergoing epithelial–mesenchymal transition (EMT) and those of non-epithelium origin such as mesothelioma cells.

In conclusion, cell-based liquid biopsies have a variety of advantages over cell-free liquid biopsies. Most importantly, cell-based liquid biopsies potentially provide the molecular characterization of tumor cells not only at the genomic level (e.g., genomic alterations in tumor cells) but also at the cellular level (e.g., expression of tumor-specific antigens on tumor cells). Effective capture and detection of CTCs remain a technical challenge, which can be overcome by the novel "universal" CTC-chip. In future studies, we will analyze the molecular profile of captured cells including gene mutations and expression of tumor-specific antigens.

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Part V
Molecular Genetics

Chapter 12
Recent Advances in the Genomic and Proteomic Researches on Mesothelioma: What Are Novel Insights into Mesothelioma Biology?

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Abstract Malignant mesothelioma is an aggressive tumor that has been associated with exposure to asbestos fibers. The discovery that germline heterozygous mutations of the gene encoding the deubiquitylase BRCA-associated protein 1 (BAP1) leads to inheritable higher susceptibility to mesothelioma underscores the relevance of gene x environment (GxE) interactions. Carriers of *BAP1* germline mutations are affected by the BAP1 cancer syndrome, a high penetrance Mendelian disorder, characterized by earlier development of mesothelioma and specific types of other cancers. Numerous next-generation sequencing (NGS) analyses have been recently conducted searching for both germline and somatic alterations in patients affected by mesothelioma and associated cancers, and their relatives. *BAP1* resulted in the more frequently germline mutated gene; however, other genes involved in DNA repair and homologous recombination were also identified. The pattern of chromothripsis, or chromosome staggering, which has been somatically identified in mesothelioma by several groups, may explain the frequent occurrence of noncontiguous biallelic genome alterations. Moreover, transcriptome studies in mesothelioma showed also the occurrence of fusion transcripts involving tumor suppressor genes. The complete knowledge of the genetic background associated with the GxE interactions involved in the pathogenesis of mesothelioma will be further improved by future genetic and genomic studies, allowing to develop better strategies for the prevention and treatment of this malignancy.

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Keywords Mesothelioma · *BAP1* · NGS · GxE interaction · Chromothripsis

1 Introduction

Malignant mesothelioma is an aggressive tumor whose pathogenesis is associated closely with occupational exposure to asbestos. The populations of workers handling asbestos, such as miners, manufacturing, or shipyard workers displayed a higher incidence of mesothelioma than the general population [1, 2].

The latency period between the exposure to mineral fibers to the development of asbestos-associated pleural mesothelioma is on average of 30–60 years [3]. Therefore, the incidence of mesothelioma is still increasing despite the legal bans on the use of asbestos in the Western countries at the end of the last century [4]. The majority of emerging countries are still using asbestos in their manufacturing activities, thus mesothelioma incidence in these counties is expected to keep increasing in the future [5].

Asbestos refers to a family of six mineral fibers that were used commercially until the 1970s and 1980s, which are classified into two subgroups: the amphiboles, a group of rod-like fibers including amosite, or brown asbestos, crocidolite or blue asbestos, anthophyllite, actinolite, and tremolite; and the serpentine group, consisting of chrysotile or white asbestos [6]. Exposure to the naturally occurring asbestos-like mineral fibers, such as erionite, antigorite, and others, as well as irradiation, account for further environmental risk factors for mesothelioma.

It has been observed that human mesothelial cells are particularly susceptible to cytotoxicity induced by asbestos, of which major mechanism of cell death appears to be in the form of necrosis rather than apoptosis. Then, a large amount of high mobility group box 1 (HMGB1) protein that belongs to the damage-associated molecular protein (DAMP) family, gets released by mesothelial cells, recruiting macrophages to sustain chronic inflammation [7]. Owing to the prolonged chronic inflammation microenvironment, surviving mesothelial cells accumulate genetic alterations after prolonged asbestos exposure. The accumulation of such genetic alterations might cause those mesothelial cells to develop mesothelioma after long latency [8]. However, the observation that among the workers with a long history of exposure to asbestos, only ~5% developed mesothelioma led to speculate that genetic component may also confer addition to occupational and environmental risks [5].

2 Germline Mutations of the *BAP1* Gene

About 20 years ago, Michele Carbone discovered apparent autosomal dominant transmission of mesothelioma susceptibility in some Turkish families, who have resided and have been exposed to erionite in the soil for a long time [9, 10].

Furthermore, Carbone and coworkers discovered germline mutations in the gene encoding the BRCA1-associated Protein 1 (BAP1), located in chromosome 3p21.3, in families with a high incidence of both pleural and peritoneal mesothelioma as well as uveal melanomas (UVMs), cutaneous melanoma, and clear cell renal carcinoma [11]. Subsequently, families of similar phenotypes with *BAP1* germline mutations have been reported in various ethnicities with an elevated risk of developing several other malignancies, such as cholangiocarcinoma, basal cell carcinoma, meningioma (reviewed in [12]). These findings established the concept of the "BAP1 cancer syndrome," as an autosomal familial cancer syndrome. An extended family with over nine generations inheriting mesothelioma, UVM, and other cancers since the 1700s established the inheritance mode of BAP1 cancer syndrome [11].

BAP1 encodes a nuclear ubiquitin carboxy-terminal hydrolase (UCH) functioning as a deubiquitinating enzyme. BAP1 is unique among UCH family members because of its long C-terminal tail, which contains two nuclear localization signals [13]. Both nuclear localization and deubiquitinating activity of BAP1 protein are postulated to be necessary for the maintenance of tumor suppressor activity [14]. BAP1 is implicated in the regulation of cell cycle, cellular differentiation, gluconeogenesis, chromatin remodeling, gene transcription, and DNA repair [12].

At the clinical level, the discovery of the BAP1 cancer syndrome emphasizes the necessity for genotyping the DNA of patients with mesothelioma for mutations, to determine the presence of germline mutations in the *BAP1* gene and other yet unidentified additional genes to acquire more complete information on the inherited predisposition to cancers like mesothelioma.

3 NGS Analysis in the Search for Germline Mutations in Other Genes

Several Next-Generation Sequencing (NGS) studies have been performed following the identification of *BAP1* in mesothelioma and other cancers to investigate germline variants individuals at risk of mesothelioma or in patients with this aggressive cancer [12]. Patients (n = 89) who developed pleural mesothelioma because of ascertained cumulative exposure to asbestos were screened for the presence of germline pathogenic truncating nonsense or frameshift variants (PTVs), by targeting 94 genes known for predisposition to cancer. *BAP1* germline PTVs were identified in four patients with mesothelioma, while germline PTVs were found also in *CDKN2A* or DNA repair genes. The asbestos exposure was significantly higher in patients with familial mesothelioma and PTVs in tumor suppressor genes than the patients with no germline variants in the 94 cancer-predisposing genes [15, 16].

A different approach, aimed at studying the inheritance of germline mutations of *BAP1* or other genes, was used to select a cohort of 79 individuals to be investigated. This population consisted of 52 unrelated probands with familial mesothelioma and their 27 first- and second-degree relatives, and was selected for

possible genetic predisposition, based on the following four criteria: (1) mesothelioma in first- or second-degree relatives; (2) diagnosis of cancers typical of *BAP1*$^{+/-}$carriers (uveal melanoma, cutaneous melanoma, clear-cell renal cell carcinoma) in the probands or at least one first- or second-degree relative; (3) family history of multiple cancers; and (4) early cancer onset less than 50 years old. *BAP1* Sanger sequencing and tNGS of more than other additional 50 cancer susceptibility genes were performed in this population. The results of this study showed that most of the patients were carriers of *BAP1*$^{+/-}$ with familial mesothelioma (43/79). Germline PTVs involving the following cancer susceptibility genes other than *BAP1* were also identified in this group: *ARID1A*, *ARID2*, *BAP1*, *CREBBP*, *KDR*, *MLH1*, *NCOR1*, *RAD50*, *RBM6*, *SETD2*, *SMARCA2*, *SMARCA4*, *SMARCE1*, *SMO*, *TP53*. Survival of 77 patients were compared with data from the mesothelioma in general, using dataset of the Surveillance, Epidemiology, and End Results (SEER) cohort (https://seer.cancer.gov), revealing a significant improvement of survival and earlier age at diagnosis (5 years and 54 years of age, respectively) in the selected population compared with the SEER cohort (8 months and aged 72 years, respectively). In the selected patients with familial mesothelioma and wild-type *BAP1*, survival was even more favorable (9 years) and diagnosis occurred earlier (45 years). These data point at the selected criteria as helpful in identifying patients and family members who are more susceptible to develop additional cancers [17].

Another study performed targeted NGS (tNGS) in 198 germline DNAs from patients with different types of mesothelioma, analyzing 85 cancer susceptibility genes. Germline mutations of *BAP1* other genes involved in homologous recombination (HR) and DNA repair were found in 12% of cases. Age, cancer diagnosis, and asbestos exposure were examined by multivariate analysis, revealing that young age and a second diagnosis of cancer were significantly associated with the occurrence of germline mutations in cancer susceptibility genes, for which minimal or no asbestos exposure turned out to be the most significant predictor [18].

The joint effort of two large centers of the National Cancer Institute (NCI) and the University of Chicago (UC) allowed studying the relationship of germline mutations in tumor suppressor or DNA repair genes with responsiveness to platinum-based chemotherapy in 385 patients with different types of mesothelioma. A multi-gene panel BROCA v10, containing 73 target genes associated with DNA repair and/or with inherited predisposition to develop solid cancers was used for genotyping. The analysis of the NCI/UC cohort identified at least a mutation in one of the targeted genes in 12% of patients. *BAP1* was the most altered gene (16 mutations), while the other 12 mutations involved the following genes: *CHEK2*, *PALB2*, *BRCA2*, *MLH1*, *POT1*, *TP53*, and *MRE11A*. In patients with pleural mesothelioma (not with peritoneal type) mutated *BAP1*, or a mutation in the other targeted genes, was significantly associated with improved overall survival (OS), compared with wild-type patients [19].

Interestingly, within a large exon tNGS study of 168 genes associated with hereditary cancer in a cohort of more than 600 patients with different cancers, the results obtained in 12 mesotheliomas revealed the highest frequency of pathogenic variants (7/12, 58%) in genes regulating HR DNA repair, with the genes of the

Table 12.1 NGS studies of germline mutations in patients with mesothelioma

Study	Target genes (*n*)	Adopted criteria	Mutated genes (no. of patients)	Total patients
(a)	Cancer-predisposing genes (94)	• Truncating variants • Asbestos exposure	*BAP1* (4), *ATM*, *BRCA1*[a], *BRCA2*, *CDKN2A*, *FANCC*, *FANCF*, *FANCI*[a], *PALB2*, *PMS1*, *SLX4*, *XPC* (1 each)	89
(b)	Cancer linked genes (56)	• Allele frequency • CADD[b] score > 20 • Family history of cancers • Early diagnosis	*BAP1* (43/79[c]), *MLH1* (3), *SMARCA2* (2), *ARID1A*, *ARID2*, *CREBBP*, *KDR*, *NCOR1*, *RAD50*, *RBM6*, *SETD2*, *SMARCA4*, *SMARCE1*, *SMO*, *TP53* (1 each)	45
(c)	Cancer-predisposing genes (85)	• Allele frequency • ACMG/AMP[d] guidelines	*BAP1* (6), *BRCA2* (3), *CHEK2* (3), *CDKN2A* (2), *ATM* (2), *BRCA1*, *MRE11A*, *TP53*, *MSH6*, *TMEM127*, *SDHA*, *VHL*, *WT1* (1 each)	198
(d)	DNA repair and/or cancer-predisposing genes (73)	• Protein damaging variants	*BAP1* (16), *CHECK2* (5), *PALB2* (2), *BRCA2*, *MLH1*, *POT1*, *TP53*, *MRE11A* (1 each 1)	239
(e)	Hereditary cancer genes (168)	• Allele frequency • ACMG/AMP[d] guidelines	*BAP1*, *BRCA2*, *FANCA*, *FANCC*, *FANCD2*, *FANCM*, *XPC* (each 1)	12

(a) Betti et al., Cancer Lett 405:38–45, 2017
(b) Pastorino et al., J Clin Oncol 36:3485–3494, 2018
(c) Panou et al., J Clin Oncol 36:2863–2871, 2018
(d) Hassan et al., Proc Natl Acad Sci U S A 116(18):9008–9013, 2019
(e) Bertelesen et al., NPJ Genom Med 4:13, 2019
[a]Occurring in the same patient
[b]Combined Annotation Dependent Depletion
[c]16 $BAP1^{+/-}$ patients +27 relatives
[d]American College of Medical Genetics/Association for Molecular Pathology

pathway of Fanconi anemia (*BRCA2* or *FANCD1*, *FANCA*, *FANCC*, *FANCD2*, and *FANCM*) particularly represented [20].

The results of all these studies (summarized in Table 12.1) clearly indicate that at least 10%–12% of mesothelioma cases were associated with germline mutations in *BAP1* or in other HR genes and displayed better prognosis and chemosensitivity than patients with wild-type genetic background.

4 Somatic Mutations of *BAP1*

Frequent somatic mutations in BAP1 have been observed in highly metastatic uveal melanomas, 26 of 31 (84%) metastasizing tumors [21]. The majority (63.6%) of sporadic mesotheliomas contain somatic *BAP1* mutations/inactivation [22]. These

findings confirmed our previous data on *BAP1* inactivation in epithelioid type mesothelioma accompanied by loss of heterozygosity (LOH) [23], and are supported by two NGS studies of the mesothelioma genome that revealed that *BAP1* was somatically mutated in 41% [24] and 58% [25] of mesotheliomas, respectively. Therefore, the *BAP1* gene undergoes biallelic inactivation in tumors, thus, meeting the criteria of classical two-hit inactivation theory for tumor suppressor genes.

5 Chromothripsis in Mesothelioma Genome

Frequent observation of loss of heterozygosity on 3p21 in malignant mesothelioma led us and others to focus on *BAP1* as a target gene of somatic inactivation. In 2011 a study found that *BAP1* was inactivated by somatic mutations in mesothelioma [26], while in metastatic clear cell renal carcinoma the minimal common deletion region at 3p21.1 contained *BAP1* and *PBRM1* at 3p21 [27]. The genomic pattern of peritoneal mesothelioma is similar to that of pleural mesothelioma [28]. We performed a comprehensive tumor genome analysis targeting the 3p21 region by performing high-density array comparative genomic hybridization (CGH; average probe interval: 254 bp) detecting multiple minute simultaneous biallelic deletions in this region, especially in *BAP1* (8/33, 24%), *SETD2* (7/33, 21%), *PBRM1* (3/33, 9%), and *SMARCC1* (2/33, 6%) [29]. Overall, 46 genes in this region were found to contain biallelic deletions in at least one biopsy specimen out of 33 mesothelioma specimens examined. Breakpoints of these genomic deletions were different in different cases. Many of these deletions were not contiguous but alternated with segments showing oscillating copy number changes along the 3p21 region. This may be because of chromothripsis (derived from the Greek word "chromos" for chromosome and "thripsis" for shattering into pieces) [30], a phenomenon characterized by numerous genomic rearrangements caused by a single catastrophic event in multiple cancer samples. The catastrophic genetic event known as chromothripsis consists of the fragmentation of a segregated single chromosome that is then rearranged leading to incorrect reassembling or loss of certain DNA sequences. Therefore, a single chromothripsis event may cause a high number of alterations in the genome after a short number of cell replications, leading to oncogenic activations or to loss of tumor suppressor functions, eventually favoring tumorigenesis [30].

Interestingly, noncontiguous biallelic genome alterations with the characteristic pattern of chromothripsis have been observed in mesothelioma [29], later confirmed by other groups [31], also with the potential consequence of neoantigen expression and tumor immunogenicity [31].

NGS alone hardly detects larger-sized DNA deletions (>30 bp). Conventional array CGH alone cannot detect smaller-sized deletions (<3000 bp). In other words, these analyses overlook genomic alteration in the size range of 30–3000 bp. Our comprehensive genome analysis combining high-density array CGH (average probe interval: 254 bp in the 3p21 region) and targeted NGS disclosed to or at higher frequencies than frequencies of sequence-level mutations [29]. Genomic alterations in

mesothelioma usually include genomic rearrangements that induce complex and multiple deletions. Digital MLPA, which analyzes the copy number of approximately 600 exons simultaneously by using NGS-based MLPA, shall become a reliable method for high-throughput detection of multiple segmental deletions in small amounts of DNA in mesothelioma specimens to complement NGS analysis.

6 LOH, CDKN2A, NF2

The chromosomal changes of malignant mesothelioma are complex and heterogeneous, and more losses than gains of genetic material are observed. Losses of chromosomes 1p, 3p, 4q, 6q, 9p, 13, 14q, and 22 were detected in the majority of the abnormal cases [32–34]. Homozygous deletion of 9p21.3 is most frequently detected for the genetic alteration of mesothelioma and occurs in more than 90% of established cell lines. Deletion region involves *CDKN2A*, *CDKN2B* (cyclin-dependent kinase inhibitor 2B), and often adjacent *MTAP* (methylthioadenosine phosphorylase) and *MIR31* genes. The *CDKN2A* gene generates at least three alternatively spliced variants encoding distinct proteins: p16INK4A, p16gamma, and p14ARF. These products encoded by this gene play an essential role in cell cycle and senescence regulation through two major tumor-suppressing pathways of retinoblastoma protein (RB) and p53 in the cell. Fluorescence in situ hybridization (FISH) of *CDKN2A* would be useful for the diagnosis of mesothelioma because this analysis could differentiate pleural mesothelioma cells from reactive mesothelial cells [35, 36]. Accumulating information shows that the homozygous deletion of *CDKN2A* is a predictor of poor survival [37].

The *NF2* (Neurofibromin 2) gene responsible for neurofibromatosis type 2 familial cancer syndrome was shown to be the target gene of 22q12 loss. This gene is inactivated by homozygous deletion or heterozygous deletion/point mutation in a total of 40–50% of mesotheliomas [38, 39]. NF2 protein acts upstream of SAV1, LATS1/2, and yes-associated protein (YAP) in the Hippo tumor suppressor pathway. In addition to *NF2* inactivation, deletions/mutations in *SAV1* and *LATS2* genes are found in mesothelioma [40]. Hippo tumor suppressor pathway plays a vital role in controlling proper organ sizes, cell contact inhibition, stem cell function, and regeneration. Studies with this pathway would hide the possibility of causing a new therapeutic strategy.

7 Fusion Transcripts, Altered Splicing, MicroRNA

Transcriptome analysis by next-generating sequencing (n = 211) showed fusion transcripts involving tumor suppressor genes in mesothelioma: 13 fusions in *NF2*, 7 in *BAP1*, 8 in *SETD2*, 7 in *PBRM1*, 2 in *PTEN,* and 6 in others [41]. The reports on fusion transcripts in mesothelioma have been accumulating [42, 43], but the gene

pairs of fusion and the braking-region of these transcripts were different among patients with mesothelioma. Then the detection of fusion transcripts has not yet to be exploited as a diagnostic tool. Many of these fusions and aberrant splicing variants are derived from the genes in chromosomes 3p21, 9p21.3, 13q12, and 22q12, frequently deleted regions in mesothelioma. These gene regions might be fragmented by chromothripsis and lead to extensive rearrangements causing fusion genes or aberrant splicing variants. In addition, the mutation of the *SF3B1* gene, encoding subunit 1 of the splicing factor 3b protein complex, was found at ~2% of frequency (4/216) [41] and the mutations in this splicing factor gene were associated with specific alterations in mRNA splicing.

Because mutations in the genes encoding proteins associated with histone modification and chromatin remodeling, including BAP1, SETD2, and PBRM1, occur predominantly in mesothelioma, diverse gene expression changes induced by aberrant epigenetic regulation are estimated. Most of the deregulated genes in mesothelioma belong to the following pathways: angiogenesis, cell adhesion, p53 signaling, integrin signaling, MAPK signaling, apoptosis, and cell cycle regulation [44]. A special set of genes could differentiate mesothelioma from others. The set of 26 genes could distinguish pleural mesothelioma from others, normal pleura, sarcomas, renal cell carcinoma, and thymoma, with high sensitivity and specificity [45]. It was also reported that two gene sets, one including 22 genes and the other 40 genes, narrowed down from 117 genes selected from previous reports could be discriminate malignant from benign pleural proliferations [44].

MicroRNAs (miRNAs) are short noncoding RNAs of approximately 18–22 nucleotides in length, which function as posttranscriptional regulators of gene expression. It is known that miRNA expression is dysregulated in human cancer through various mechanisms, including amplification or deletion of miRNA genes, abnormal transcriptional control of miRNAs, dysregulated epigenetic changes, and defects on biogenesis components. MiR-31 expression was shown to be reduced in mesotheliomas in most cases via deletion combined with the *CDKN2A* gene at 9p21.3. MiR-34b and miR-34c, sharing a common primary transcript, were silenced by methylation in the majority (85%) of mesothelioma tumors. The miR-15/16 family has also been shown to be significantly downregulated in mesothelioma compared with those from normal pleura. MiR-193a-3p and the miR-200 family showed a statistically significant down-expression in mesothelioma tumors compared to normal pleura. The miRNAs including let-7 and miR-21 have been reported several times from different groups. These findings are reviewed in [46]. MiRNA mimics are small, double-stranded RNA molecules, designed to mimic endogenous mature miRNA molecules when transfected into cells. In order to deliver miRNAs, the minicells, known as EDVTMnanocells (EDVs) derived from asymmetric bacterial cell division were used. The therapy, dubbed TargomiRs, comprises patented miRNA mimics based on the miR-15/107 consensus sequences, packaged in EDVs that are targeted with an anti-EGFR-specific antibody. The trial was designed to test TargomiRs in patients with pleural MM or advanced NSCLC (ClinicalTrials.gov Identifier: NCT02369198). The drug showed early signs of activity [47].

Comprehensive molecular profiling, including exome sequencing, copy-number arrays, mRNA sequencing, noncoding RNA profiling, DNA methylation, and

Table 12.2 Association between prognosis and the four distinct integrated subtypes of pleural MM by the multiplatform molecular profiling

iCluster	Enriched histology	Molecular profiling characteristics	Prognosis
1	Epithelioid	Low somatic copy-number alteration, low *CDKN2A* homozygous deletions, high DNA methylation, high *BAP1* alterations	Best
2	Epithelioid	Low *BAP1* alteration, low DNA methylation	
3	Biphasic	High *CDKN2A* homozygous deletion, low *CLDN1* expression	
4	Biphasic & Sarcomatoid	High *MSLN* promoter methylation, high *LATS2* mutations, high *CDKN2A* homozygous deletions, gene expression showing epithelial-mesenchymal transition (high mRNA expression of *VIM*, *PECAM1*, and *TGFB1*, and low miR-200 family expression)	Worst

reverse-phase protein arrays, identified four distinct integrated subtypes of mesothelioma [48]. The results of the study (summarized in Table 12.2) indicate that survival was significantly different across the 4 clusters ($P < 0.0001$) [48]. Cases in the poor-prognosis subset showed higher *AURKA* mRNA expression and upregulation of the PI3K and mTOR signaling pathways. This study showed a strong expression of the immune-checkpoint gene *VISTA* in epithelioid pleural mesothelioma. These new findings integrated into the biology of mesothelioma could lead to new therapeutic strategies.

8 Conclusions

Since the discovery of *BAP1* as a predisposition gene to mesothelioma and a number of other different cancers, grouped in the BAP1 cancer syndrome, numerous germline analyses were performed in patients with mesothelioma and in subjects individuals who have experienced environmental or occupational exposure to

carcinogenic fibers and are therefore at high risk of developing mesothelioma. The knowledge of the molecular mechanisms underlying the pathogenesis of malignant mesothelioma will benefit from the future results of further studies required to complete the information on the prevalence of germline and somatic variants present in cancer susceptibility genes.

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Chapter 13
Genetic Predisposition to Mesothelioma: What Are the Biological Mechanisms and What Are the Clinical Characteristics of These Mesotheliomas?

Michele Carbone, Michael Minaai, Sandra Pastorino, and Haining Yang

Abstract Mesothelioma has been for many years the example of a malignancy induced exclusively by exposure to the environmental carcinogen asbestos. In recent years additional fibers, erionite and antigorite for example, and therapeutic ionizing radiation have been shown to cause mesothelioma. Most importantly, molecular genetic studies conducted by our team revealed that inactivating mutations of the *BAP1* gene predispose individuals to mesothelioma. At times these mutations cause mesothelioma in combination with exposure to asbestos or to other carcinogens. Recent studies revealed that at least 12% of mesotheliomas develop in carriers of germline *BAP1* mutations or, less frequently, of mutations of other tumor suppressor genes: these patients have a prolonged survival of 5 or more years. Thus mesothelioma has now become the preferred model to study gene x environment (GxE) interaction in human cancer. Genetic testing has become routine for mesothelioma patients in most major research hospitals and it is hoped that soon all patients will be tested to identify possible germline mutations as well tumor-specific mutations that can inform therapy. Moreover, family members of patients carrying *BAP1* mutations can be tested, and if positive for mutations, they can be enrolled in early detection clinical trials that are often life-saving.

Keywords BAP1 · Germline mutations · Mesothelioma · Asbestos · Erionite

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1 Introduction

Mineral fibers are inhaled and are caught into the lung filter. Depending on the fiber type, their size, and bio-durability, some fibers via the lymphatics reach the pleura where they cause chronic inflammation that leads to scarring, known as "pleural plaques." Some develop mesothelioma. When the asbestos load is high, the fibers can reach the peritoneum, via lymphatic and possibly the bloodstream, where asbestos causes chronic inflammation, adhesions, and eventually mesothelioma [1]. It is estimated that ~80% of pleural mesotheliomas have been caused by exposure to asbestos or by other carcinogenic mineral fibers [1–3]. The association of peritoneal mesothelioma with asbestos is much less frequent [1]. Historically, about 50% of peritoneal mesotheliomas occurred in individuals occupationally exposed to asbestos, especially among those with heavy exposure, as asbestos overloaded the lung filter and reached the peritoneum [4]. In the past, most peritoneal mesothelioma occurred in asbestos workers. Presently, in the USA where asbestos use has been strictly regulated for the past 40–50 years, most peritoneal mesotheliomas develop in patients without clear evidence of asbestos exposure [1, 2]. In one US series of 64 patients undergoing surgery, only 8% were listed as asbestos-exposed [1, 5]. The infrequent association of peritoneal mesothelioma with asbestos, a cluster of peritoneal mesotheliomas in Chinese women with no evidence of asbestos exposure [6], etc., points to additional causes [6]. Genetics plays a role as an increasing number of peritoneal mesotheliomas is being reported in young adults who carry germline mutations of *BAP1* and of other tumor suppressor genes, mostly genes involved in the regulation of DNA repair and cell death [1]. In some instances, germline mutations and asbestos exposure synergize in causing mesothelioma: gene x environment (GxE) interaction [1].

2 Asbestos and Mineral Fiber Carcinogenesis

In spite of stringent regulations introduced in the 1970s and 1980s to limit asbestos exposure, the incidence of mesothelioma reached 3200 cases/year in the USA in 2003 and did not increase or decline since then [3]. The incidence of mesothelioma continues to increase worldwide [1]. About 20–60 years elapse between initial exposure to asbestos or other carcinogenic fibers mineral fibers and the development of mesothelioma [1]. This long latency provides potential opportunities for prevention, early detection, or intervention at the early stages of tumor growth when mesothelioma is more susceptible to therapy [7].

There are over 400 mineral fibers in nature. Six of them, crocidolite, amosite, anthophyllite, actinolite, tremolite, and chrysotile were used commercially, and millions of people were exposed. For simplicity, although they have very different mineralogical characteristics, these six commercial fibers were collectively called "asbestos" [8]. In the late 1970s in the USA and soon after in Western Europe, the

use of these six mineral fibers was first regulated and later almost totally banned. Besides this family of six regulated asbestos minerals, there are about 400 additional nonregulated fibers that possess physical and chemical structures that are similar to asbestos fibers, and they may be carcinogenic [9–11].

While occupational exposure to asbestos in the USA and some other countries has been significantly reduced following the implementation of regulatory measures, the expected significant decline in mesothelioma incidence has not been observed [1, 10]. As occupational exposure to asbestos in the Western World is decreasing, environmental exposure caused by human development of areas containing geological deposits of asbestos and other carcinogenic fibers is increasing [8–12].

Crocidolite is the most potent type of mesothelioma-causing asbestos [7]. Erionite, a zeolite mineral fiber, is the most potent fiber that has been known so far to cause mesothelioma [12]. The different potency of mineral fibers in causing mesothelioma has been linked to their ability to induce chronic inflammation [13–16], genotoxcity [17], biopersistence [1, 15], and ability to activate specific signaling pathways [13, 14]. However, not all fibers are necessarily carcinogenic and a number of evidences are required in order to identify a fiber, or other agents, as a human carcinogen [18]. For example in vitro and in vivo experiments revealed that palygorskite, a fiber that is abundantly present in the desert areas in California and Nevada does not cause inflammation or changes in the signaling pathways linked to mesothelioma nor causes mesothelioma upon injection in mice [19]. In mice, where exposure can be tightly controlled and monitored, there is a well-documented dose-response effect between the amount of asbestos exposure and the development of mesothelioma [20]. In humans, the issue of dose-response is less clear as it is difficult to study because it is not easy to quantify exposure, which often involves exposure to different types of fibers over the course of many decades [1]. An exception is represented by asbestos miners, who are exposed for years to the same type of fibers and where exposure can be quantified based on the time of employment in the mine. A study among South African asbestos miners revealed that 4.7% of deaths among them were caused by MM [21]. Specifically, Sluis-Cremer et al. followed a cohort of 7317 white asbestos miners (3212 amosite miners, 3430 crocidolite miners, 675 exposed to both, crocidolite and amosite) of which only 8% had been exposed for more than 10 years. There were 1225 deaths during the period of observation, an excess of 331 over controls. Thirty of those deaths were due to MM, 20 among crocidolite miners (423 total deaths, 4.7%), 4 among amosite miners (648 total deaths, 0.6%), and 6 among miners with mixed exposures (154 total deaths, 3.9%). Twenty-eight of 30 MM occurred 10 or more years since first employment (latency) and only 1 occurred in a worker with “only” 8 years of exposure. The exposure time in the remaining case of MM could not be evaluated because this miner grew up in an area contaminated with asbestos. None of these 30 miners who developed MM had a period of exposure of less than 3 months (amosite and mixed exposure) or 12 months (crocidolite). Among the cases of crocidolite-associated MM, 6 developed in individuals exposed for a period ranging from 12 to 95 months, 6 in miners exposed for 96–191 months, and 8 in miners exposed for more than

192 months [21]. The fact that the majority of workers who were exposed to the same type and amount of asbestos did not develop mesothelioma after a similar latency suggested to us that some individuals were more susceptible than others [22].

Asbestos activates the NLRP3 inflammasome, which in turn induces IL-1β secretion triggering the early inflammatory reaction caused by asbestos [23]. Although these reactions are NLRP3-dependent, it is not clear whether NLRP3 activation plays a role in asbestos-induced mesothelioma [24].

Recent studies have linked the release of a protein associated with native immunity, high-mobility group box protein 1 (HMGB1), with mesothelioma development [1, 7, 25]. When asbestos is deposited in the lung and in other tissues, it causes chronic inflammation [25] the production of mutagenic reactive oxygen species is largely triggered by the release of HMGB1 in the extracellular space by mesothelial cells and macrophages [1, 7, 16, 17, 25]. Physiologically HMGB1 is present in the nucleus, where it regulates nucleosome assembly and chromatin structure [25]. Extracellular HMGB1 initiates and perpetuates the inflammatory response [25]. HMGB1 can gain entry to the extracellular space by passive release from necrotic cells or by active secretion by macrophages and mesothelioma cells. Mesothelioma develops from an HMGB1-rich environment, and therefore, mesothelioma cells are "addicted" and require HMGB1 for malignant growth: in fact, anti-HMGB1 therapies significantly reduce the growth of mesothelioma in mice [26, 27].

Downstream HMGB1 mediators include tumor necrosis-α (TNF-α and NF-κB, that contribute directly to the growth of asbestos-induced mesothelioma [28, 29]. Extracellular secretion of HMGB1 requires HMGB1 acetylation that in turn prevents HMGB1 nuclear translocation. Therefore, HMGB1 accumulates in the cytoplasm and is secreted extracellularly. Non-acetylated HMGB1 locates in the nucleus where it binds chromatin. When cells die of programmed necrosis following asbestos exposure, they passively release the non-acetylated nuclear HMGB1 [25, 30].

HMGB1 is found in the sera of mesothelioma patients [26, 30–32]. Different isoforms of HMGB1 are produced in response to asbestos exposure and in patients with mesothelioma [30]. In the near future, it is hoped that serum detection of HMGB1 alone or in combination with proteomic studies will help detect mesothelioma at an early stage when can be managed clinically more effectively [30, 33].

3 Genetic Predisposition to Mesothelioma

Studying an epidemic of mesothelioma in three villages in Cappadocia, Turkey where 50% of villagers die of mesothelioma, we proposed that the cause was GxE interaction. This research, that we conducted over a period of over 14 years, is described in great detail and accuracy in an article by our former collaborator Dr. Salih Emri [34]. Here we will summarize the studies in Cappadocia and then describe our discovery that carriers of heterozygous *BAP1* mutations develop

mesothelioma and other malignancies, and the mechanisms involved. Initially studying the epidemic of mesothelioma in Cappadocia, where erionite exposure was widespread [35] we noted that mesothelioma clustered in certain families but not in others in spite of the fact that all villagers were exposed to the same environmental carcinogen. Studying these families, we demonstrated that susceptibility to develop mesothelioma was transmitted in a Mendelian fashion in certain families [36]. This was the first demonstration that genetics cause the development of mesothelioma in some families [36]. With this information, and together with our Turkish collaborators Drs. Izzetin Y. Baris, Umran Dogan, and Salih Emri, and thanks to the support of the Director of the Cancer Control at the Turkish Ministry of Health Dr. Murat Tuncer, we convinced the Turkish Ministry of Health to build two new villages, where the villagers were relocated to eliminate exposure and thus eliminate the "E" from the GxE equation, which should eliminate or reduce the incidence of mesothelioma in future generations. Presently the old villages contaminated with erionite have been abandoned and villagers relocated to modern housing. Moreover, a clinic was built in "new" Tuzkoy upon our requests to Dr. Murat Tuncer who coordinated the rapid implementation of this project, where villagers are now treated close to their homes [34, 35]. In summary, the researchers worked together with the State authorities to implement measures to prevent or at least delay the incidence of mesothelioma in future generations and at the same time to provide much-improved living and health conditions to these villagers [34, 35].

We proposed the existence of a gene/s that when mutated increased susceptibility to asbestos and erionite and caused mesothelioma, reviewed in [34, 35]. We won NCI-P01 funding (M. Carbone Principal Investigator) to try to identify the putative mesothelioma susceptibility gene, and we studied families from Cappadocia and from the USA with high incidence of mesothelioma. Four and half years into the grant we had produced only negative data, in spite of a tremendous amount of laboratory work—in pre-NGS era, all we had to localize the gene were array-CGH, linkage analyses, and Sanger sequencing. We were underpowered: there were too many possible chromosomes where the mutations could hide and not enough time and resources to study them all. A patient who developed both UVM and mesothelioma, pointed us in the right DNA segment, as mesothelioma and UVM often carry deletions in 3p. We focused our sequencing efforts on 3p, and we discovered that in 2/2 families with high incidence of mesothelioma, all affected family members had inherited BRCA1-associated protein 1 (*BAP1*) heterozygous mutations which thus were the cause of mesothelioma and uveal melanoma in those patients [37]. This was the first Identification of a gene that when mutated in the germline causes mesothelioma, as well as the first identification that acquired *BAP1* mutations in sporadic mesothelioma could be easily detected by loss of BAP1 nuclear staining—a test that has now entered the routine of most pathology laboratories. We named this condition "The BAP1 Cancer Syndrome" [1, 38, 39]. To test our hypothesis that germline *BAP1* mutations increased the susceptibility to asbestos [35, 36] we used a mouse BAP1 heterozygous deletion model. We found that heterozygous BAP1 deletion lowered the minimal threshold exposure to asbestos required to cause mesothelioma

[20]. Germline *BAP1* mutations are also associated with clear cell renal cell carcinoma, breast carcinomas, and various types of skin cancer, and less frequently with other malignancies [1, 40–43]. Germline *BAP1* mutations are highly penetrant as close to 100% of mutant carriers developed one or more cancers during their lifetime, about 1/3 of them mesotheliomas [1, 40–43]. Clinically, mesotheliomas that develop in a background of *BAP1* germline mutations have a significantly prolonged survival of >5 years [43–46]. However, biallelic somatic *BAP1* mutations, which are present in 2/3 of all mesotheliomas, are not associated with a similarly improved survival [1].

The origin of *BAP1* mutations in several families was traced to a Swiss couple that emigrated to the USA from Germany in the early 1700s. An ~80,000 individuals pedigree of relatives of this family allowed identification of branches of the family carrying the *BAP1* mutation and prompted the implementation of annual screening for cutaneous and uveal melanoma that resulted in early detection and curative resection in some patients [41]. These results underscore the value of identifying carriers of germline *BAP1* mutations, as family members can be screened and those who carry mutated *BAP1* can be followed for early detection, which can be life-saving [1, 7].

Carriers of heterozygous germline *BAP1* mutations starting at a young age, often develop benign melanocytic skin tumors that were initially identified as atypical Spitz tumors [47]. However, these benign tumors have unique histological characteristics that set them apart from atypical Spitz tumors and we named them melanocytic *BAP1*-mutated atypical intradermal tumors (MBAITs) [38] a finding confirmed by others [40, 48]. MBAITs (Fig. 13.1) provide physicians with a visual clue to identify *BAP1* mutation carriers who can then be followed for early cancer detection [1, 7, 40, 48].

In parallel studies we, and others, initially demonstrated that acquired, somatic *BAP1* mutations were present in 22–23% of mesotheliomas [37, 49]. However, those initial studies underestimated the incidence of biallelic inactivating *BAP1* mutations in mesothelioma. In subsequent studies we demonstrated that >60% of sporadic mesotheliomas carry somatic inactivating biallelic *BAP1* mutations, easily detected by lack of BAP1 nuclear staining in the tumor cells [50], a finding widely accepted [1, 51, 52]. So, why did we miss over half of *BAP1* mutations in earlier studies? We discovered that about 50% of somatic acquired mutations encompass DNA segments of about 100–3000 kb, and thus can be detected by high sensitive custom made array-CGH (i.e., array-CGH with a probe every 250 base pairs) and also by immunohistochemistry; but these minute deletions are easily missed, by next-generation sequencing (NGS), a technique developed to detect nucleotide level mutations [53]. Numerous studies have expanded the tumor types containing somatic inactivating biallelic *BAP1* mutations to include about 90% of metastatic UVM, almost any type of skin cancer, 40% of squamous cell carcinomas of the esophagus, 15% of clear cell renal cell carcinomas, etc., reviewed in [1]. There is a significant overlap among the cancer types that develop in carriers of heterozygous germline *BAP1* mutations and those that contain somatic *BAP1* mutations.

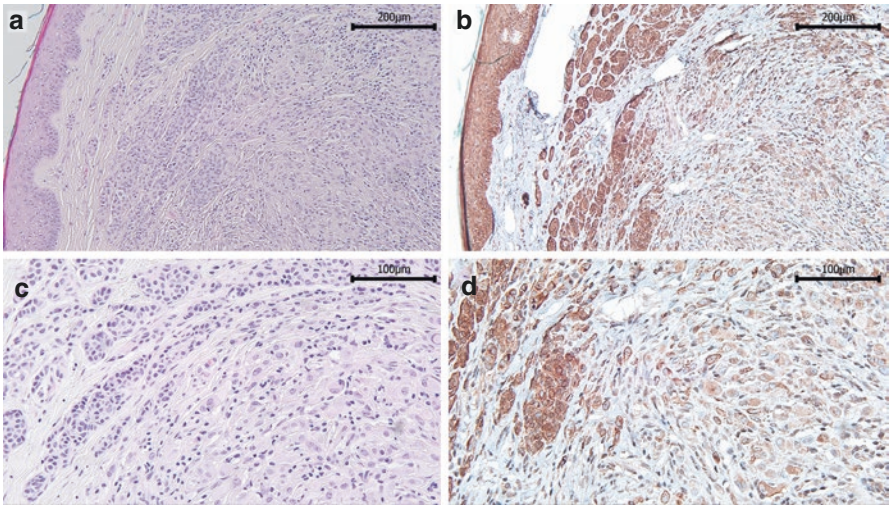

Fig. 13.1 MBAITs lesion in germline *BAP1* mutant carrier [38]. Representative images of MBAITs. (**a** and **c**) Hematoxylin Eosin staining; (**b** and **d**) BAP1 staining. Note: absence of nuclear staining in MBAITs cells, which is evidence of biallelic BAP1 inactivation, whereas nearby cells and infiltrating mononuclear phagocytes show positive nuclear BAP1 staining. MBAITs show large epithelioid clonal cells that resemble those found in SPITZ nevus and in AST (that are at times confused with MBAITs). However, the cells in MBAITs are present only in the dermis (there is no epidermal component). In this case, as in almost all MBAITs, it is possible to detect—in the top left corner below the epidermis—remnants of a nearby conventional dermal nevus formed by smaller BAP1—positive cells in close proximity to the large BAP1 negative epithelioid cells that characterize the MBAITs. This feature is rarely found in SPITZ or ASTs lesions. In contrast to SPITZ nevi there is no maturation toward the deeper part of the dermis, and in contrast to AST, Ki67 stain—not shown—reveals that that are no mitotic figures. In addition, no Kamino bodies—common in SPITZ tumors—are detected in the MBAITs. These lesions were described in Ref. [38]. Magnification: (**a**) and (**b**) 100X; (**b**) and (**c**) 200X

4 Mechanisms: How Does BAP1 Work and Why Reduced Levels of BAP1 Cause Mesothelioma and Other Cancers?

BAP1 is a de-ubiquitylating enzyme (DUB) that is present in both the nucleus and the cytoplasm [39]. BAP1 deubiquitylates itself in order to get access into the nucleus [54]. This is because the ubiquitin-conjugating enzyme UBE2O induces BAP1 sequestration in the cytoplasm by multi-monoubiquitination of its nuclear localization signal (NLS) [54]. Nuclear BAP1 promotes DNA double-strand break repair by facilitating homologous recombination (HR), an error-free repair mechanism [55, 56]. Moreover, nuclear BAP1 regulates transcription [57]. All *BAP1* mutations identified to date result either in a truncated protein lacking the NLS or affect the catalytic subunit [1]. Cancers in carriers of germline *BAP1* mutations as well as acquired *BAP1* mutations in various types of human cancers, including

mesothelioma, show biallelic inactivation [1]. Almost all *BAP1* mutations are either truncating mutations or mutations in the catalytic domain of BAP1 and thus they prevent BAP1 nuclear translocation, resulting in negative BAP1 nuclear staining, with possible cytoplasmic staining when mutated inactive BAP1 accumulates in the cytoplasm [1].

We discovered [58] that in the cytoplasm, BAP1 is found only in the endoplasmic reticulum (ER) where BAP1 deubiquitylates, stabilizes, and modulates the activity of the IP3R3 Ca^{2+} channel. The IP3R3 on the ER regulates Ca^{2+} release from the ER into the cytosol and mitochondria. The reduced BAP1 levels in carriers of heterozygous germline *BAP1* mutations cause reduced IP3R3 levels, and therefore, reduced release of Ca^{2+} from the ER. In turn, this leads to low levels of Ca^{2+} in the mitochondria which cause the reduced ability of *BAP1* mutant cells to execute apoptosis following DNA damage induced by asbestos, UV light, ionizing radiation, etc. (Fig. 13.2). Since DNA-damaged *BAP1* mutant cells do not die due to the reduced apoptosis, they continue to divide and over time may become malignant [58]. The importance of BAP1 in regulating cell death is underscored by the recent findings of Zhang et al. who discovered that BAP1 promotes ferroptosis. Thus, BAP1 mutations also impair this form of cell death [59].

Malignant growth is facilitated by our additional discovery that "normal" primary cells from individuals carrying germline *BAP1* mutations derive energy through aerobic glycolysis and thus display a set of metabolic alterations known as the "Warburg effect" which is a requirement for tumor cell growth and invasion [60]. Therefore, cells of carriers of germline *BAP1* mutations are already primed for tumor growth, and when these cells accumulate genetic damage and become transformed they are capable to grow into malignancies. These findings may lead to novel treatment options aimed at replacing BAP1 activity to ameliorate the downstream effects of BAP1 loss or at targeting specific vulnerabilities of *BAP1* mutant cells. *BAP1* mutations may also help direct current therapies: two independent recent studies proposed that *BAP1* mutations induce resistance to gemcitabine-induced apoptosis, suggesting that gemcitabine should not be used in mesotheliomas with mutated *BAP1* [61, 62].

5 Additional Considerations Regarding Genes and Factors that May Contribute or Cause Mesothelioma

CDKN2A and NF2 somatic mutations are present in up to 50% of mesotheliomas [63–66]. CDKN2A encodes p16INK4A, which binds and inhibits the catalytic activity of the CDK4/cyclin D enzymes inducing G1 cell-cycle arrest by inhibiting the phosphorylation of pRb. CDKN2A also encodes p14ARF, which is required for the activation of p53. Thus, deletions of CDKN2A inhibit both the pRb and p53 tumor suppressor pathways [67]. The incidence of p16/p14 mutations is higher in mesothelioma-derived cell lines, suggesting that these mutations favor the establishment of mesothelioma in tissue culture [67].

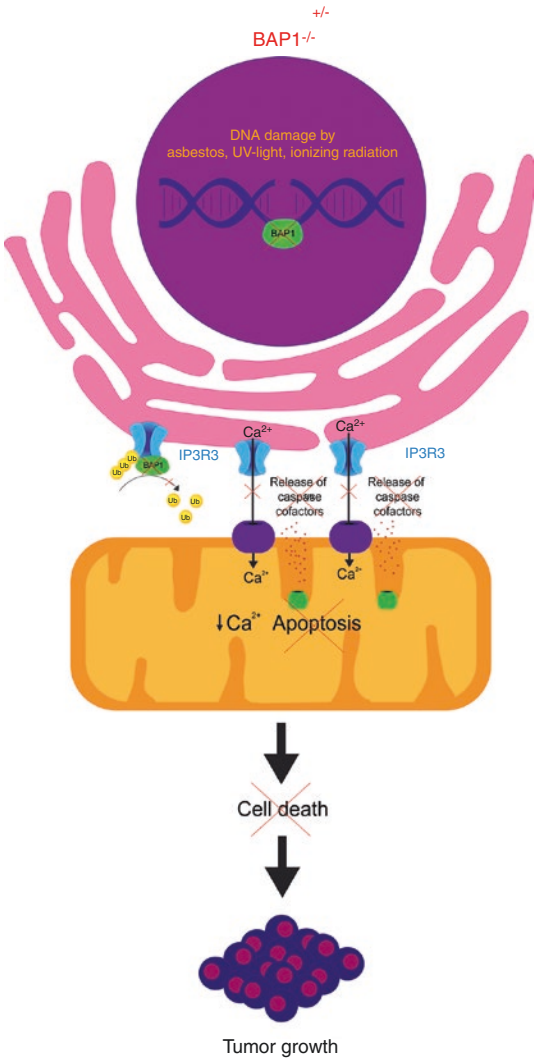

Fig. 13.2 Nuclear BAP1 regulates DNA repair and cytoplasmic BAP1 regulates cell death. Normal cells accumulate mutations each time they divide. DNA damage can be more extensive upon exposure to direct mutagens, such as ultraviolet light and ionizing radiations, or to indirect carcinogens such as asbestos that cause DNA damage by eliciting the production of mutagenic reactive oxygen species by cells in the microenvironment, mostly macrophages. Nuclear BAP1 contributes to the orchestrated process that results in DNA repair. When nuclear BAP1 levels are reduced or absent the cells accumulate more DNA damage. Cells in which the DNA damage is not repaired undergo cell death by apoptosis, and also by ferroptosis, programmed cell necrosis, etc. These cells are eliminated so that genetically damaged cells do not proliferate and give rise to malignancies. BAP1, by controlling the stability of the IP3R3 calcium channel that modulates the transfer of Ca^{2+} from the endoplasmic reticulum to the mitochondria plays a key role in regulating apoptosis [58]—and through other mechanisms in regulating ferroptosis [59]. Cells with reduced or absent cytoplasmic BAP1, such as normal cells of carriers of germline BAP1 mutations, have significantly reduced ability to execute cell death, thus their cells accumulate genetic damage and are prone to malignant transformation, resulting in the "BAP1 cancer syndrome," reviewed in Ref. [1]

The neurofibromatosis gene NF2 codes for merlin. Merlin downregulates the phosphorylation of focal adhesion kinase, a key component of cellular pathways that regulate cell migration and invasion [65]. Surprisingly, 92% of the same mesothelioma biopsies express merlin by immunohistochemistry [65], suggesting that NF2 mutations are often of minor biological significance. Mutations other than BAP1, CDKN2A, and NF2 in mesotheliomas are in the single digits [63–66].

Several studies have investigated the possible role of microRNAs (miRNAs) in mesothelioma and as possible diagnostic/therapeutic targets. The results of these studies are often discordant and require validation; reviewed in [68]. Additional carcinogens have been linked alone or with asbestos to mesothelioma. Simian virus 40 (SV40) DNA sequences have been detected in human mesothelioma, lymphoma, bone, and brain tumors; these same tumor types are induced when SV40 is injected into hamsters [69, 70]. SV40 was shown to be a co-carcinogen with asbestos [71]. However, in human tumors, the SV40 DNA, or fragments of SV40 DNA were detected in an episomal state. This raised concerns about possible laboratory contamination because in rodents SV40 was always integrated in the tumor cells DNA. This issue was never sorted out and differences of opinion persist, reviewed in [72, 73]. The discovery of a novel mechanism of viral latency that allows SV40 to persist episomally in mesothelial and brain cells may account for some of these discrepancies [74, 75].

Ionizing radiation has also been linked to mesothelioma. Statistically significant increases in the risk of mesothelioma have been reported in nuclear power workers, and in patients who received therapeutic external beam radiation or Thorotrast [76–78]. Radiation therapy increases the risk of both pleural (hazard ratio (HR) 1.34) and peritoneal mesothelioma (HR 2.20); higher risks are associated with longer latency periods [78]. Mesotheliomas in previously radiated lymphoma patients develop at a mean latency of 21.4 years and often have unusual histologic features [77]. These patients, similarly to those carrying germline mutations of *BAP1* or mutations of other tumor suppressor genes, are more likely to be younger, female, and may have a better survival than patients with asbestos-induced mesothelioma [1, 77, 79].

6 Conclusions

The discovery that germline *BAP1* mutations cause mesothelioma has infused new energy into mesothelioma research. Mesothelioma has become the preferred cancer model in which to study GxE in human cancer. Genetic screening to identify patients and family members carrying germline BAP1 mutations have already resulted in benefits to patients and family members [1, 80–82].

Acknowledgments We thank all the family members affected by the “BAP1 cancer syndrome” and the patients with *BAP1*-mutated tumors that donated their specimens. They allowed us to study and discover how this gene functions in different cancer types and to translate this knowledge to improve cancer prevention, diagnosis, and prognosis [already being implemented), and in the near future, we hope, to improve therapies for patients with *BAP1*-mutated cancers.

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Chapter 14
Frequent NF2 Inactivation in Mesothelioma: How Can We Treat Mesothelioma with Targeted Therapies for Molecular Aberrations?

Yoshitaka Sekido

Abstract The *NF2* gene was initially identified as a causative gene for neurofibromatosis type 2 cancer syndrome. Soon thereafter, *NF2* was shown to be frequently mutated in malignant mesothelioma (MM). Recent genomic profiling confirmed that approximately 40% of mesothelioma cases show inactivating alterations of *NF2*. *NF2* encodes moesin–ezrin–radixin-like (merlin), a protein that regulates multiple cell-signaling cascades including the Hippo tumor-suppressive signaling pathway. MMs also exhibit genetic or epigenetic inactivation of Hippo pathway components including MST1/2 and LATS1/2, which indicates that merlin–Hippo pathway dysregulation plays a key role in MM development and progression. Hippo pathway inactivation leads to the constitutive activation of the YAP1/TAZ transcriptional coactivators, thereby conferring malignant phenotypes to mesothelial cells. Critical YAP1/TAZ target genes include prooncogenic cell cycle promoter genes such as *CCDN1* and growth factor/cytokine genes including *CTGF* and *IL1B*. Meanwhile, YAP1/TAZ may also act as a tumor suppressor under specific cellular contexts; for example, YAP1 promotes regulated cell death known as ferroptosis. These data indicate that the merlin (*NF2*)–Hippo pathway may be a therapeutic target for the treatment of MM and support the development of new strategies to effectively kill MM cells depending on the dysregulated (or regulated) status of this pathway.

Keywords Malignant mesothelioma · NF2 · Hippo pathway · YAP1/TAZ

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1 Introduction

Genomic analysis of malignant mesothelioma (MM) over two decades has identified frequent somatic alterations of characteristic and selective tumor suppressor genes in MM. The representative genes are *CDKN2A*, neurofibromin 2 (*NF2*), BRCA1-associated protein 1 (*BAP1*), and *TP53*. Recent comprehensive genomic profiling revealed a more detailed genomic landscape of MM. Compared to other solid malignancies, MMs harbor a relatively small number of somatic mutations. The newly identified genes from those analyses include those involved in histone modification and RNA processing [1, 2]. Other characteristics of genomic alterations of MM are extensive chromosome loss termed "genomic near-haploidization" [2]. Only very rare mutations in other well-known oncogenes are detected. Therefore, traditional approaches that target activated oncogene products with specific kinase activity are not utilized for MM; thus, different concepts need to be established for the development of new molecular target therapy against this formidable disease.

2 NF2

NF2, which is localized at chromosome 22q12 region, was initially identified as a gene responsible for familial cancer syndrome. The protein encoded by *NF2*, moesin–ezrin–radixin-like (merlin), is a member of the Band 4.1 family of cytoskeletal linker proteins [3]. Although patients with *NF2* family cancer syndrome do not have an increased risk of MM, approximately 40% of MM tumor samples show *NF2* inactivation [4]. *NF2*-inactivating alterations detected in MM tumors include nonsense/missense mutations or small/large deletions, resulting in bi-allelic loss of function. RNA sequencing revealed that gene rearrangements of *NF2* that cause inactivating alterations have also been found in MMs [1, 2]. *NF2* mutations seem to be observed more frequently in sarcomatoid rather than in epithelioid types of MM.

Besides genetic/epigenetic alterations of *NF2* itself, merlin can also be inactivated via other mechanisms. Protein kinase C-potentiated phosphatase inhibitor of 17 kDa (CPI-17) inhibits the myosin phosphatase (MYPT-1-PP1δ), which dephosphorylates/activates merlin at Ser518; thus, increasing CPI-17 expression inactivates merlin by phosphorylation. A carboxyl-terminus *NF2* (isoform 2) splicing variant may not have tumor-suppressive activity, although this mechanism remains controversial. Upregulation of *NF2*-targeting microRNAs, such as has-miR-885-3p, can also inhibit *NF2* (Fig. 14.1). Physiologically, merlin is regulated by extracellular signaling from CD44, adherence junction, and receptor tyrosine kinases (RTKs) (Fig. 14.1).

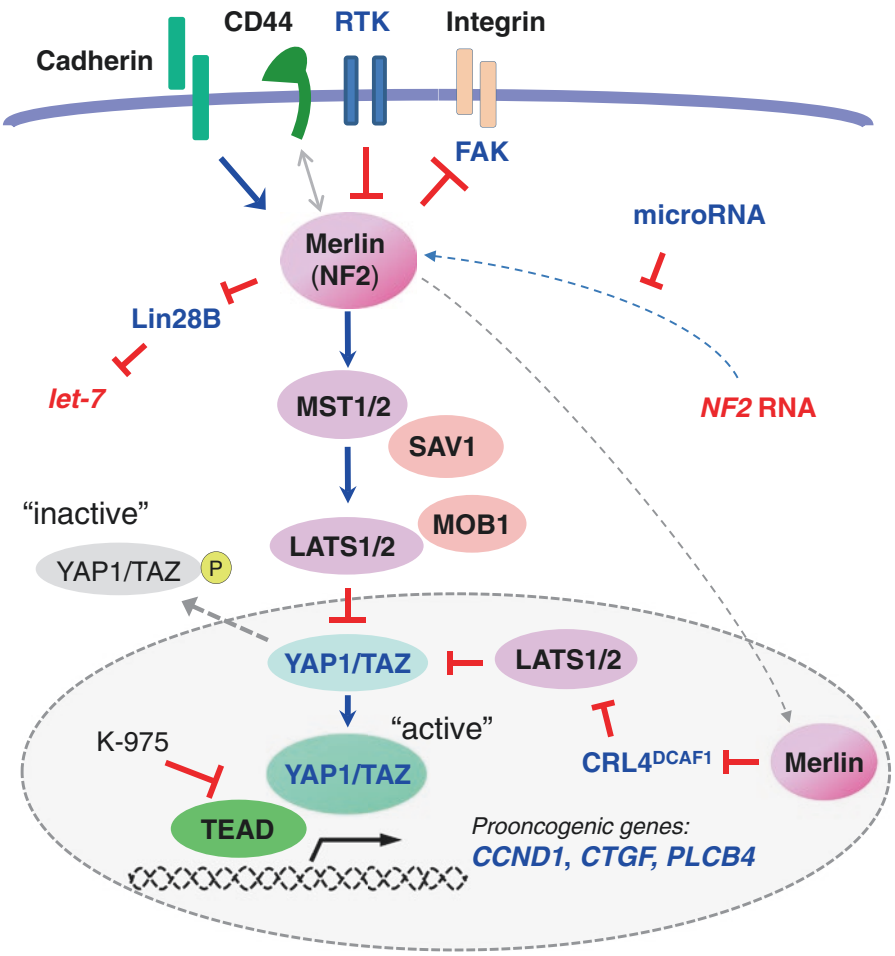

Fig. 14.1 Merlin (*NF2*)–Hippo pathway. Activated merlin regulates the Hippo signaling cascade and suppresses the YAP1/TAZ transcriptional coactivators. Inactivation of NF2 or Hippo pathway components results in constitutive underphosphorylation (activation) of YAP1/TAZ, which enhances the transcription of prooncogenic genes

2.1 Tumor-Suppressive Activity of NF2/Merlin

Transduction of *NF2* can inhibit the malignant phenotypes of *NF2*-deficient MM cells. Specifically, merlin suppresses focal adhesion kinase (FAK) activity, thereby disrupting the interaction between FAK and its binding partners, Src and p85, the regulatory subunit of phosphatidylinositol-3-kinase (PI3K) [5] (Fig. 14.1). Thus, merlin inactivation is likely related to the upregulation of FAK activity.

Nf2-knockout mouse models have also confirmed the significance of *Nf2* inactivation on MM pathogenesis in vivo. For example, asbestos-exposed *Nf2* (+/−) knockout mice exhibited markedly accelerated MM tumor formation compared to that in their asbestos-treated wild-type littermates [6]. Conditional knockout mouse models of mesothelium-specific losses of the *Nf2*, *Bap1*, and/or *Cdkn2A* combination showed an increased incidence of MM development, with the triple knockout mice developing high-grade and very invasive tumors and the shortest survival times [7].

Besides functioning under the cytoplasmic membrane, the underphosphorylated form of merlin can translocate to the nucleus (Fig. 14.1). Merlin binds to the E3 ubiquitin ligase $CRL4^{DCAF1}$ and inhibits $CRL4^{DCAF1}$ ubiquitination of its target proteins [8]. Accordingly, merlin exhibited tumor-suppressive activity via $CRL4^{DCAF1}$-suppression in MM cell lines [8]. Since $CRL4^{DCAF1}$ directly binds to LATS1/2 to direct ubiquitination and degradation [9], de-repressed $CRL4^{DCAF1}$ in *NF2*-deficient cells promotes LATS1/2 degradation and, thus, activates YAP1.

Merlin also exhibits a cell-density-dependent, but Hippo pathway-independent, tumor-suppressive activity to inhibit its downstream target, Lin28B, a *let-7* microRNA inhibitor [10](Fig. 14.1). As *let-7* microRNAs act as tumor suppressors by silencing the expression of *MYC* and *RAS* oncogenes, inhibition of *let-7* by Lin28B promotes cell growth.

2.2 Hippo Pathway

One of the best-characterized downstream signaling cascades regulated by merlin is the Hippo pathway. This pathway is involved in critical biological processes including organ-size control, development, differentiation, and cancer development [11]. The four core components in this pathway comprise MST1 and MST2 kinases, SAV1 (also termed WW45), MOB1, and LATS1 and LATS2 kinases (Fig. 14.1).

Under Hippo pathway activation, MST1/2 kinases phosphorylate (activate) LATS1/2. LATS1/2 then phosphorylates the YAP1 and TAZ transcriptional coactivators. The phosphorylated YAP1/TAZ are retained within the cytoplasm or degraded; thus, phosphorylated YAP1/TAZ are the inactivated forms. Conversely, when the Hippo pathway is not active, underphosphorylated YAP1/TAZ enter the nucleus and act as transcriptional coactivators. YAP1/TAZ interact with several distinct transcription factors including TEA domain (TEAD) transcription factors [12].

Besides *NF2*, MMs show frequent inactivation of other Hippo pathway components. *LATS2* mutations or deletions were observed in 7–11% of MM cases [13]. A comprehensive genomic analysis of MM samples revealed frequent copy number loss among various Hippo pathway genes, including *MST1* and *LATS1* [1]. Epigenetic alteration of the promoter regions of the components has also been reported.

2.3 YAP1 and TAZ Transcriptional Coactivators

YAP1 expression is observed in ~70% of primary MM tissues, with most positive cases showing increased YAP1 staining in the nucleus compared to that in the cytoplasm. Activated YAP1/TAZ induces the transcription of multiple cancer-promoting genes in MM cells, including those encoding cell cycle promoters such as cyclin D1 (*CCND1*) [14], connective tissue growth factor (*CTGF*) [15], and phospholipase-C beta 4 (*PLCB4*) [16], as well as cytokines such as interleukin 1β (*IL1B*) [17].

The effects of YAP1/TAZ activation were also tested in immortalized human mesothelial cells. Exogenously transduced wild-type *YAP1,* and even more so, an activated mutant *YAP1 S127A,* stimulated immortalized mesothelial cells to form mesothelioma-like tumors when the cells were inoculated into nude mice [16]. An activated mutant *TAZ S89A*, but not wild-type *TAZ*, also induced a similar phenotype [17].

CTGF is a secretory extracellular matrix-associated matricellular protein that regulates cell-extracellular matrix (ECM) interactions, cell proliferation, migration, fibrosis, and inflammation. CTGF expression was shown to be associated with abundant extracellular matrix formation in MM tissues [15], and CTGF expression was further enhanced in response to TGF-β stimulation [15] and β-catenin-TCF-LEF signaling [18]. CTGF was highly expressed in sarcomatoid-type MMs and mediated the epithelial-mesenchymal transition (EMT) in MM [18].

3 NF2 Mutation Status for Clinical Application

NF2 loss has been investigated as a possible diagnostic and prognostic/predictive biomarker for MM patients. *CDKN2A* FISH and BAP1 immunohistochemical staining are currently considered the most effective molecular methods of differentiating between MMs, other malignancies, and reactive mesothelial hyperplasia. A recent study that added genetic *NF2* screening reported improved MM diagnosis sensitivity or specificity [19]. Both low merlin expression and high Survivin labeling index were shown to be indicators of poor prognosis in patients with malignant pleural mesothelioma (MPM) [20]. Likewise, a combination of homozygous *CDKN2A* deletions and hemizygous *NF2* loss in peritoneal mesotheliomas was an independent negative prognostic factor for both progression-free and overall survival [21].

A FAK inhibitor, VS-4718, inhibited proliferation and induced apoptosis in MM cells lacking merlin expression [22]. However, a phase-II (COMMAND) trial of another selective FAK inhibitor, VS-6063 (defactinib), in patients with mesothelioma did not show any clinical benefits as maintenance therapy for advanced MPM after first-line chemotherapy [23]. Thus, whether FAK inhibitors can provide a clinical benefit for patients with merlin-negative MM cells remains unclear. Meanwhile, another study reported that E-cadherin expression was correlated with the resistance of merlin-negative MM cells to FAK inhibitor treatment [24].

3.1 Direct Targeting of the Hippo Pathway

The merlin–Hippo pathway is an attractive molecular target for the development of novel therapeutic approaches to MM. However, reintroducing a tumor suppressor gene to the pathway components and activation in whole cells within tumor tissues is technically challenging. One rational approach is to block YAP1/TAZ interactions with their target transcription factors. Since TEADs are thought to be involved in the prooncogenic functions of YAP1/TAZ in MM cells, the disruption of YAP1/TAZ and TEAD interaction may be one approach. The first small molecule shown to inhibit YAP1-TEAD binding was verteporfin (Visudyne), which is used clinically as a photosensitizer in photodynamic therapy for neovascular macular degeneration. Verteporfin treatment suppressed YAP1 activity along with the viability, invasion, and tumorsphere formation of MM cell lines in vitro [25]. Mammalian vestigial-like 4 (VGLL4) was also identified as a natural YAP1 antagonist and a synthetic peptide (48-mer) that mimicked its key interaction domain suppressed gastric and lung cancer cells by competing with YAP1 to bind to TEADs [26].

K-975, a recently identified small molecule, was shown to interrupt YAP1-TEAD interaction [27]. K-975 covalently bound to Cys359 of TEAD1, a palmitoylation site important for YAP1-TEAD interaction. K-975 suppressed MM cell line growth in vitro and in vivo. The combination of K-975 and chemotherapeutic drugs prolonged the survival of mice orthotopically implanted with MM cells. Thus, K-975 appears to be a very effective drug to suppress the growth of YAP1-activated MM cells. However, K-975 also caused severe kidney damage in vivo, indicating the need for future development to overcome this adverse effect.

3.2 Indirect Targeting of the Hippo Pathway

Cellular metabolic status is linked to Hippo signaling. Statins, which inhibit the mevalonate cholesterol biosynthesis pathway, had anticancer effects in MM cell lines and were also effective against MM in vivo when combined with doxorubicin [28]. Notably, their effects on the mevalonate pathway were shown to control YAP1/TAZ activity, such that statins inhibited both YAP1/TAZ nuclear localization and transcriptional responses [29]. Consistent with these data, statins exhibited greater antiproliferative activity in MM cells with Hippo pathway inactivation [30].

Merlin is a negative regulator of the mammalian target of rapamycin complex 1 (mTORC1). Merlin-deficient MM cells were selectively sensitive to the growth-inhibiting effects of the allosteric mTOR inhibitor, rapamycin [31]. However, the results of a phase-II study of an mTOR inhibitor, everolimus, suggested that everolimus had limited clinical activity in advanced MPM [32]. In comparison, a phase-I study of the dual class-I PI3K and mTOR kinase inhibitor apitolisib (GDC-0980) showed a partial response in patients with pleural and peritoneal mesothelioma [33].

The targeting of gene products whose expressions are induced by YAP1/TAZ might be another rational strategy. As described above, *CTGF* is a well-known YAP1 target gene and *IL1B* is also enhanced by TAZ. The human anti-CTGF monoclonal antibody pamrevlumab (FG-3019) is currently undergoing clinical testing for idiopathic pulmonary fibrosis (IPF) [34]. As pamrevlumab is effective against high-grade serous ovarian cancer [35], it should also be investigated as a potential therapeutic agent for MM. A recent study reported that FG-3019 significantly inhibited mesothelioma growth in an orthotopic mice model [36]. Finally, knockdown of *IL1B* and IL1 receptor antagonists strongly inhibited mesothelioma cell proliferation [17].

3.3 Other Hippo Pathway Targeting Strategies

Several tyrosine kinase inhibitors (TKIs), including dasatinib and pazopanib, have also been shown to inhibit YAP1/TAZ [37, 38]. Although dasatinib suppressed MM cell growth in vitro and in vivo, clinical trials showed no clinical benefit to patients with MPM. Meanwhile, the anti-parasitic agent ivermectin was shown to inhibit YAP1, thereby inhibiting MM cell proliferation [38].

The induction of synthetic lethality is another promising therapeutic strategy. The best example is the poly(ADP-ribose) polymerase (PARP) inhibitor olaparib, which induces cytotoxicity in breast cancer cells harboring *BRCA1/2* mutations. This phenomenon occurs due to the suppression of endogenous DNA damage repair systems in these cells due to BRCA1 inactivation and PARP inhibition. In this regard, when PARP inhibitors were also tested for MM cell lines, they unexpectedly exerted inhibitory effects on all analyzed lines, regardless of their *BAP1* mutation status [39]. In any event, the identification of genes or agents that induce synthetic lethality in *NF2*-deficient MM cells may provide new therapeutic approaches to MM.

4 YAP1/TAZ as Tumor Suppressors

Since the discovery of the Hippo pathway, YAP1/TAZ have been proposed to be both prooncogenic and tumor-suppressive, depending on the cellular context. We and other groups have mainly focused on the prooncogenic roles of YAP1/TAZ activation for mesothelioma development and progression.

An interesting finding has recently been reported [40] (Fig. 14.2). Ferroptosis is a cell death process driven by cellular metabolism and iron-dependent lipid peroxidation. The enzyme glutathione peroxidase 4 (GPX4) is a central regulator of ferroptosis and protects cells by neutralizing lipid peroxides. Notably, mesenchymal and metastatic cancer cells are susceptible to ferroptosis. Wu et al. demonstrated that ferroptotic cell death is mediated by cell contact such as E-cadherin and the

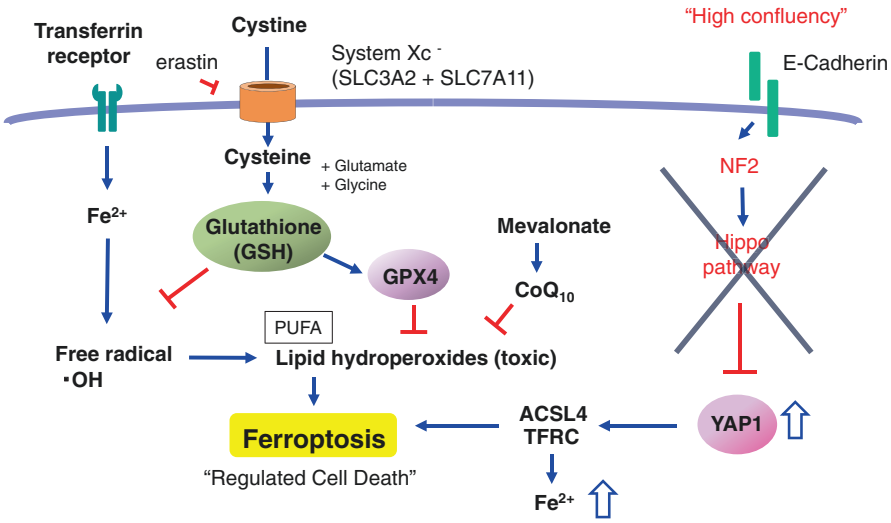

Fig. 14.2 Under merlin (*NF2*)–Hippo pathway inactivation, activated YAP1 contributes to the promotion of ferroptosis. Polyunsaturated fatty acids (PUFA) are susceptible to lipid peroxidation and are necessary for ferroptosis. ACSL4, acyl-CoA synthetase long-chain family member 4; GPX4, glutathione peroxidase 4; TFRC, transferrin receptor

merlin (*NF2*)–Hippo pathway [40]. They found that *NF2* inactivation sensitizes cancer cells to ferroptosis in vitro and in vivo because YAP1 overexpression leads to the upregulation of the ferroptosis modulators ACSL4 and TFRC, indicating that YAP1/TAZ are tumor suppressors that induce ferroptosis. This finding suggested that alterations in merlin (*NF2*)–Hippo signaling could predict the responsiveness of cancer cells to ferroptosis-inducing therapies.

5 Conclusions

Targeting of the merlin (*NF2*)–Hippo pathway, including the approaches described above, represents a promising therapeutic strategy for patients with MM. Since the discovery of the small compound, K-975, which directly inhibits YAP1-TEAD binding, more direct and preclinical studies are expected to analyze how the Hippo pathway can be targeted. One issue to overcome is that the current methodologies including immunohistochemistry are neither very sensitive nor specific for evaluation of merlin (*NF2*)–Hippo pathway status. More precise assays and more effective MM biomarkers are needed to accurately determine which MM cases are associated with activated YAP1/TAZ.

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Part VI
Clinical Features and Management

Chapter 15
Tunneled Catheters or Pleurodesis: How Can We Palliate Effusions for Patients with Malignant Pleural Mesothelioma?

Shamus R. Carr and Joseph S. Friedberg

Abstract Pleural effusions are common in patients with malignant pleural mesothelioma. They can cause dyspnea, decreased quality of life, and may even contribute to weight loss. Palliation, whether as a bridge to treatment or part of a definitive plan, should always be considered. Options for management reside in either pleurodesis or placement of a tunneled pleural catheter. Herein, we discuss the advantages and limitations of each along with current and evolving treatment paradigms to provide evidence to aid in the comprehensive decision management of these patients.

Keywords Pleurodesis · Pleural catheter · Palliation

1 Introduction

Dyspnea related to recurrent pleural effusion is one of the most common presenting symptoms for patients with malignant pleural mesothelioma (MPM). As with most malignant effusions, palliation is almost always indicated, either to save the patient's life or to enhance quality of life. Serial thoracenteses are generally only appropriate for patients who have a life expectancy measured in days or weeks, or with an underlying problem expected to resolve with initiation of treatment—which is not the case with MPM. Consequently, for MPM, a tube-mediated approach is typically indicated. The two options are the placement of a tunneled catheter or pleurodesis.

Establishing a diagnosis for MPM is commonly intertwined with palliation of an effusion. Pleural fluid, obtained during thoracentesis and sent for cytology, is often

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reported as "negative" in a significant percentage of cancer-related effusions, up to 40% in some series [1]. With MPM the inaccuracy of pleural fluid in establishing a diagnosis is further complicated by the fact that sarcomatoid cells tend to shed less into the pleural fluid than the epithelioid cells [2]. Thus, cytology is a notoriously inaccurate way to establish a diagnosis of MPM, either because of a completely false-negative interpretation of the cytology, or subclassification of MPM as epithelioid, when it is actually a biphasic subtype where the sarcomatoid cells were not represented in the pleural fluid. The subtype of MPM is very important, as it not only impacts prognosis, but it may determine whether the patient is a candidate for specific clinical trials and, in many centers, whether the patient is a candidate for surgery-based treatments.

The most accurate way to establish the diagnosis of MPM, including the subtype, is with a thoracoscopic biopsy. This offers the advantage of both establishing a diagnosis and palliating a recurrent pleural effusion, at the same time. This procedure can typically be accomplished through a single one-centimeter incision with a 5-mm video thoracoscope and standard thoracoscopic instrumentation. This procedure, conducted under general anesthesia with a double-lumen endotracheal tube, allows for complete drainage of the effusion, disruption of any loculations, accurate and deep biopsies that capture the interface between the tumor and normal underlying tissue (sometimes necessary to establish invasion as a criteria for diagnosis), and to allow for insufflation of the lung to determine if the lung is entrapped. This last point may be of particular value in helping to decide between tunneled catheter placement or pleurodesis, particularly if intraoperative talc poudrage is being considered. The reason for this is that pleural–pleural apposition is required to affect a pleurodesis and lung entrapment is very common with MPM. Lung entrapment will not only preclude a successful pleurodesis, but if talc is instilled and the pleural space gets infected, then the patient will have an imbedded infected foreign body that results in an intractable empyema that could potentially become a greater threat to their life than the underlying cancer. "Medical" thoracoscopy is another option for obtaining adequate biopsies, though it lacks the robustness of surgical thoracoscopy and is typically performed under conscious sedation and will not allow the same degree of entrapment assessment.

Definitive palliation of an effusion is likely to enhance a patient's quality of life in multiple ways. The most obvious is that, if caught before the lung is completely entrapped, re-expansion of the lung can result in dramatic relief of dyspnea. In some patients, particularly those who are in good enough shape to tolerate a very large effusion, there can actually be mediastinal displacement and tamponade-like physiology contributing to their dyspnea. Patients will often complain of pain, particularly when bending over or with Valsalva maneuvers. The pain is frequently described as vague and "in the back" or maybe to the shoulder, more suggestive of being diaphragmatic in origin. Draining the fluid, even if it only modestly improves breathing, may relieve this symptom. Lastly, especially in patients with large effusions, weight loss sometimes appears attributable to early satiety, presumably from the weight of several liters of fluid being transmitted across the diaphragm and compressing the stomach (Fig. 15.1).

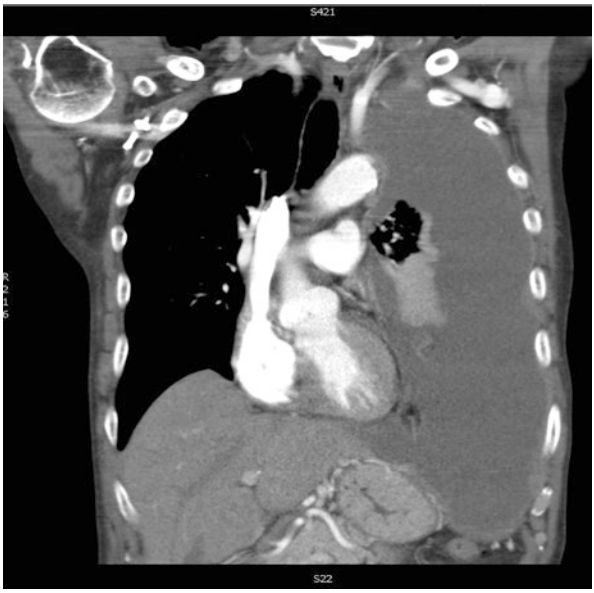

Fig. 15.1 A large left pleural effusion with nearly complete compression of the lung, contralateral displacement of the mediastinum, and eversion of the diaphragm with compression of the stomach

2 Pleurodesis Versus Tunneled Catheter

Once it has been determined that a patient requires durable palliation, the choice is between tunneled catheter placement or pleurodesis. With respect to pleurodesis, talc still appears to be the most popular and, arguably, the most effective agent that is available worldwide [3]. Tunneled catheters have been a major advance in the treatment of this disorder, a study out of China reported the overall response rate of achieving pleurodesis with talc in MPM patients was only 68% [4]. This is similar to separate results published out of Australia [5]. A recent randomized trial found the success rate of pleurodesis with all malignant effusions, regardless of etiology, was 78% [6].

While talc appears to be quite successful in achieving pleurodesis, it does have some issues to consider. First, while rare, a systemic inflammatory response can occur that leaves a patient hypotensive that requires supportive care, sometimes with vasoactive drugs. Talc has also been associated with ARDS. This appears most common with the more finely milled talc, with smaller particle sizes, used in the United States. This appears to be much less common with the more coarsely ground talc, used in Europe [7]. Lastly, as opposed to chemical sclerosants, talc is a permanent foreign body and, should it become infected, can serve as a nidus for infection and potentially result in an intractable empyema. Additionally, after pleurodesis, patients typically require several days of hospitalization for suction therapy until removal of the chest tube.

With the development of the indwelling, cuffed pleural drain (e.g., Aspira, PleurX), talc pleurodesis has become less common. After placement of the catheter, the patient is able to be discharged usually the same day. They can then commence drainage of the effusion as an outpatient. While the risks of placing the catheter and the time in the hospital after placement are less compared to talc pleurodesis, there are a few issues of concern. First, there is a risk of infection that is reported to be between 2 and 8% while the catheter is in place [8–11]. Also, the catheter can be in for a considerable amount of time. While there is no data about the average length of time an indwelling catheter is in for patients with MPM, data for all MPE is around 50 days to achieve pleurodesis [12]. When spontaneous pleurodesis does occur, it occurs in over 50% of cases [11, 13].

The combination of using talc with an indwelling pleural catheter has been proposed as a clinical trial of using talc for outpatient pleurodesis with indwelling catheters (TOPIC Trial). While this idea carries merit to try and decrease the length of time that the catheter is in place and achieve pleurodesis, it has potential pitfalls with clogging the catheter, infection rates, and side effects of the talc. This trial is currently open and results are anticipated.

The choice between pleurodesis and a tunneled catheter needs to be individualized for each patient. If the lung is significantly entrapped, but the patient derives some symptomatic relief from a thoracentesis, then a tunneled catheter is pretty clearly the correct choice. If the lung fully expands, either with thoracentesis or intraoperatively, then both options are reasonable and should be based on preoperative discussion with the patient about the relative merits and risks of both approaches. If the patient is a potential surgical candidate, then pleurodesis does not summarily exclude the patient from surgery, but our preference is for the patients to be palliated with a tunneled catheter.

3 When a Diagnosis Has Not Been Established

When a patient presents with recurrent unilateral pleural effusion and there is clinical concern on axial imaging (e.g., CT Scan) for a thickened parietal pleura, regardless of possible asbestos exposure, a biopsy can help secure the diagnosis. In these cases, we prefer to perform a video-assisted thoracic (VATS) biopsy. We place the anterior port of the camera at a position along a potential thoracotomy. This allows for resection of the port site, as port site recurrence is not uncommon with MPM [14–16]. We attempt the place the surgical telescope and the biopsy forceps through the same incision to minimize possible areas of port site recurrence, and this incision rarely needs to be more than a centimeter in length, if a 5-mm 30-degree thoracoscope is employed.

After the fluid is drained, collected, and sent for cytology, pleural biopsies are taken. This also allows for evaluation of the extent and burden of disease, which may help with discussions with the patient and other members of the treating team such as medical and radiation oncology. It is important that “lesional” tissue be

confirmed by the pathologist on the frozen section. While it is unlikely that a definitive diagnosis can be confirmed on the frozen section, the procurement of sufficient specimens to establish a diagnosis on permanent pathological evaluation can, and should be, confirmed. This is to avoid the "need more tissue" request from the pathologist, which appears more likely to occur with MPM than some other malignancies, as the tumor can have a very bland appearance. Furthermore, especially if the pathologist is unsure if the specimen is malignant on the frozen section, it is important to capture the interface between the tumor and the underlying tissue as demonstration of invasion may be necessary to establish a diagnosis of malignancy.

After the biopsies are completed, an indwelling, tunneled pleural catheter is placed. The technique to place the catheter may hinge on where the chest was entered, which may have been dictated by the safest site of entry as determined by fluid location. Most tunneled catheter kits provide both a removable trocar, that can be used for tunneling, or a Seldinger kit, with a breakaway sheath. Either can be used, at the surgeon's discretion, as long as care is taken to place the catheter exit site at a location that can be easily accessed by the patient and, if the patient is a future surgical candidate, ideally be placed in a location that might lend itself to incorporation into a future incision. This latter point speaks to the propensity for MPM to seed incisions. Regardless, the indwelling portion of the catheter should be positioned in a location where it would be expected to maximally drain the effusion. A general principle, especially in particularly thin patients, is to make sure the catheter is tunneled for several centimeters and bring it out through a separate site, rather than trying to incorporate the catheter into the single VATS incision. This will help avoid leakage and will also allow for more accurate closure of the VATS port. Some advocate placing the polyester cuff closer to the chest entry site, while other advocate placing the cuff closer to the exit site. Theoretically, the former approach would decrease the likelihood that fluid would track along the tube until the cuff is encountered. The disadvantage of this approach is that a counter-incision to remove the tube may need to be performed. If the cuff is placed a centimeter distal to the exit site, then the tube can be easily removed in an office setting, numbing the exit incision with local anesthesia and dissecting free the cuff while the tube is maintained under tension to deliver the cuff to the exit site. Regardless of which approach is employed, it is important for the surgeon to note the location of the cuff in their operative report to guide the planned extraction when the tube has run its course. With only anecdotal evidence, it is our practice to irrigate the VATS incision with povidone-iodine and peroxide, prior to closure, in an effort to decrease the chance of tumor seeding into the incision.

4 How Often to Drain

Recommendations on how often to drain range from changing frequency based upon the drainage amount to a more set schedule. The optimal regimen is unclear. The AMPLE-2 trial [17] did not demonstrate any differences between daily and

symptom-guided drainage regimens for symptom relief. However, daily drainage appeared to be more effective in achieving spontaneous pleurodesis, which may improve the quality of life. In a separate trial, the ASAP (Impact of Aggressive versus Standard Drainage Regimen Using a Long-Term Indwelling Pleural Catheter) trial evaluated daily versus every other day drainage in the time to pleurodesis. While there was no difference in adverse events, autopleurodesis did occur faster in patients drained daily [18].

We currently recommend beginning with daily drainage. We then decrease the frequency of drainage to every other day when the amount drops to less than 100 ml per drainage. When the drainage is below 25 cc per drainage on three consecutive drainages, we stop and assess a week later with a chest radiograph. At that time, if there is no fluid on imaging, the tube is removed.

5 Clogged Drains

There are no published results in the literature about the optimal way to deal with a pleural catheter that is clogged. There are reports, however, of various techniques that have been successful [19–21]. The first thing that needs to be done is confirm that there is fluid present and that autopleurodesis has not occurred. This is usually done with a two-view chest radiograph. However, CT scan can also be used. If there is significant fluid, management begins with flushing the catheter with 10–30 cc of normal saline. This sometimes helps to clear the fibrin deposits that can occur in the channels along the drain. If the catheter flushes easily and there is still minimal return, a trial of tissue plasminogen activator (TPA) combined with dornase (i.e., pulmonzyme) can be instilled. We inject 5 mg of TPA in 50 ml of normal saline combined into a single syringe with 5 mg of dornase. After leaving the tube clamped for an hour, we then attempt drainage. It is important to note the character of the fluid and if there are any concerns for infection to send the fluid for microbiological analysis. If the drain is infected, it should be removed. In our experience, most of the time a clogged drain can be flushed and regain function.

6 Cost Analysis

A final point to consider is the cost of the two modalities. While this has been evaluated in a few articles in the literature [22–25], the data available in the literature is fraught with limitations. At the present time, taking all variables into consideration there is no data that definitively proves whether pleurodesis or pleural catheter is overall more cost-effective. The variable that seems to drive up the cost of indwelling pleural catheters are visiting home nursing time and if the catheter is in for longer than 14 weeks. While indwelling pleural catheter does result in fewer overall hospital days, but this finding is of unclear clinical importance [26].

7 Consideration for Sclerosant Choice

Over the years many different substances other than talc have been tried to achieve pleurodesis after instillation into the pleural cavity. However, today there are only a limited number available and this does differ slightly by country. There does seem to be some differences in achieving pleurodesis with different substances. However, talc routinely has been shown to have a very high rate of successful pleurodesis while its direct cost is very low. Recently, implications of talc-induced malignancy have come to light which raises additional legal concerns. Despite these concerns, purified, graded talc preparations have been shown to be both safe and effective and led to talc being the most commonly used sclerosant [27].

8 Conclusion

Palliation of pleural effusions represents a key component in the care of many MPM patients. Pleurodesis, primarily with talc, was the only long-term palliative option for many years. The development of tunneled catheters has been a significant advance and this has become the most popular option. If a lung is significantly entrapped, then pleurodesis is not an option and a tunneled catheter should be placed, assuming a test thoracentesis results in symptom relief. If the lung does expand, such that pleural–pleural apposition can occur for pleurodesis, then both options are viable. Each approach has its relative merits and risks and a candid conversation with the patient is likely the best way to decide which to employ.

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Chapter 16
Advanced Minimally Invasive Approach with Medical Thoracoscopy for Mesothelioma: What Are the Roles in Diagnosis?

Satoru Ishii

Abstract Medical thoracoscopy can be carried out by an internist under local anesthesia without the need for intubation. Thoracoscopic findings of mesothelioma was various; it has reported nodules, pleural thickening, pachypleuritis, masses, and inflammation. It has recently been reported that cryobiopsy is useful for the diagnosis of hypertrophic type.

Keywords Medical thoracoscopy · Epitheloid type · Sarcomatoid type
Cryoprobe

1 Introduction

Even with examinations such as cytological diagnosis of specimens collected by thoracentesis, the etiology of approximately 15% of pleural effusions remains undiagnosed [1]. Diseases such as malignant mesothelioma are difficult to diagnose. Although cytological diagnosis and blind pleural biopsy with thoracentesis are easy procedures to evaluate pleural disease, the diagnostic yields are reported to be low [2, 3]. Cytological diagnosis is common for carcinomatous pleurisy, the positive cytology ratio is reportedly only 62%, the positive ratio of a blind pleural biopsy is 42%, and the diagnostic yield of the combination of both reaches 74%. The

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diagnostic yields with thoracoscopy under local anesthesia, on the other hand, have been reported as being as high as 79%–96% [4–7].

Thoracoscopy provides a high diagnostic yield. Medical thoracoscopy and video-assisted thoracoscopic surgery (VATS) are two techniques that can be used in the evaluation of undiagnosed exudative pleural effusion.

VATS is performed by a surgeon under general anesthesia. But if lesions are localized in the parietal pleura and diaphragm, medical thoracoscopy can be carried out by an internist under local anesthesia without the need for intubation. McDonald have reported VATS was associated with higher procedure-related average cost than medical thoracoscopy (VATS Canadian dollars $7962 versus medical thoracoscopy Canadian dollars $2815) [8].

The first medical thoracoscopy was introduced in 1910, the first publication appeared in the 1960s [9]. Medical thoracoscopy is less invasive and is frequently performed by internist in recent years. This procedure enables direct visualization of the parietal pleura, while biopsy allows for safe and accurate diagnosis of pleural diseases. With a short examination time of about half an hour, and hence, is more popular. Moreover, the complication rate is also lower, with major complications (pyothorax, pneumothorax, etc.) reportedly occurring in 1.8%, and minor complications (generation of heat, sharp pain, etc.) in 7.3% of patients, indicating that it is a relatively safe procedure [10–12].

2 Procedure of Medical Thoracoscopy

Medical thoracoscopy examinations were performed in the endoscopy room or operating room. For the purpose of pain control, 15 mg of pentazocine was injected intramuscularly before the procedure. After the establishment of a peripheral intravenous line, the patient was placed in the lateral decubitus position, with the side of the pleural effusion being uppermost. In all patients, chest ultrasonography was performed before thoracoscopy to evaluate the pleural effusion. In the world, many hospitals use to injection midazolam for patient comfort sedation [13, 14]. In general, following skin disinfection, local anesthesia is performed above the midaxillary line between the 5th and 7th intercostal spaces, and an incision is made in the skin with 15 to 20 ml of 1% lidocaine. A thin diameter thoracic videoscope (LTF 240 or LTF-Y0032, Olympus Medical Systems, Japan) was used for visualization of the thorax. After examining the thorax, approximately 7 pleural biopsies were performed. At the end of the examination, a 22F chest tube was placed for drainage of the effusion.

The thoracic videoscope is flexible only at the tip. If the fiber is advanced forward with the tip bent, it will leave the biopsy site. Loosen the curve, tilt the fiber slightly, and advance it to the target, and then apply the curve again. If the fiber is tilted too much, the ribs can be damaged, so care must be taken and technical skill is required. Now the LTF-Y0032 enables observation with a maximum angle of curvation of 180°, allowing visualization of the area near the insertion site [15].

Although the diagnostic yield of pleuroscopy under local anesthesia for pleural effusion of unknown origin is high, however, conventional thoracic videoscope has a limited bending angle, resulting in blind spots. LTF-Y0032 bends to a bending angle of 180 degrees and the blind spot decreases.

3 Malignant Mesothelioma

Malignant mesothelioma is exudative pleural effusion. Diagnostic rate is malignant pleural mesothelioma of 20.7% from blind pleural biopsy, 38.7% from a combination of pleural effusion cytology and pleural biopsy, and 98% from medical thoracoscopy have been reported [16, 17]. Malignant mesothelioma is divided into three histologic subtypes; epithelioid (50–70%), sarcomatoid (10–20%), and biphasic type. Malignant mesothelioma (epithelial type) shows hypertrophic changes of the entire pleura and demonstrating unevenness, the vascular irregularities (Fig. 16.1).

Malignant mesothelioma (sarcomatoid type) shows masses. The LTF-Y0032 is capable of observations at a maximum curvation of 180°when directed fully upward, enabling the observation of masses at the introducer insertion site and close to the introducer (Fig. 16.2).

Thoracoscopic findings of mesothelioma was various; Boutin has reported nodules was 39.6%, multiple lesions 32%, pleural thickening 11.2%, pachypleuritis 10.4%, masses 5.6%, inflammation 1.2% [17]. It is difficult to clarify the difference between the sarcomatoid type and epithelium type by thoracoscopic findings. It is important to observe visceral pleura for the staging of mesothelioma [18].

Malignant pleural mesothelioma is divided not only into histologic subtypes but bosselation type and hypertrophic type. Bosselation type is easy to biopsy tissue, hypertrophic type is difficult because thickened pleura includes the adipose tissue.

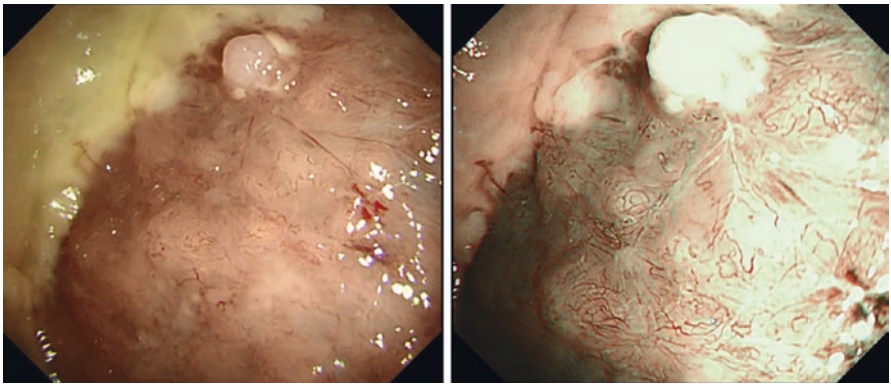

Fig. 16.1 Malignant mesothelioma (epithelioid type). Unevenness of the parietal pleura was observed using LTF-Y0032, and narrow-band imaging demonstrated that bosselated lesion was seen with a network of blood vessels

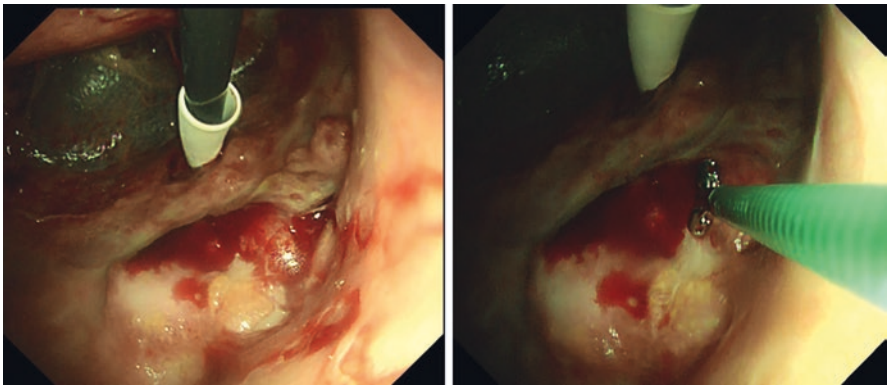

Fig. 16.2 Malignant mesothelioma (sarcomatoid type). LTF-Y0032 is capable of observations at a maximum curvature of 180° when directed fully upward, enabling the observation of masses at the introducer insertion site and close to the introducer. Biopsy forceps were inserted and moved near the mass, which was close to the introducer

It has recently been reported that cryobiopsy is useful for the diagnosis of hypertrophic type [19, 20]. ERBE 1.9 mm cryoprobe (ERBE CRYO2 system; Erbe Elektromedizin GmbH, Tubingen, Germany) cooled by nitrous oxide or carbon dioxide can rapidly freeze surrounding tissue. Thickened flat type lesions of malignant pleural mesothelioma cannot be diagnosed with conventional biopsy forceps. It is necessary to collect not only the pleural surface but also the subpleural fat tissue, and a cryoprobe is useful for that purpose.

4 Conclusion

Medical thoracoscopy enables lesions to be observed and biopsied with direct observation, improving the diagnostic yield. The main objectives of medical thoracoscopy are to observe the thoracic cavity in cases of pleural effusion and to obtain a confirmed diagnosis by parietal pleural biopsy. It has recently been reported that cryobiopsy is useful for the diagnosis of hypertrophic type of mesothelioma.

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Chapter 17
Imaging Evaluation of Local Tumor Growth in Malignant Pleural Mesothelioma: What Is the Role of Imaging Modalities in Curative Intent Surgery for Mesothelioma?

Kazunori Okabe

Abstract Malignant pleural mesothelioma (MPM) is well known as a dreadful disease with very poor prognosis. The available options for the treatment of MPM include either one or a combination of chemotherapy, surgery, radiotherapy, and immune checkpoint inhibitor. Extrapleural pneumonectomy (EPP) or pleurectomy/decortication (P/D) is indicated for operable MPM. However, it is difficult to decide if a case is operable or inoperable. The decision may vary among thoracic surgeons and medical oncologists, and usually factors such as imaging diagnosis, blood tests, pulmonary function, cardiac function, performance status, and past medical history should be taken into consideration. Of these, imaging diagnosis is one of the most important factors.

Our first-line treatment strategy for operable MPM has been upfront EPP with adjuvant hemithoracic radiotherapy followed by chemotherapy. The use of this strategy on 29 consecutive cases of epithelioid MPM between 2011 and 2018 was shown to have a 5-year survival rate of 43% and a median survival period of 58.9 months. Moreover, there have been significant improvements in prognosis, with a median survival of almost 5 years. In this chapter, the images of four long-term survivors are shown. For curative intent surgery for MPM, minute image reading of the computed tomography (CT) and ^{18}F-fluorodeoxyglucose-positron emission tomography (^{18}F-FDG PET)/CT is important to evaluate resectability.

Keywords Malignant pleural mesothelioma (MPM) · Extrapleural pneumonectomy (EPP) · Image diagnosis · Imaging · Resectablility

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1 Introduction

MPM is a dreadful disease with very poor prognosis [1]. Extrapleural pneumonectomy (EPP) [2–8] or pleurectomy/decortication (P/D) [7, 9] can be performed for operable MPM. Imaging diagnosis [10, 11] is one of the most important factors to decide the operability and staging [12, 13]. The decision is tough not only for radiologists and medical oncologists but also for thoracic surgeons. International Mesothelioma Interest Group (IMIG) guidelines [14] concluded that EPP or P/D for MPM should be selected on the basis of disease distribution, institutional experience, and surgeon preference and experience. The judgment on disease distribution is mainly based on imaging diagnosis, but it can be difficult even for experienced thoracic surgeons.

According to the International Association for the Study of Lung Cancer mesothelioma database [15], the median survival of stage I MPM was much better after EPP than after P/D (40 months vs. 23 months). Our standard treatment strategy for operable MPM has been upfront EPP with adjuvant hemithoracic radiotherapy at 45–50.4 Gy followed by chemotherapy with cisplatin plus pemetrexed. In cases wherein EPP is inappropriate, P/D with adjuvant chemotherapy is usually performed. Although the prognosis of MPM has been widely known to be dismal, it has been significantly improving at our hospital. In fact, analysis of 29 consecutive epithelioid MPM patients who underwent EPP between 2011 and 2018 showed a 5-year survival rate of 43% and a median survival period of 58.9 months. In this chapter, the images of four long-term survivors who underwent our standard treatment, including EPP, are shown.

2 Figure 17.1 (a, b), **Left Epithelioid MPM, 58-year-old Man**

A 58-year-old man visited another hospital because of back pain. Chest X-ray and CT showed pleural effusion and thickening on the left. Video-assisted thoracic surgery (VATS) with pleural biopsy on the left revealed a pathologic diagnosis of epithelioid MPM. The patient was referred to our hospital, where repeat CT scan revealed left pleural thickening and para-aortic lymph node swelling (Fig. 17.1 (a,b)). He subsequently underwent left EPP for a total operation time of 6 h and 56 min. No blood transfusion was needed during the EPP. He developed mild interstitial pneumonia as a postoperative complication. The pathologic diagnosis and IMIG stage [10] were epithelioid MPM and T3 (pericardium) N0M0, stage III. He completed 45 Gy hemithoracic radiotherapy followed by chemotherapy. At 6 years and 7 months after the EPP, he eventually died due to mesothelioma.

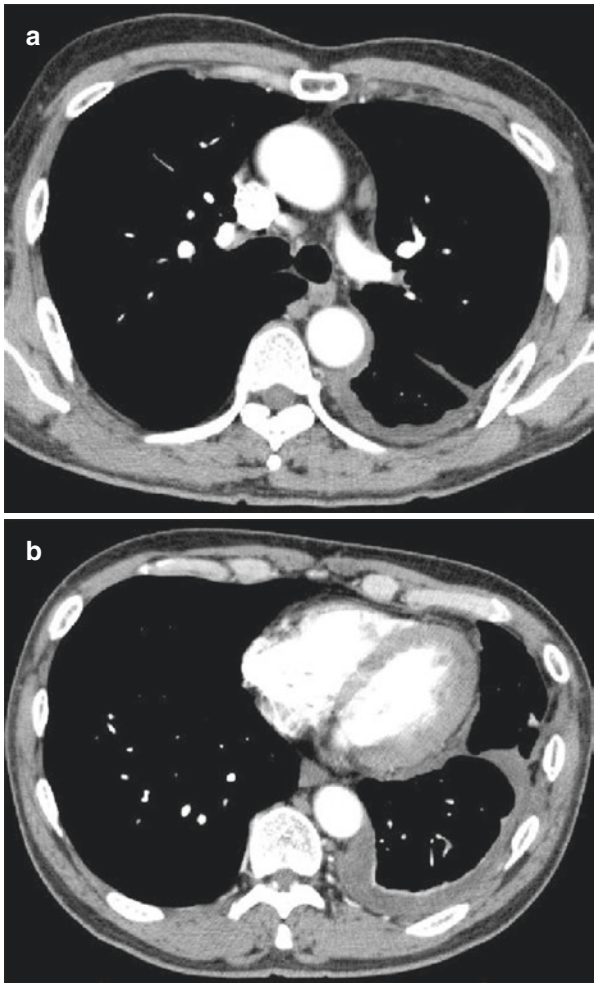

Fig. 17.1 (**a**, **b**) CT of left epithelioid MPM which revealed left pleural thickening and para-aortic lymph node swelling

3 Figure 17.2 (a,b), **Right Epithelioid MPM, 60-year-old Woman**

A 60-year-old woman visited another hospital because of cough. Chest X-ray and CT showed right pleural effusion and mild thickening. The pathologic diagnosis of the right-sided VATS pleural biopsy was epithelioid MPM. She was referred to our hospital, where repeat CT scan revealed right pleural thickening (Fig. 17.2 (a, b)). She underwent right EPP for a total operation time of 6 h 43 min. No blood transfusion was needed during the EPP. Postoperatively, she developed atrial fibrillation,

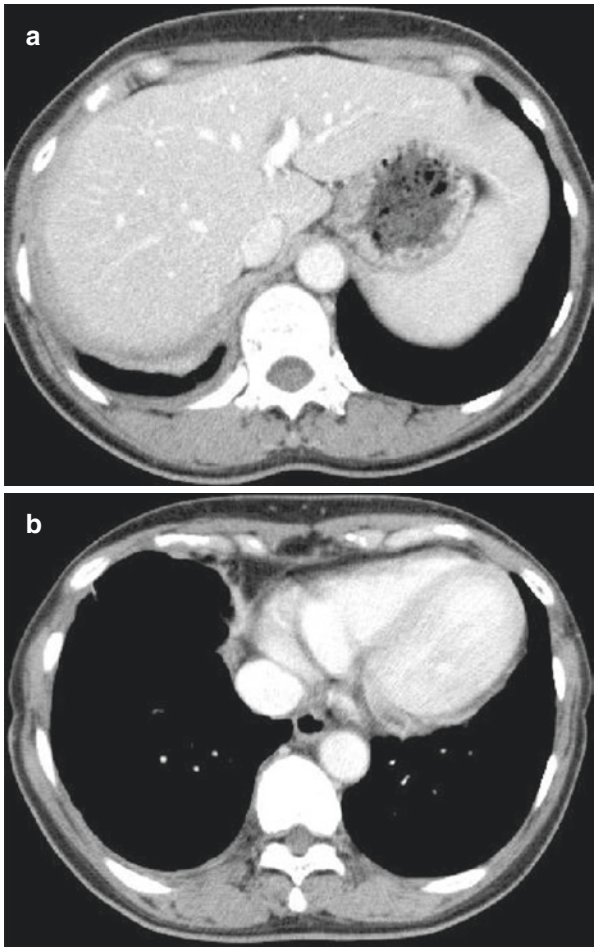

Fig. 17.2 (**a**, **b**) CT of right epithelioid MPM which showed right pleural thickening

which was medically controlled. The pathologic diagnosis and IMIG stage [10] were epithelioid MPM and T3 (pericardium) N0M0, stage III. She completed 45 Gy hemithoracic radiotherapy followed by chemotherapy. She passed away due to mesothelioma at 5 years and 9 months after the EPP.

4 Figure 17.3 (a,b), **Left Epithelioid MPM, 51-year-old Woman**

A 51-year-old woman visited another hospital because of chest pain. Chest X-ray and CT showed left pleural effusion and thickening. The pathologic diagnosis of left-sided VATS pleural biopsy was epithelioid MPM. She was referred to our

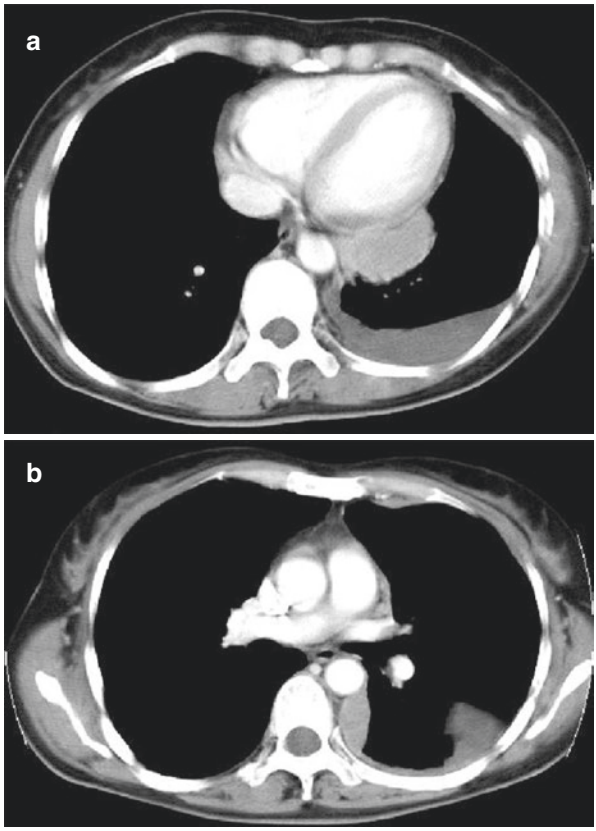

Fig. 17.3 (**a**, **b**) CT of left epithelioid MPM which revealed left pleural effusion, tumor, and thickening

hospital, where repeat CT scan revealed left pleural effusion, tumor, and thickening (Fig. 17.3 (a,b)). She underwent left EPP for a total operation time of just 7 h. No blood transfusion was needed during the EPP. Her postoperative course was uneventful without any complication. The pathologic diagnosis and IMIG stage [10] were epithelioid MPM and T3 (pericardium) N0M0, stage III. She completed 45 Gy hemithoracic radiotherapy followed by chemotherapy. At 12 years and 2 months after the EPP, she is alive and in excellent condition without recurrence.

5 Figures 17.4 (a,b) and 17.5 (a,b), **Right epithelioid MPM, 61-year-old man**

A 61-year-old man, who was a construction worker and was exposed to asbestos fibers, visited another hospital with mild fever. Chest X-ray and CT showed right pleural effusion without pleural thickening or tumor. Thoracentesis revealed right bloody effusion with a cytologic diagnosis of class I. VATS pleural biopsy was not

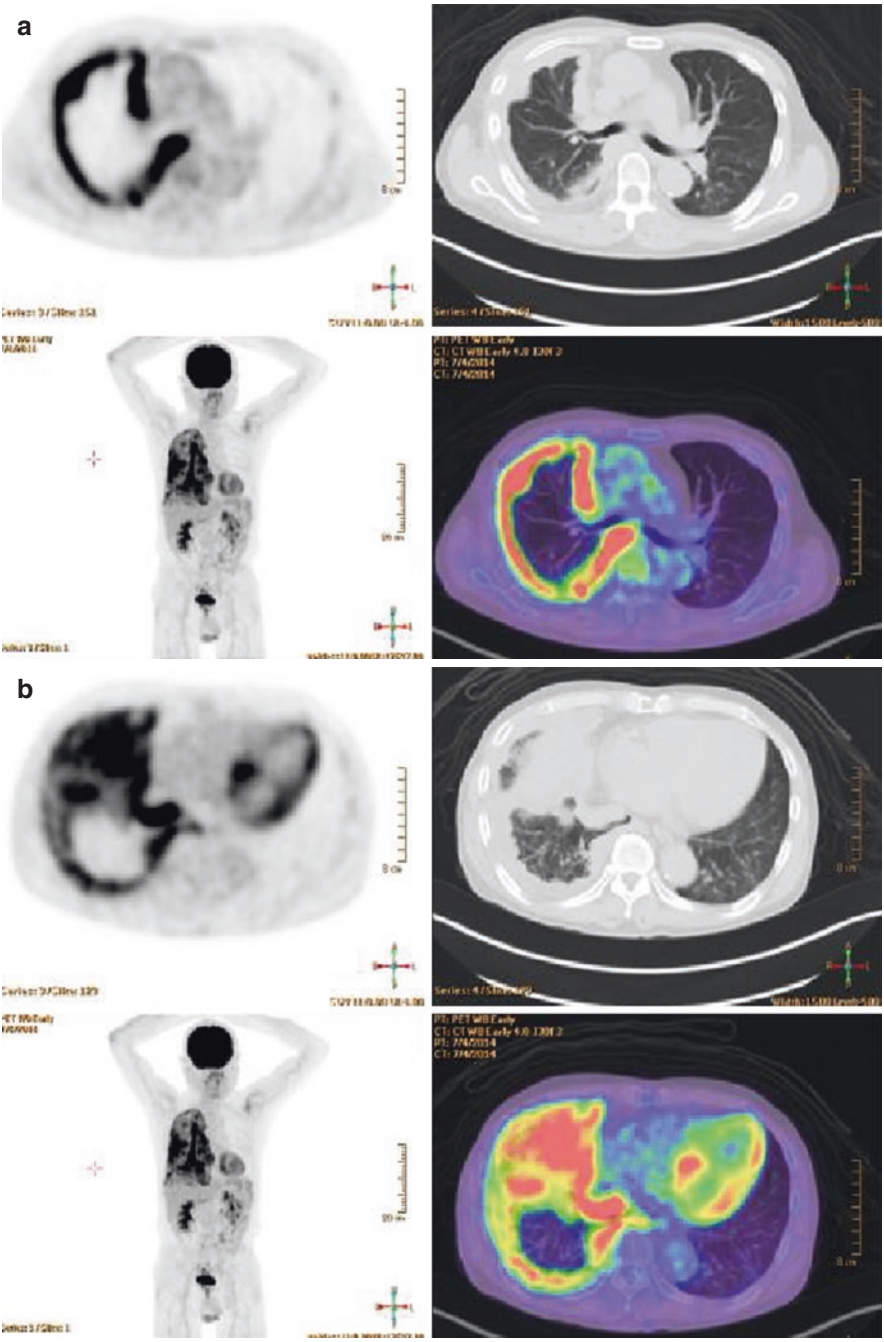

Fig. 17.4 (**a**, **b**) PET/CT of right epithelioid MPM which showed very thick pleural tumor and enlarged subcarinal (#7) lymph node, and both were PET positive

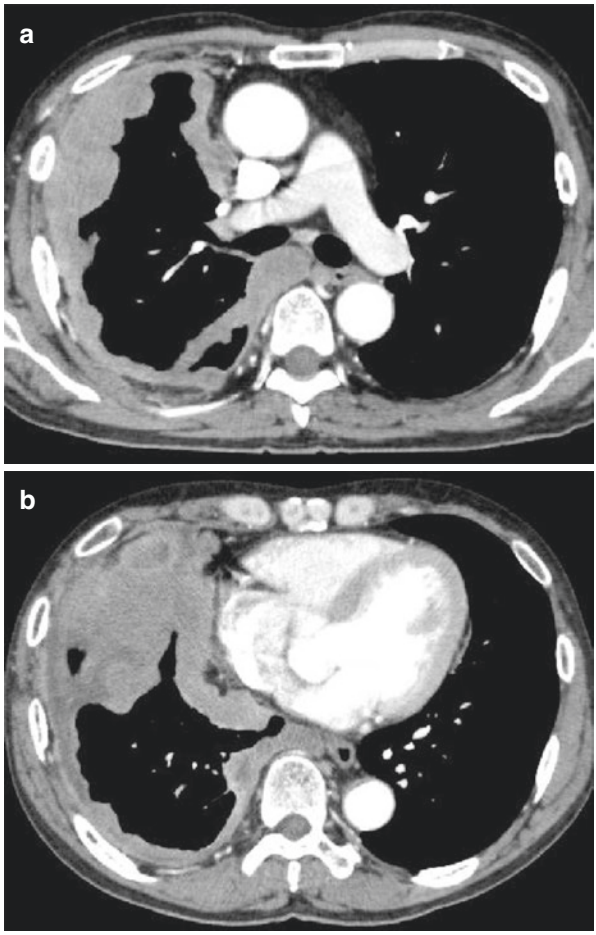

Fig. 17.5 (**a**, **b**) CT of right epithelioid MPM which revealed more than 3-cm right pleural thickening, more than 8-cm right pleural tumor, and enlarged subcarinal (#7) lymph node

performed at that time. After 6 months, he developed bilateral leg edema. Further examination revealed high urine protein and low serum albumin, and he was diagnosed with nephrotic syndrome. Kidney biopsy showed membranous nephropathy. Steroid therapy was started and resulted in remission but did not cure the nephrotic syndrome. Subsequently, he developed steroid-induced diabetes mellitus and atrial fibrillation, which was treated with warfarin.

After 1 year, follow-up CT and ^{18}F-FDG PET/CT showed remarkable pleural thickening on the right. At this time, serum albumin was 2.7 g/dL (normal range, 3.7–5.2 g/dL) and HbA1c was 7.1% (normal range, 4.6%–6.2%). Because of a strong suspicion of MPM, he was referred to our hospital. We suspected that his nephrotic syndrome was a paraneoplastic syndrome. Review of the PET/CT conducted in the preceding month revealed very thick pleural tumor on the right and enlarged subcarinal (#7) lymph node, and both were PET-positive (Fig. 17.4 (a,b)). The CT scan done at our hospital showed more than 3-cm right pleural thickening,

more than 8-cm right pleural tumor, and enlarged subcarinal (#7) lymph node (Fig. 17.5 (a,b)). On admission to our hospital, urinalysis showed 3+ glycosuria sugar and 2+ proteinuria. At this time, he was on prednisolone 20 mg once a day for nephrotic syndrome. Subsequently, right-sided VATS pleural biopsy was performed, and the pathologic diagnosis was epithelioid MPM.

In this case, although performing a major surgery seemed very risky because of nephrotic syndrome, diabetes mellitus, atrial fibrillation, and chronic brain infarction, we judged that EPP was indispensable for the treatment of the MPM, paraneoplastic syndrome, and even diabetes mellitus. Therefore, he underwent right EPP, which was regarded as macroscopic complete reduction, for a total operation time of 7 h and 57 min. Transfusion of 12 units of red blood cells was given during the EPP. He was extubated in the operating room, and he began drinking, eating, and standing up on the second postoperative day. The pathologic diagnosis and IMIG stage [10] were epithelioid MPM and T3 (pericardium) N2 (#7 and pericardial) M0, stage III. Five station #7 lymph nodes were dissected, and all of them were metastatic. Moreover, one pericardial lymph node was positive. The asbestos body count was 159,579 per 1 g of dried lung.

The postoperative complications were recurrent atrial fibrillation, mild carbon dioxide narcosis, and mediastinal shift to the contralateral side; all these were treated successfully. One week after EPP, the proteinuria resolved and the nephrotic syndrome was cured. There was improvement of diabetes mellitus after tapering and discontinuation of the steroid. He completed 50.4 Gy hemithoracic radiotherapy followed by chemotherapy. At 6 years after the bloody pleural effusion was detected and at 4 years and 9 months after the EPP, he passed away due to mesothelioma. Despite the very advanced MPM with mediastinal lymph node metastases, he survived for a long time.

6 Conclusions

Our standard treatment strategy of upfront EPP with adjuvant hemithoracic radiotherapy followed by chemotherapy has improved the prognosis of operable MPM. For epithelioid MPM, this strategy was shown to have a 5-year survival rate of 43% and a median survival period of 58.9 months. In fact, successful treatment and survival for more than 5 years were possible even in patients with very advanced MPM. As demonstrated by the four long-term survivors presented in this chapter, minute image reading of the CT and ^{18}F-FDG PET/CT by thoracic surgeons, as well as radiologists and physicians, would be necessary to evaluate tumor invasion and assess resectability during curative intent surgery for MPM.

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Chapter 18
18F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography in Malignant Pleural Mesothelioma: What Is the Role in Mesothelioma Detection and Treatment Response Assessment?

Kazuhiro Kitajima, Hiroshi Doi, and Kozo Kuribayashi

Abstract Integrated positron emission tomography/computed tomography (PET/CT) with 2-[^{18}F]fluoro-2-deoxyd-glucose (^{18}F-FDG) has emerged as a powerful tool for combined metabolic and anatomic evaluations in clinical oncologic imaging. ^{18}F-FDG PET/CT is also a useful tool to manage patients with malignant pleural mesothelioma, including diagnosis, initial staging, and treatment response assessment. However, a further improvement about PET is desirable.

Keywords FDG · PET/CT · Malignant pleural mesothelioma · Diagnosis Staging · Treatment response assessment

1 Introduction

Malignant pleural mesothelioma (MPM) is the most common primary malignancy of the pleura. MPM arises from mesothelial cells that cover the lung and chest wall and is strongly associated with asbestos exposure, with latency periods ranging from 20 to 50 years. The patient prognosis is poor, with a median survival of 9–17 months, and the disease is often fatal in 4–8 months if untreated [1]. There is

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https://doi.org/10.1007/978-981-15-9158-7_18

no universally accepted standard therapeutic regimen. Advances in the treatment of patients with MPM over the past few years include a unified staging system, novel targeted agents, improved radiation therapy techniques for local control, and decreased morbidity and mortality in patients who undergo curative surgical resection [2]. In addition, the failure of single-modality therapy has led to the increasing use of multimodality regimens combining chemotherapy, radiotherapy, and surgery.

MPM involves the parietal and visceral pleural layers and can spread along the interlobar fissures, diaphragm, mediastinum, pericardium, and into the peritoneum. MPM can invade the lung directly or by the interstitial and alveolar spread. Local invasion can involve the extrapleural fat with extension into the chest wall soft tissues and ribs. Lymphatic dissemination is common, and mediastinal nodes are involved in 50% of cases [3]. Extrathoracic metastatic disease was observed at autopsy in 50–80% of cases [4]. Distant hematogenous metastases are common and can involve the lungs, liver, spleen, adrenal glands, lymph nodes, bones, and brain.

Knowledge of the strengths and limitations of computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET)/CT with 2-[^{18}F]fluoro-2-deoxy-d-glucose (^{18}F-FDG) is essential in the appropriate management of patients with MPM (diagnosis, initial staging, therapy planning, treatment response assessment, re-staging, and prognostication). This review discusses the current and future roles of ^{18}F-FDG PET/CT in the management of MPM, focusing diagnosis, initial staging, and treatment response assessment.

2 Diagnosis

Cytologic evaluation of pleural fluid and needle aspiration pleural biopsy show poor sensitivity for the diagnosis of MPM (26 and 20.7%, respectively) [5]. If malignant cells are found in these specimens, differentiating MPM from adenocarcinoma or severe atypia can be difficult. In contrast, improved diagnostic accuracy has been shown with image-guided core needle biopsy (77% with ultrasound guidance and 83% with CT guidance) [6]. When a larger diagnostic specimen is needed, options include Cope needle biopsy, video-assisted thoracoscopic surgery (VATS), and open biopsy. VATS has a diagnostic rate of 98%, but can only be performed when the visceral and parietal pleural surfaces do not adhere. The rate of chest wall seeding is 50% for VATS compared with 22% for image-guided biopsies [5, 6].

Increasingly, ^{18}F-FDG PET/CT is often used for the diagnosis MPM. PET/CT findings in MPM commonly include unilateral circumferential or near-circumferential pleural and fissural thickening that shows ^{18}F-FDG avidity (Figs. 18.1, 18.2, 18.3). Using a visual analysis or semiquantitative measurements (maximum standardized uptake value [SUVmax]), many groups have demonstrated the clinical utility of ^{18}F-FDG PET and PET/CT for discriminating MPM from

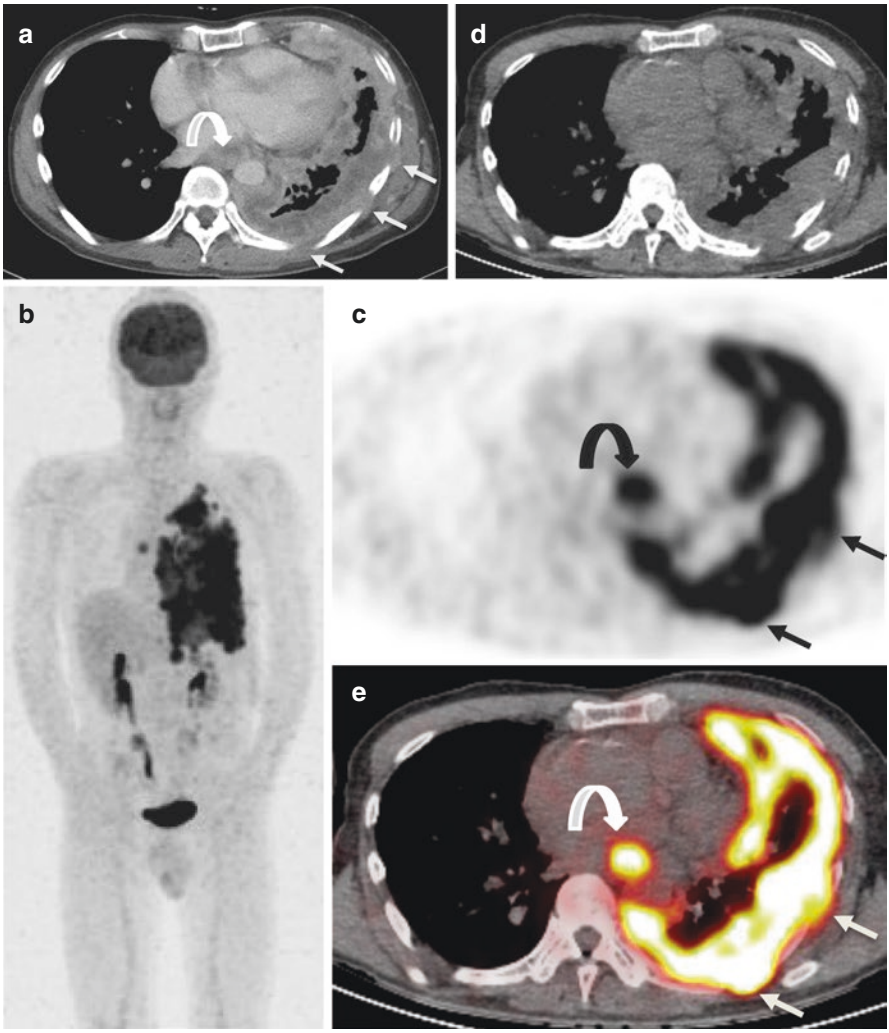

Fig. 18.1 A 56-year-old man with malignant pleural mesothelioma and mediastinal lymph node metastasis (cT4N2) at initial staging. (**a**) Contrast-enhanced CT shows nodular pleural thickening in the left hemithorax with invasion of the left anterolateral chest wall and the left rib (arrows) (arrows). A swollen lymph node (21 × 30 mm) is seen in the mediastinum (curved arrow), suggesting the presence of nodal cancer spread. (**b**) A maximum intensity projection (MIP) of the ^{18}F-FDG PET image shows multiple intense uptakes in the left hemithorax and mediastinum. (**d**) ^{18}F-FDG PET, (**c**) CT and (**e**) fused PET/CT show intense ^{18}F-FDG uptakes corresponding to nodular pleural thickening in the left hemithorax and swollen mediastinal node (curved arrow), confirming nodal metastases. Invasion of the left anterolateral chest wall and the left rib (arrows) is not so apparent relative to contrast-enhanced CT

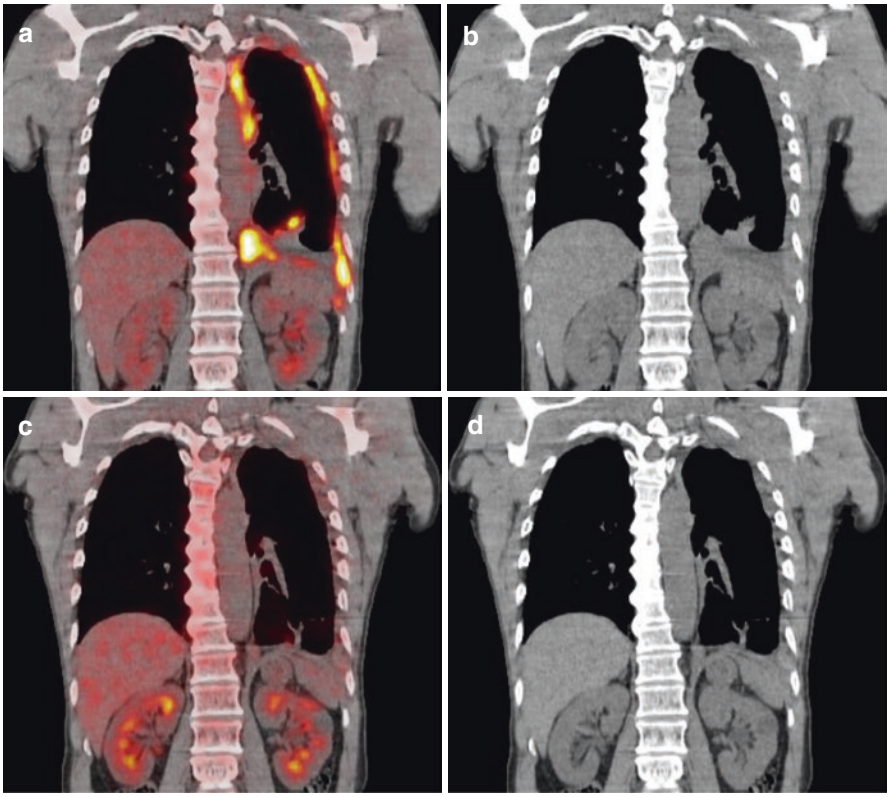

Fig. 18.2 A 66-year-old man with malignant pleural mesothelioma at the chemotherapy response evaluation. Initial coronal reformatted fused (**a**) ^{18}F-FDG PET/CT and (**b**) the CT portion show moderate ^{18}F-FDG uptakes corresponding to nodular pleural thickening in the left hemithorax. Coronal reformatted fused (**c**) ^{18}F-FDG PET/CT and (**d**) the CT portion after three cycles of chemotherapy show decreased left pleural thickening and almost no ^{18}F-FDG uptake. ^{18}F-FDG PET/CT clarifies the great response achieved with chemotherapy (complete metabolic response: CMR)

inflammatory conditions and benign pleural tumors with a sensitivity of 60–100%, specificity of 62–100%, and accuracy of 84–98% [7–17] (Table 18.1). Unfortunately, ^{18}F-FDG PET imaging has poor sensitivity for subcentimeter cancers, low volume MPM (Fig. 18.4), low-grade variant of MPM because of the limited spatial resolution of current PET/CT cameras, which is around 5–6 mm [18]. Specificity can also be altered. ^{18}F-FDG uptake is observed in several inflammatory conditions including pleuritis, chronic granulomatous inflammation, benign asbestosic plaque, parapneumonic effusion, talc pleurodesis, and some benign tumors, such as a solitary fibrous tumor [19, 20]. National Comprehensive Cancer Network (NCCN)

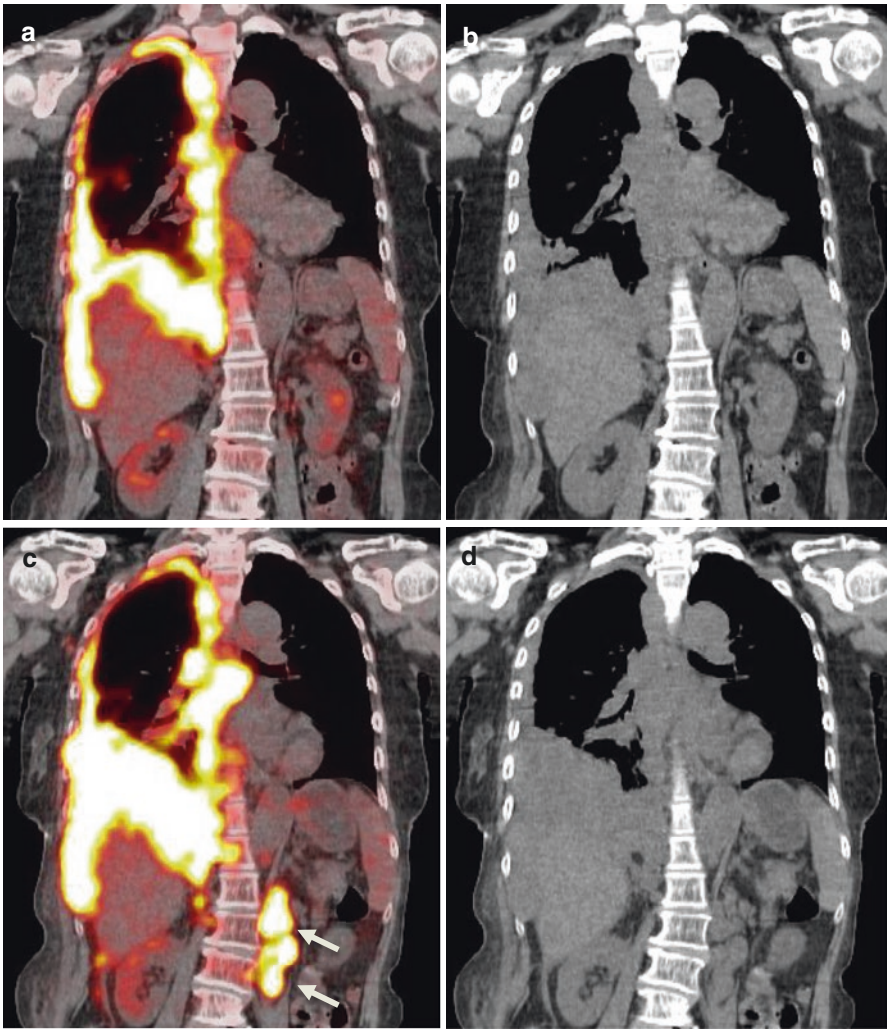

Fig. 18.3 A 65-year-old woman with malignant pleural mesothelioma at the chemotherapy response evaluation. Initial coronal reformatted fused (**a**) ^{18}F-FDG PET/CT and (**b**) the CT portion show strong ^{18}F-FDG uptakes corresponding to nodular pleural thickening in the right hemithorax. Coronal reformatted fused (**c**) ^{18}F-FDG PET/CT and (**d**) the CT portion after three cycles of chemotherapy show right pleural thickening and strong ^{18}F-FDG uptake. Several abdominal nodal swelling with strong ^{18}F-FDG uptake appear (arrows). ^{18}F-FDG PET/CT clarifies the progressive response to chemotherapy (progressive metabolic disease: PMD)

Table 18.1 Studies evaluating the discrimination malignant pleural mesothelioma from benign and inflammatory pleural disease by 18F-FDG PET(/CT)

Authors	Reference	Year	N	Cut-off	Sen	Spe	Acc	18F-FDG PET or PET/CT
Bury	[7]	1997	25	Visual	100	78	92	PET
Bénard	[8]	1998	28	2.0	91	100	93	PET
Carretta	[9]	2000	14	Visual	92	100	92	PET
Gerbaudo	[10]	2002	15	Visual	97	80	94	PET
Kramer	[11]	2004	32	Visual	95	92	94	PET
Duysinx	[12]	2004	98	Visual	97	89	94	PET
Yildirim	[13]	2009	31	2.2	94	100	96	PET/CT
Orki	[14]	2009	83	3.0	100	95	98	PET/CT
Terada	[15]	2012	76	3.5	60	93	NA	PET/CT
Elboga	[16]	2012	50	Visual	92	62	84	PET/CT
Abe	[17]	2012	90	2.0	97	88	91	PET/CT (Early)
					100	88	92	PET/CT (Delay)

N: number of patients, Sen: sensitivity, spe: specificity, Acc: accuracy, 18F-FDG: 2-[18F]fluoro-2-deoxy-d-glucose, PET: positron emission tomography/computed tomography, NA: not available

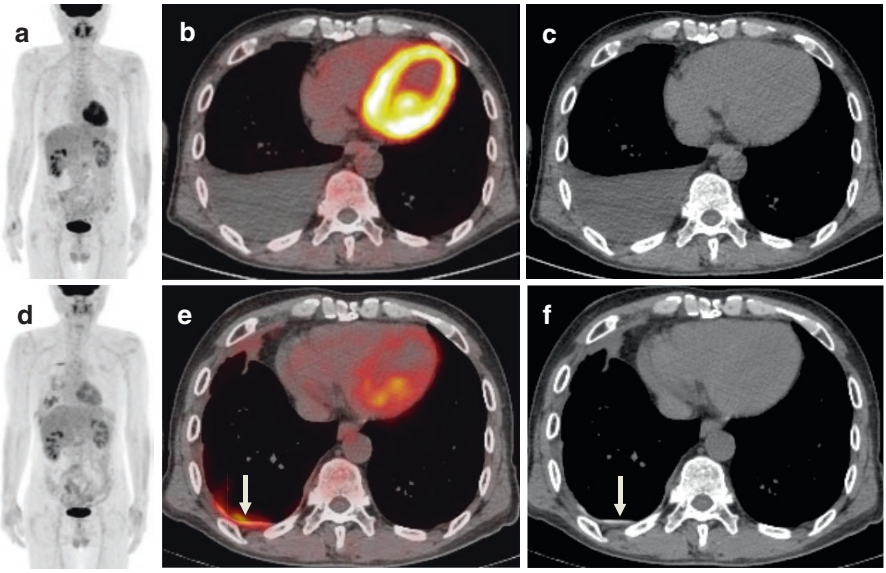

Fig. 18.4 A 68-year-old man with malignant pleural mesothelioma (cT1N0M0) who underwent ^{18}F-FDG PET/CT scans before and after talc pleurodesis and neoadjuvant chemotherapy (NAC). (**a**) MIP of ^{18}F-FDG PET before talc pleurodesis and neoadjuvant chemotherapy (NAC) shows no abnormal FDG uptake of the right pleura. Initial axial (**b**) fused ^{18}F-FDG PET/CT and (**c**) the CT portion before talc pleurodesis and NAC show pleural effusion with no abnormal FDG uptake. (**d**) MIP of ^{18}F-FDG PET after talc pleurodesis and NAC shows several abnormal FDG uptakes of the right pleura. (**e**) Axial ^{18}F-FDG PET/CT and (**f**) the CT portion after talc pleurodesis and NAC shows moderate ^{18}F-FDG uptake in the high attenuation area of right pleural thickening (arrow), reflecting nonspecific ^{18}F-FDG uptake for benign granulomatous inflammatory processes due to talc pleurodesis

guidelines suggest that staging with ^{18}F-FDG PET should be performed before the enforcement of pleurodesis [2]. The use of dual time point ^{18}F-FDG PET, which includes a delayed acquisition at 90–120 min after injection, has been reported to increase sensitivity and specificity [17]; however, the result is not satisfactory. The SUVmax of sarcomatoid histologic MPM was significantly higher than that of epithelioid histologic MPM [15].

Moreover, ^{18}F-FDG PET/CT can be used to plan image guided and surgical biopsies because the sites of greatest ^{18}F-FDG uptake and/or the most accessible sites can be identified and targeted for tissue sampling.

3 Staging

A clinically and pathologically accurate staging system is essential in the selection of homogeneous groups of patients with similar prognoses for entry into clinical trials to better assess new treatment options. The staging system proposed by the International Mesothelioma Interest Group was accepted by the International Union Against Cancer and the American Joint Commission on Cancer [2, 21]. This system describes the tumor extent according to the tumor-node-metastasis (TNM) classification. An important role of imaging is identifying unresectable disease, and thus avoiding unnecessary surgical procedures. This includes the distinction between T3 (resectable; a solitary focus of chest wall involvement, the involvement of the endothoracic fascia, mediastinal fat extension, or nontransmural pericardial involvement) and T4 (unresectable; diffuse tumor extension or multiple chest wall foci, direct extension into the mediastinal organs, spine, internal pericardial surface, or contralateral pleura, and transdiaphragmatic invasion) diseases, identifying N3 node involvement, and identifying distant metastasis.

Contrast-enhanced CT remains the primary imaging modality used to evaluate MPM, and efficiently demonstrates the extent of the primary tumor, intrathoracic lymphadenopathy, and extrathoracic spread (Fig. 18.1). ^{18}F-FDG PET/CT can accurately demonstrate extrathoracic lymphadenopathy and metastatic disease (Fig. 18.1). ^{18}F-FDG PET/CT has been shown to improve the accuracy of staging compared with CT alone, primarily by identifying additional lymph node involvement and sites of distant metastasis. In a prospective study of 29 patients, Erasmus et al. [22] noted that the use of ^{18}F-FDG PET/CT precluded surgery in 41% of patients by identifying locally advanced tumors and extrathoracic metastases not seen on conventional imaging. ^{18}F-FDG PET/CT upstaged 70% of patients being evaluated for surgery in one study [23] and changed treatment planning for 33% of patients in another study [24]. Whereas, in the diagnosis of nodal metastases, both contrast-enhanced CT and ^{18}F-FDG PET/CT have several limitations.

4 Therapy Response Evaluation

Currently, contrast-enhanced CT is the gold standard imaging technique in the treatment response assessment of MPM. However, since the pleural lining has a complex shape, CT has difficulties in measuring tumor burden on anatomic imaging. To address the problem associated with the nonspherical morphology of MPM, Byrne, and Nowak [25] proposed a modified Response Evaluation Criteria for Solid Tumors (modified RECIST). However, its use did not completely overcome the difficulties in response interpretation.

^{18}F-FDG PET, a possible tool for metabolic evaluation, has an emerging role in the evaluation of chemotherapy response (Figs. 18.2, 18.3) (Table 18.2). In addition, because changes in metabolic activity generally occur earlier than changes in tumor size during chemotherapy, ^{18}F-FDG PET may be able to detect a response to chemotherapy before there is a measurable change on CT and may allow for the earlier detection of responders and non-responders to chemotherapy. Ceresoli et al. [26] evaluated the predictive value of ^{18}F-FDG PET to assess treatment response after

Table 18.2 Studies evaluating the chemotherapy treatment response of malignant pleural mesothelioma by 18F-FDG PET/CT

Authors	Reference	Year	N	Chemotherapy	PET/CT timing	PET/CT parameters	Response criteria
Ceresoli	[26]	2006	20	Pemetrexed based	Baseline and after 2 cycles of chemotherapy	ΔSUV	RECIST, EORTEC
Francis	[30]	2007	23	Cisplatin and gemcitabine	Baseline and after 1 cycles of chemotherapy	ΔSUV, ΔMTV	mRECIST, EORTEC
Veit-Haibach	[31]	2010	41	Pemetrexed and platinum-based	Baseline and after 3 cycles of chemotherapy	ΔSUV, ΔMTV, ΔTLG	mRECIST, EORTEC
Schaefer	[32]	2012	41	Pemetrexed and platinum-based	Baseline and after 3, 6, 9, 12, 15 cycles of chemotherapy	ΔSUV, ΔMTV, ΔTLG	mRECIST, EORTEC
Tsutani	[28]	2013	50	Cisplatin and pemetrexed	Baseline and after 3 or 4 cycles of chemotherapy	ΔSUV	mRECIST
Lopci	[27]	2015	131	Pemetrexed based	Baseline and after 2 cycles of chemotherapy	ΔSUV, ΔTLG	
Kanemura	[35]	2017	82	Pemetrexed and platinum-based	Baseline and after 3 cycles of chemotherapy	ΔSUV	mRECIST, EORTEC

18F-FDG: 2-[18F]fluoro-2-deoxy-d-glucose, PET: positron emission tomography/computed tomography, Δ: percentage change, SUV: maximum standardized uptake value, MTV: metabolic tumor volume, TLG: total lesion glycolysis, RECIST: Response Evaluation Criteria for Solid Tumors, EORTEC: European Organization for Research and Treatment of Cancer, m:modified

two cycles of single-agent pemetrexed or pemetrexed in combination with carboplatin in 20 patients with MPM and showed a significant correlation ($p < 0.05$) between an early metabolic response defined as a 25% decrease in the SUV and a median time to tumor progression of 14 months for metabolic responders compared with 7 months for non-responders, but no correlation was found between the time to tumor progression and anatomic response as assessed by CT. The same group evaluated the predictive value of ^{18}F-FDG PET to assess treatment response after two cycles of pemetrexed-based chemotherapy in 131 patients with MPM and showed percentage change in SUVmax (ΔSUVmax) was significantly correlated with progression-free survival (PFS) in the entire population ($p = 0.02$) and with both PFS and overall survival (OS) in 65 patients not undergoing talc pleurodesis ($p < 0.01$ for PFS, $p = 0.03$ for OS) [27]. From Japan, Tsutani et al. [28] evaluated the usefulness of the metabolic response by ^{18}F-FDG PET/CT and CT after neoadjuvant chemotherapy to predict prognoses for patients with resectable MPM who underwent extrapleural pneumonectomy and clarified that metabolic responders (an SUVmax decrease of ≥30%) were significantly correlated with OS, whereas no correlation was observed between a modified RECIST response and OS.

Because the SUVmax approach is based only on a single pixel, the use of SUVmax for a diffuse, complex, and often heterogeneous tumor geometry is limited, and moreover considers the total tumor volume. On the other hand, volume-based quantitative PET/CT parameters metabolic tumor volume (MTV) and total lesion glycolysis (TLG), in which TLG can be calculated by multiplying the MTV by the mean SUV, which weights the volumetric burden and metabolic activity of the tumors, are three-dimensional measurements that incorporate total tumor volume and metabolic activity and may potentially be more sensitive than a single pixel approach (SUVmax) [29].

In a prospective study of 23 MPM patients after one cycle of chemotherapy, a Cox regression analysis showed a statistically significant relationship between a decrease in total glycolytic volume and improved patient survival [30]. Neither a reduction in the SUVmax nor CT demonstrated a significant association with patient survival. In a study of 41 patients with MPM [31] proposing new thresholds (< −25%, −25% to −75%, and > −75% reduction) for PET parameters, a decrease in MTV and TLG after three cycles of chemotherapy was associated with improved survival ($p = 0.002$ and $p = 0.01$, respectively). Although CT response after chemotherapy was also significantly related to OS ($p = 0.001$), neither SUVmax nor SUVmean was correlated significantly with OS. The same group [32] studied 41 patients after continued pemetrexed and platinum-based chemotherapy treatment and compared modified RECIST criteria, European Organization for Research and Treatment of Cancer (EORTC) score, SUVmax, TLG, and MTV. They showed that SUVmax had a high variance over time among individual patients, and variations in SUVmax did not predict OS. The morphological response on CT using modified RECIST criteria had the highest correlation with overall and predicted survival up to the 15th cycle of chemotherapy. TLG and MTV, compared to the pre-therapeutic scan, predicted a continuous response and significantly longer overall survival, but these parameters only predicted overall survival up to the sixth cycle.

5 CT and PET Response Criteria

5.1 Modified Response Evaluation Criteria for Solid Tumors (Modified RECIST)

Modified RECIST response criteria using CT in MPM patients were published in 2004 [25] (Table 18.3). In this scheme, the overall tumor burden is assessed by summing a total of six measurements of the maximal tumor thickness perpendicular to the chest wall, with two measurements performed at reproducible landmarks on three different slices at least 1 cm apart. Modified RECIST defines complete response (CR) as the disappearance of all target lesions, partial response (PR) as more than 30% tumor reduction and progressive disease (PD) as more than 20% tumor progression. Stable disease (SD) is defined as −30% to 20% tumor change. For follow-up assessment, all measurements were taken in the same position and same level.

Table 18.3 Summary of response classification of modified RECIST, EORTECT, and PERCIST

	Modified RECIST [25]	EORTEC [33]	PERCIST [34]
Measurable lesions	10 mm at CT	The most FDG uptake lesions by SUVmax normalized by body surface area	Minimum tumor SUL 1.5 times the mean SUL of the liver
Number of lesions	Up to six lesions	Not specified	Up to five lesions
CR/CMR	Disappearance of all target lesions	Complete absence of FDG uptake	Disappearance of all metabolically active tumors (< mean liver activity and indistinguishable from background)
PR/PMR	Decrease in target lesion diameter sum >30%	Decrease in SUVmax >25%	Decrease in SULpeak >30%
SD/SMD	Does not meet other criteria	Does not meet other criteria	Does not meet other criteria
PD/PMD	Increase in target lesion diameter sum >20% or appearance of new lesions	Increase in SUVmax >25% or appearance of new lesions	Increase in SULpeak >30%, increase in TLG >75%, or appearance of new FDG-avid lesions

RECIST: Response Evaluation Criteria for Solid Tumors, EORTEC: European Organization for Research and Treatment of Cancer
PERCIST: Positron emission tomography Response Criteria in Solid Tumors, FDG: fluorodeoxyglucose
SUVmax: maximum standardized uptake value, SUL: standardized uptake lean body mass, TLG: total lesion glycolysis

5.2 European Organization for Research and Treatment of Cancer (EORTC)

EORCT PET response criteria were published in 1999 [33] (Table 18.3). Response was classified on each scan according to the four categories defined in the criteria. Complete metabolic response (CMR) was a complete resolution of ^{18}F-FDG uptake within all lesions, making them indistinguishable from surrounding tissue. Partial metabolic response (PMR) was a reduction in SUVmax of at least 25% after more than one treatment cycle. Progressive metabolic disease (PMD) was an increase of at least 25% in SUVmax or a new ^{18}F-FDG–avid lesion. Stable metabolic disease (SMD) was a response between PMR and PMD.

5.3 Positron Emission Tomography Response Criteria in Solid Tumors (PERCIST)

PET Response Criteria in Solid Tumors (PERCIST) response criteria were published in 2009 [34] (Table 18.3). Peak standardized uptake value (SUVpeak) was calculated in a 1.2-cm-diameter region of interest (ROI) placed on the hottest site of the tumor, then was normalized to SULpeak (SUVpeak × [lean body mass]/[total body mass]). SULpeak was also used to determine whether that value for the tumor was greater than 1.5 times that of mean liver SUL value +2 SD (3-cm-diameter spherical ROI in the normal right lobe of liver). The therapeutic response shown by PERCIST was considered to be a complete resolution of FDG uptake within the lesion at a level less than the mean liver activity and indistinguishable from the surrounding background with no new FDG-avid lesions in a pattern typical of cancer and was determined as CMR. Reduction of a minimum of 30% of SULpeak in the target volume in the same lesion as compared to the baseline measurement was considered to be PMR. PMD was defined as a 30% increase in the SULpeak of FDG uptake, a greater than 75% increase in TLG, or advent of new FDG-avid lesions that were typical of cancer and not related to treatment effect or infection. SMD was defined as disease that did not qualify as CMR, PMR, or PMD. If multiple lesions were present, up to 5 of the hottest lesions were evaluated and the worst objective response was chosen for determination by PERCIST.

Although several groups have compared modified RECIST and EORTC, the superiority of these two criteria is controversial. In the evaluation of the treatment response after 3 cycles of pemetrexed and platinum-based chemotherapy in 41 MPM patients, Veit-Haibach [31] demonstrated that the number of responders/SD/PD were 10/30/1 for modified RECIST and 14/23/4 EORTC. Moreover, Schaefer [32] evaluated continued pemetrexed and platinum-based chemotherapy treatment

response and demonstrated that EORTEC did not predict OS, whereas modified RECIST had high correlation with OS and could predict survival up to the 15th cycle of continued chemotherapy. Kanemura and Kuribayashi et al. [35] compared modified RECIST and EORTEC criteria in the evaluation of the treatment response after three cycles of pemetrexed and cisplatin, or pemetrexed and carboplatin in 82 MPM patients. Although modified RECIST criteria showed PR/SD/PD = 15/62/5 patients, 62 modified RECIST SD patients were classified as metabolic responders (CMR/PMR/SMD = 2/18/24) and metabolic non-responders (PMD = 18) They clarified that median time to progression (TTP) for 44 metabolic responders (CMR/PMR/SMD) was significantly higher than that for 18 metabolic non-responders (PMD) (13.7 months versus 10.0 months, $p < 0.001$) and concluded that FDG-PET/CT may be used to identify non-responders among modified RECIST SD patients. There have been no reports evaluating PERCIST response criteria in MPM patients.

The current methods of ^{18}F-FDG uptake measurement are diverse, and the timing with respect to chemotherapy and thresholds used to define responses vary. Talc pleurodesis has been reported to increase ^{18}F-FDG uptake in the high attenuation areas of pleural thickening (Fig. 18.4), making it difficult to distinguish between benign granulomatous inflammatory processes and malignancies, which may therefore interfere with the post-chemotherapy disease evaluation on FDG-PET/CT [36]. Therefore, further study is required to address these major issues before it is possible to draw definite conclusions on ^{18}F-FDG PET as a tool for monitoring the therapeutic response.

6 Conclusion

^{18}F-FDG PET/CT can allow combined metabolic and morphological assessments of tumors with significant improvements in diagnostic accuracy and considerable impacts on MPM patient management, including diagnosis, initial staging, and treatment response assessment. Further analyses on the development of new PET cameras with higher spatial resolutions and new radiotracers beyond ^{18}F-FDG, the best method of chemotherapy response evaluation, are needed.

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Chapter 19
Palliative Treatment in Mesothelioma: How to Manage Clinical Symptoms in Mesothelioma

Helen Clayson

Abstract Malignant pleural mesothelioma carries a high symptom burden. This chapter discusses palliative care options for the alleviation of suffering due to breathlessness, pain and other symptoms that are related to this devastating disease.

Keywords Mesothelioma · Breathlessness · Pain · Symptoms · Palliation

1 Introduction

Malignant pleural mesothelioma is a tragic condition: despite research into oncological treatments it is still universally fatal, usually within 18 months from diagnosis, and it has a high symptom burden [1]. Reports of the patient's experience of mesothelioma reveal that suffering is multidimensional: the disease impacts on physical, psychological and social domains [2, 3].

Respected international organisations and a recent literature review emphasise the importance of palliative care to people with mesothelioma in order to improve quality of life and reduce distress. Palliation can be provided in parallel with any active oncological treatments [4, 5, 6]. This paper discusses the most common symptoms in mesothelioma and offers suggestions for their alleviation through palliative care.

Definition of palliative care:

> The World Health Organization (WHO) defines palliative care as 'an approach that improves the quality of life of patients and their families facing the problems associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial, and spiritual' [7].

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2 Symptoms in Malignant Pleural Mesothelioma

Breathlessness and/or pain are presenting symptoms in most patients with malignant pleural mesothelioma and pain in this condition tends to increase throughout the disease [1, 2, 6, 8]. A quality of life assessment conducted as part of a chemotherapy trial revealed scores in mesothelioma were worse than those in lung cancer in the following areas: dyspnoea, pain, insomnia, cough, anorexia and fatigue [9]. A retrospective documentary review of medical records of 80 patients with malignant pleural mesothelioma in the UK reported the recorded incidence of physical symptoms as shown in Table 19.1 [2]:

2.1 Breathlessness

The most common presenting symptom is breathlessness. Initially, this is most likely due to a pleural effusion and aspiration relieves symptoms. Pleural effusions tend to recur and pleurodesis is the most effective way of preventing recurrence. Pleurodesis is most effective if performed early in the disease process. If pleurodesis is ineffective a tunnelled indwelling pleural catheter can be used. This can be managed at home and sometimes allows a collapsed lung to re-expand. Pleurectomy or partial pneumonectomy is indicated if there is extensive pleural involvement or if 'fixed lung' has developed [1, 2, 10]. Patients may have comorbidities such as chronic obstructive pulmonary disease or cardiac failure that contribute to breathlessness, and treatment of these conditions should be optimised. A case review of 80 patients who had malignant pleural mesothelioma revealed that 75% had one or more comorbidities and 69% had a history of smoking [2]. Occasionally breathlessness is caused by pericardial effusion due to either primary pericardial

Table 19.1 Incidence of physical symptoms [2]

Symptom	Incidence as % (n = 80 patients)
Breathlessness	96
Pain	91
Cough	41
Weight loss	41
Anorexia	25
Sweating	18
Nausea	14
Fatigue	13
Dysphagia	11
Constipation	10
Ascites	9
Vomiting	6
Painful metastases	6

mesothelioma or extension from the pleura. This can be a medical emergency and is associated with a short survival time [11].

Palliation of breathlessness requires a multimodal approach involving airflow or oxygen, opioids, sedatives such as benzodiazepines and psychological interventions [12, 21].

Breathlessness is a bio-psychosocial phenomenon. The 'lived experience' of breathlessness encompasses physical sensations and psychosocial/emotional factors. It is essential to explain the reasons for breathlessness to patients and restore a sense of mastery in order to counter feelings such as loss of control, stigma and fear of imminent death [2, 12, 13].

Non-pharmacological interventions are helpful. Patients can learn self-help techniques such as pursed lips breathing, use of a handheld fan, pacing activities and use of walking aids when required. For patients who are well enough, pulmonary rehabilitation can be useful [13, 14, 15].

The flow of air from a handheld fan can reduce the sensation of breathlessness in some patients with mesothelioma [16]. Oxygen is not recommended for palliation of breathlessness unless oxygen saturation is low and even then it might not be effective [17].

Morphine is effective in reducing the sensation of breathlessness [18]. It has been traditional to start with a low dose in opioid-naïve patients and then convert to long-acting morphine when stabilised. However an alternative regime has been shown to be safe and effective: starting with a long-acting oral preparation of 10 mg morphine/24 hours and increasing, if required, to 30 mg/24 hours [19]. In patients who are already on morphine for the pain it is normal practice to use the breakthrough dose for breathlessness, although lower doses have been shown to be equally effective [20]. In patients with significant comorbidities, for example, renal impairment, lower doses of morphine should be prescribed e.g. 1 mg orally 4-hourly or less frequently or, alternatively, sublingual or subcutaneous fentanyl or alfentanil can be used because these are not renally-excreted [21].

Breathlessness causes anxiety that exacerbates breathlessness and a vicious cycle develops. Complementary therapies such as relaxation, visualisation, distraction and creative activities can be beneficial and some patients will find counselling helpful. Short-acting benzodiazepines, such as oral lorazepam or intra-nasal midazolam, can be effective [19]. It is important to educate carers about breathlessness and how to help the patient when breathing becomes a problem e.g. with positioning, fetching medication or through reassurance or distraction [1, 2, 12, 21].

Breathlessness is often accompanied by a cough. This might be due to an infection, comorbidities, pleural or pericardial effusion or occasionally due to diaphragmatic irritation by the tumour. Investigations including a chest X-ray should reveal treatable conditions. Cough often persists for a week or two after radiotherapy. If all treatable causes have been excluded simple techniques such as using traditional cough sweets or drinking water when feeling like coughing might be helpful. Cough suppression with oral morphine might be required for refractory cough having excluded treatable conditions [21].

Refractory distressing breathlessness at end of life is managed by the use of centrally acting sedatives such as midazolam, usually administered by continuous subcutaneous infusion to achieve smooth symptom control. Haloperidol and levomepromazine are useful in the more agitated patient [21].

2.2 Pain

Pain occurs in most cases of malignant pleural mesothelioma ([1], [12], and see Table 19.1). In the 1960s Dame Cicely Saunders, pioneer of modern palliative and hospice care, developed the concept of 'total pain' as the suffering that encompasses all the physical, psychological, social, spiritual, and practical problems experienced by a patient [22]. This concept emphasises the need for a multidimensional approach to the palliation of pain and underscores the holistic ethos of palliative care. Mesothelioma is usually a rapidly progressive disease and due to the complex nature of pain in mesothelioma, it can be challenging to treat effectively therefore patients need timely and comprehensive attention to increasing pain with early referral to palliative care [1, 23].

Patients with malignant pleural mesothelioma can experience any or all of various types of pain: localised chest wall pain, diffuse ache, dull background discomfort, and/or severe sharp stabbing pleuritic chest pain; the symptom of pain typically increases in severity as the disease progresses. The pathogenesis of pain in malignant pleural mesothelioma is multifactorial. Pain in mesothelioma is often due to a combination of nociceptive and neuropathic pain. Nociceptive pain develops from the inflammatory malignant process that activates nociceptive receptors in innervated tissues such as pleura, lungs or surrounding soft tissues. Neuropathic pain develops when nerve tissues, for example, the intercostal nerves, are affected by infiltration, compression or destruction by the encroaching tumour. Pain can also occur as a result of invasive procedures such as thoracentesis or decortication when tumour subsequently seeds along the tracks through the chest wall and forms painful chest wall nodules at port sites or surgical scars [1, 6, 8, 12].

The WHO Analgesia Ladder for Cancer Pain Relief was the accepted guidance for cancer pain for many years (Fig. 19.1) [24].

However, newer proposed models such as the Pain Pyramid and the Pain Platform illustrate multimodal approaches to pain control in the light of modern advances in analgesia [12, 25].

In the early stages of malignant pleural mesothelioma, simple analgesics such as paracetamol and/or non-steroidal anti-inflammatory drugs might be sufficient to control pain but pain escalates throughout the illness and, due to its complex nature, usually requires opioid +/− adjuvant analgesics [1, 2, 6].

Morphine is a safe and effective medication when prescribed according to palliative care guidelines. In all cases when prescribing morphine the patient's beliefs and fears concerning morphine need to addressed, low doses used initially and reassurance given that in the context of advancing cancer addiction is unlikely to be a concern. Alternative routes of administration of opioid analgesia such as

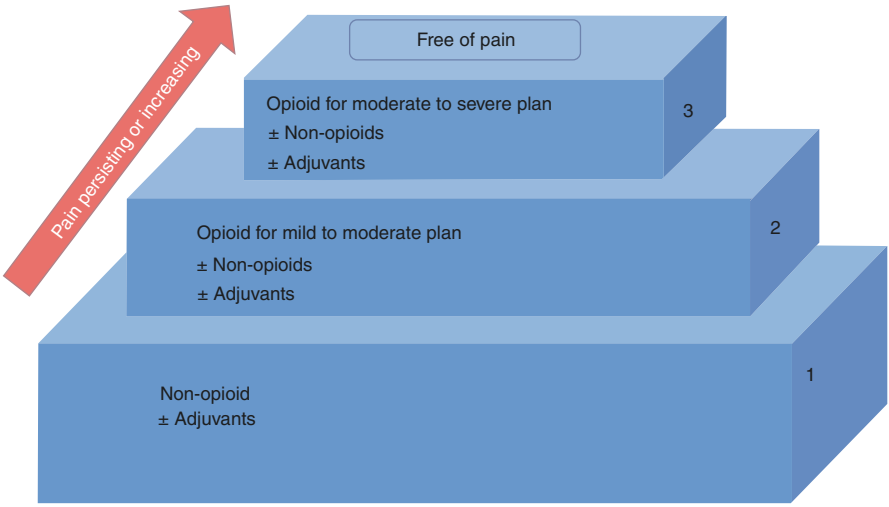

Fig. 19.1 The WHO pain relief ladder. (World Health Organisation 1986 [24])

transdermal fentanyl patches or continuous subcutaneous infusions of morphine or other opioid are useful when the oral route is no longer possible, and especially at end of life. Care is needed when switching opioids to be aware of their comparative strengths and conversion tables are available for reference. Patients on regular opioid medications should always be prescribed an additional short-acting opioid to use for episodic breakthrough pain that creates anxiety and distress and has a negative impact on the quality of life [26, 27].

Adjunct analgesics such as nortriptyline or gabapentin might be prescribed for neuropathic pain, often in combination with morphine or other opioids. However, Bennett et al. (2011) have drawn attention to the relative inefficiency of medications traditionally used for neuropathic pain and emphasise the need to monitor these medications closely and withdraw them if benefit is not evident within 4–8 days [28]. Methadone and ketamine are powerful analgesics with a broad range of action including blocking the NMDA receptor channel but, due to their complex nature, risk of side effects, and interactions with other medications, they should only be used with advice from specialist palliative medicine colleagues [27].

Bone pain is often inflammatory in nature. Non-steroidal anti-inflammatory drugs are useful and radiotherapy is effective and well tolerated. Palliative radiotherapy is also effective for relieving pain from tract site metastases and where there is a chest wall or nerve root involvement [1, 12, 29].

Interventional analgesia can be used for localised control of neuropathic pain, for example, intercostal nerve blocks, preferably under ultrasound guidance to reduce the risk of a pneumothorax [12].

For intractable unilateral pain that is not controlled with medications, there is the option of percutaneous cervical cordotomy in which the contralateral spinothalamic tract is ablated. However, this procedure is not widely available and not all patients with severe pain are suitable for this intervention [30].

A small study investigated the use of radiotherapy to localised chest wall pain using a 20Guy dose in 5 fractions and showed that of 30 patients assessed at week 5 there was an improvement in pain in 47% cases and 5 patients achieved complete pain relief [31]. A randomised control study is in progress to determine the optimum regime [32].

Due to the complex nature of pain and the rapid progression of mesothelioma it is essential to review the patient's analgesia frequently and to respond to changes quickly. Involving the specialist palliative care service is advisable when pain control is challenging or in the face of multiple symptoms or confounding comorbidities including psychosocial distress. Family/informal carers need education and support in managing someone with cancer pain. Clinical nurse specialists, complementary therapies and hospice support can all be helpful to both patients and their families [1, 2].

2.3 Gastrointestinal Symptoms: Anorexia, Weight Loss, Nausea, and Vomiting

These common symptoms can be due to the illness, treatments or both. The cancer cachexia syndrome encompasses anorexia, weight loss, asthenia and anaemia and is related to the interaction of tumour and host factors associated with hypermetabolism, chronic inflammation and hormonal abnormalities [33].

Non-pharmacological approaches include addressing dietary preferences that often change due to the malignancy, eating small portions at frequent intervals, avoiding foods or cooking that have strong smells, using a small plate and seeking advice from a dietician can be helpful. Edible ginger and acupuncture can be effective.

The choice of anti-nauseants/anti-emetics depends on the likely cause of the symptoms. Constipation is a common problem. Investigation of vomiting includes checking blood biochemistry and medication side effects (including opioids). Metoclopramide is often used for its pro-kinetic and central effect on nausea and gut motility. If persistent nausea and/or vomiting occurs it is important to recognise that medications for pain and other symptoms will not be absorbed and so parenteral medications should be used. In many cases, more than one anti-emetic is needed. Standard palliative care guidelines are available online for further advice on management [34].

3 Other Physical Symptoms in Malignant Mesothelioma

Guidance on these symptoms is readily available on evidence-based websites such as the Scottish Palliative Care Guidelines [35].

3.1 Dysphagia

A frequent cause of dysphagia in a debilitated patient with mesothelioma is a candidal infection that is treated with oral nystatin. In some cases, the tumour causes compression of the oesophagus and imaging should be performed to investigate this. Treatment will depend on the nature of the compression and the condition of the patient.

3.2 Sweating

Occasionally unilateral sweating occurs in mesothelioma—the Harlequin syndrome—due to infiltration of the sympathetic nervous supply. If troublesome a stellate ganglion block can be performed [10].

3.3 Constipation

3.3.1 Ascites

Accumulation of excessive peritoneal fluid occurs either in pleural mesothelioma when tumour cells pass through the diaphragm or in primary peritoneal mesothelioma. Management in the palliative setting can be either through paracentesis when required or by insertion of a permanent indwelling peritoneal catheter. This can be managed at home with support from community nursing services [23].

3.3.2 Painful Metastases

Although 55% of patients with mesothelioma were found to have metastases in a large post-mortem series they are rarely clinically significant. Management depends on the site and symptom burden [8].

4 Psychological and Social Suffering

Patients with mesothelioma frequently experience a wide range of psychological difficulties; anxiety, depression and traumatic stress symptoms are common. A full discussion is outside the scope of this paper.

Whilst facing a terminal illness and dealing with multiple physical symptoms, patients (and their families) often struggle with attribution of the illness, anger at

negligent employers or their own past denial of asbestos risk, fear of having contaminated other family members, uncertainty and lack of control, fragmented care, fear of an agonising death, loss of identity as well as frustration with complex medico-legal procedures related to mesothelioma as an industrial disease [2, 3, 36–39].

A recent study of the social impact of mesothelioma reported that the loss of physical functioning and mobility are devastating for patients and create high dependency on carers; this situation takes a toll on both patient and family caregiver in terms of social isolation. Inadequate information about this relatively rare disease is often a problem for patients and their families. Self-help groups and clinical nurse specialists can help to counter social isolation and provide support and reliable information [2, 36–39].

5 Summary

Palliation in mesothelioma is complex and demands a timely and holistic approach. It is imperative to offer the full range of palliative care interventions at an early stage, with specialist palliative care services when necessary, in order to alleviate suffering in patients with this devastating illness.

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Chapter 20
Supportive Care for Advanced Mesothelioma: What is the Role of Mesothelioma Nurses in Clinical Practice?

Yasuko Nagamatsu

Abstract Malignant pleural mesothelioma is a rare asbestos-induced aggressive malignancy of the pleural surface that is incurable, symptomatic, and has a poor prognosis. It causes physical, social, emotional, and spiritual distress on patients as well as their families. To maintain the quality of life of patients and their families, supportive care is essential. Supportive care for advanced mesothelioma includes symptom management, decision-making support, coordination of care, emotional support, victim care, family care, and prevention of asbestos-related diseases. For successful supportive care for advanced mesothelioma, a multidisciplinary team including, pulmonologist, surgeon, oncologist, palliative care specialist, radiologist, psychologist, oncology nurse, home care nurse, pharmacologist, dietitian, lawyer, and supporter of patient support group is required. The role of mesothelioma nurses is to take the initiative in maintaining the quality of care of the patients and their families by providing supportive care as well as by empowering the multidisciplinary team to provide timely supportive care.

Keywords Supportive care · Nursing · Quality of Life · Family care · Victim care

1 Introduction

Malignant pleural mesothelioma (MPM) causes physical, social, emotional, and spiritual distress on patients as well as their families; thus, supportive care is essential. Supportive care in mesothelioma includes symptom management, decision-making support, coordination of care, emotional support, victim care, family care,

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and prevention of asbestos-related diseases. This chapter shows how mesothelioma can impact patients and their families and how mesothelioma nurses can support them to maintain their quality of life (QOL).

2 Why Patients and Their Families Need Supportive Care

Patients and their families require supportive care because MPM is

(a) "Incurable, symptomatic, and has a poor prognosis"
(b) "Rare," and
(c) "Asbestos-induced"

2.1 Incurable, Symptomatic, and Poor Prognosis

MPM is a malignancy that is hard to cure. The survival duration after diagnosis is 8–11 months [1–4]. MPM causes various symptoms such as chest pain, breathlessness, pleural effusion, cough, fatigue, anorexia, weight loss, sweats, and malaise [5–16].

2.2 Rare Disease

MPM is a relatively rare malignancy. MPM may be unfamiliar to patients and their families, healthcare providers, and the public. Reliable information based on evidence about the disease, treatment options, care, and legal benefits in MPM are limited.

Many patients and their families desire to meet other people with the same disease and their families to share their feelings, exchange information, and support each other [17, 18]. Unfortunately, these patients and their families have little opportunities to meet with the other patients. As people lack understanding of MPM, it is difficult for patients and their families to obtain support from the public [8, 17]. Finally, patients and their families tend to be isolated [18].

2.3 Asbestos-Induced

As the majority of MPM is caused by asbestos, patients and their families feel much difficulty as victims because it was not their fault to contract MPM. It is often hard for patients and their families to accept having the disease and face their lives with

the disease. The victim's feelings are often difficult to be understood by the public or even by healthcare providers because they consider that MPM is not the only fatal cancer. However, for patients and their families, asbestos-induced malignancy is extremely unacceptable.

Patients and their families express anger against the society or the company which used asbestos and long for justice [17, 19]. However, lawsuit or the legal procedure involved in securing compensation is a big burden for the patients and their families [8, 9, 17, 18].

3 The Role of Mesothelioma Nurses: Key of Supportive Care in MPM

The role of mesothelioma nurses, which are the keys for supportive care in MPM are as follows:

- Care coordination for symptom management by a multidisciplinary team
- Emotional and victim care
- Support decision-making
- Family care
- Prevention

Supportive care in MPM requires a multidisciplinary team including, pulmonologist, surgeon, oncologist, palliative care specialist, radiologist, psychologist, oncology nurse, home care nurse, pharmacologist, dietitian, lawyer, and supporter of patient support group. The role of mesothelioma nurses is to take the initiative in maintaining the quality of care of the patients and their families by providing supportive care as well as by empowering the multidisciplinary team to provide timely supportive care.

3.1 Care Coordination for Symptom Management by Multidisciplinary Specialists

MPM progresses fast and causes severe symptoms anytime. Healthcare providers prepare to apply the appropriate treatment to be provided by a palliative or pain management specialist working within a multidisciplinary team wherever the patients are. It is important to coordinate the care provided before the patients develop the symptoms.

3.2 Emotional and Victim Care

Healthcare providers must understand the difficult situation the patients and their families face and let them express their anger, sorrow, or anxiety. Genuine sympathy is the greatest support your patients and their families can receive which will enable them to face and live with MPM.

3.3 Support Decision-Making

Healthcare providers should provide a quiet environment, accurate information, a listening ear, timely advice, kind words, and warm hands to hold. Healthcare providers should also allow them to ask any questions.

3.4 Family Care

The families are the second victim. Many families get angry and feel devastated with the diagnosis of MPM.

3.5 Prevention

Prevention is not an actual clinical care. However, the prevention of exposure to asbestos is very important because there is little possibility to develop MPM if people do not get exposed to asbestos. It is crucial to educate people regarding the dangers of asbestos, as well as to promote the protection of people from asbestos by banning its use or preventing exposure.

4 Care Needs and Possible Supportive Care according to the MPM Phases

4.1 On Diagnosis

Patients and their families are anxious during the examination and the time when they are awaiting the diagnosis [9], particularly those who have lost a colleague because of MPM. When a diagnosis of MPM is confirmed, a bad premonition comes. Those who do not know the name MPM are confused by the unfamiliar diagnosis, shocked by the poor prognosis [9, 17], and devastated by the limited

choices of treatment [9, 17]. Patients and their families feel sorry and become angry about having a disease from asbestos [8, 17, 18]. For patients and their families who were just newly diagnosed, it is difficult to understand all the contents given by the physician. To enable these patients and their families to make the best decision, it would be ideal for mesothelioma nurses and other Healthcare providers to provide consultation and counseling to ensure that the patients and their families fully understand the disease and the treatment option with the benefit and risk. Suggestions for second opinion are also helpful. There should also be opportunities for the patients and their families to think about what kind of treatment they want, namely, do they want to try to cure the MPM with aggressive chemotherapy even with heavy side effects? Or do they want to have a relatively shorter life but at home under controlled symptoms? Or do they want to join a clinical trial? This process is an opportunity to think how they want to spend the rest of their life. It is hard for the patients and their families to face the severe prognosis. Treatment by a phycologist is considerable when patients and their families have the symptom of depression which often occurs. Information on legal benefits as well as patients support group should be provided.

4.2 On the Primary Treatment

Patients and their families confront surgery or chemotherapy, hoping that it would kill the tumor. On the other hand, healthcare providers should make preparations for their lives when they returned home. The care involved in chemotherapy is basically the same as that for other cancers. This chapter shows the care of extra-pleural pneumonectomy (EPP) which is a unique treatment for MPM (Table 20.1).

Table 20.1 Care for patients who undergo extra-pleural pneumonectomy (EPP)

In EPP, the surgeon removes the pleura carefully from the chest wall and extracts the diseased lung. Part of the pericardium, diaphragm, and parietal pleura is removed and reconstructed using Gore-Tex.
The following is the recommended care:
Presurgery: to ease anxiety
Postsurgery
• Apply pain management because EPP causes severe pain in a wide area.
• Keep the patient upright 30 degree and apply mouth care because infection of the
remaining lung can be fatal,
• Be cautious for palpitation because then pericardium was removed and replaced.
Rehabilitation
• Explain to the patients and their families that it is normal to have palpitation and breathlessness after EPP, which affect their activities such as eating and toileting.
• Give assurance that the side effects will subside in the future.
• Control bowel movement so as not to pressure the replaced diagraph.
• Coordinate the care after discharge.

4.3 At Home

Patients and their families can have a peaceful life after the heavy treatment, although the patients who had EPP need to manage their symptoms such as pain, palpitation, and breathlessness for months. Healthcare providers must exert all efforts to maintain the QOL of the patients and their families by timely observations and symptom management. It is advisable to emphasize with the patients and their families and allow them to do whatever they want to do before their condition worsens. It is also good for the patients and their families to meet other patients with the same disease.

4.4 Secondary Treatment

Effective treatment with evidence remains limited. The patients and their families who long for cure become impatient or devastated. Information on any available clinical trials for MPM should be given to the patients upon request.

4.5 When No More Effective Treatments Are Available to Cure MPM

At this stage, there are no other measures available to stop MPM from growing. For the patients and their families, it is extremely hard to be informed about this predicament because it indicates that death is near [17]. Some patients want to continue the treatment to survive; however, treatment should be taken only when it has possibility to cure the MPM and the risk of side effects is smaller than the benefit. Healthcare providers should accept the feelings of the patients and their families and explain that non-evidenced treatment affects QOL or can even shorten their lives.

It is extremely important to assure that the patients and their families will be cared for and supported. Healthcare providers should explain that even though there is no effective treatment to cure the MPM, there are still many treatments and care available to manage the symptoms to maintain the QOL. Moreover, end-of-life care options should be presented to allow the patients and their families to make a decision as regards the following:

- Where they want to die? At home? Hospice? Hospital?
- With whom they want to be with when they die?
- How do they want to die? Any plan?

Healthcare providers must understand that it is always hard for the patients and their families to decide at this stage. Once they choose, their decision must be respected.

Please refer to the specialist when the patients and their families have symptoms of depression.

4.6 At the End of Life

Breathlessness, pain, fatigue, and anorexia progress rapidly. Generally, the patients are conscious and are able to eat and talk until the last days before their death. At this stage, it is important to control the symptoms and establish conducive circumstances to allow a peaceful farewell with the family.

4.7 Grief

Losing a loved one because of asbestos-related disease brings a family extra pain. The pain of the familly lasts longer in MPM. There is a risk of prolonged grief [8]. Thus, grief care is very important. One of the suggestions is to share the feelings with a bereaved family of MPM.

5 Conclusion

Patients with advanced mesothelioma and their families require various forms of supportive care such as symptom management, decision-making support, coordination of care, emotional support, victim care, family care, and prevention of asbestos-related diseases. For successful supportive care of patients with advanced mesothelioma and their families, a multidisciplinary team including, pulmonologist, surgeon, oncologist, palliative care specialist, radiologist, psychologist, oncology nurse, home care nurse, pharmacologist, dietitian, lawyer, and supporter of the patient support group is required. The role of mesothelioma nurses is to take the initiative in maintaining the quality of care of the patients and their families by providing supportive care as well as by empowering the multidisciplinary team to provide timely supportive care.

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Part VII
Treatment; Current Standard and Future Perspective

Chapter 21
Antiangiogenic Therapies for Mesothelioma: What Is the Role in Mesothelioma Treatment?

Wieneke Buikhuisen and Paul Baas

Abstract Malignant pleural mesothelioma is known for its correlation between angiogenic factors and survival. In this chapter, we focus on the background of this phenomenon and present the available data of studies with antiangiogenic agents. To date, only limited signals have been found that interventions using these agents are of great impact of the disease. It is concluded that single-agent approaches are futile and should be tested in combination with chemotherapy or in a multimodality setting.

Keywords VEGF receptor · Bevacizumab · Mesothelioma · Angiogenesis

1 Introduction

There is evidence that suggests that angiogenesis, the formation of new blood vessels, is an important determinant in the development and progression of mesothelioma. The ratio of tumor angiogenesis was initially based on the observation of Judah Folkman that growth of solid neoplasms is always accompanied by neovascularization [1]. He stated that the population of tumor cells and the population of capillary endothelial cells within a neoplasm may constitute a highly integrated ecosystem. In this ecosystem, the mitotic index of the two cell populations may depend on each other. Tumor cells appear to stimulate endothelial-cell proliferation and these cells may have an indirect effect on the rate of tumor growth. The rapidity with which tumor implants are able to stimulate cell division in neighboring

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capillary endothelial cells was illustrated in the experiments of Wood [2]. Tumor cells injected into the artery supplying the ear chamber of a rabbit were observed as they entered the capillaries, traversed the capillary wall, and arrived in the extravascular space, where the cells formed a microscopic tumor nodule. Only 18 hours after their arrival, endothelial cell regeneration and the formation of new capillary sprouts were observed to originate in neighboring postcapillary venules. In 1968, it was shown that new capillary sprouts are elicited, even if a tumor implant is enclosed in a Millipore filter chamber. In the laboratory, vasoproliferative activity was consistently seen in hamster cheek pouches adjacent to tumor implants despite the separation of the tumor and its stroma by a Millipore filter, preventing the passage of cells [3]. These studies suggested that some diffusible message was released from the tumor to nearby endothelial cells. These cells are switched from a previously resting, non-regenerating, state to a rapidly dividing group of regenerating cells, capable of forming new capillary sprouts that can grow at the rate of 1 mm per day. It also has been shown that in the absence of neovascularization, most solid tumors stop growing when they are 2–3 mm in size and enter a dormant though viable state. When tumors are removed from this dormant state to an environment that is highly vascularized, however, rapid neovascularization is accompanied by rapid growth. And even after vascularization has been established, the efficiency of diffusion of nutrients diminishes with increasing distance from each capillary.

Probably one of the major "diffusible messages" that Folkman called Tumor-Angiogenesis factor (TAF) turned out to be vascular epithelial growth factor (VEGF), the most powerful endothelial cell-specific mitogen associated with neovascularization. The major components of the VEGF family are VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PIGF), as well as three receptor tyrosine kinases, VEGFR-1, VEGFR-2, and VEGFR-3. VEGF-A, usually referred to simply as VEGF, binds to endothelial cell VEGFReceptors. Binding to VEGFR-2 sets in motion a cascade of intracellular signaling pathways, leading to multiple functions required for sprouting neoangiogenesis, increased cell division, migration, changes in vascular permeability, and promotion of cell survival [4].

There is strong evidence that suggests that neo-angiogenesis is an important determinant in the development and progression of mesothelioma. Microvessel density (MVD), a means of assessing angiogenesis, is higher compared to other common tumors. Moreover, in mesothelioma a high MVD was independently related to poor survival, even if it was adjusted to other known prognostic factors, such as histological subtype and age [5–7]. In preclinical models, VEGF increased proliferation of mesothelioma and antibodies against VEGF and its receptor inhibited mesothelioma growth [5]. In a mesothelioma population, a two- to threefold higher serum levels of VEGF were observed, compared to other tumors or healthy volunteers. Patients with MPM have also been shown to have higher VEGF serum levels compared with those who had been exposed to asbestos, but who have not developed MPM [8]. Angiogenesis, however, is a complex process, regulated not only by the VEGF family but by a variety of other signaling proteins. Expression patterns of platelet-derived growth factor (PDGF) indicate that this also functions as an autocrine growth stimulator in the pathogenesis of malignant mesothelioma [9, 10].

Furthermore, preclinical studies have implicated fibroblast growth factor (FGF) and its receptor in malignant pleural mesothelioma pathogenesis, pointing to its role in cell proliferation and migration [11]. Signaling via Src and Abl kinases have also been shown to be involved in MPM cell migration [12, 13].

2 Inhibition in Angiogenesis: Monotherapy

Most of the earlier trials explored the effect of antiangiogenic therapies as single drugs, predominantly in the relapsed or recurrent setting. A few drugs were selected to be tested in the first-line setting in combination with platinum and pemetrexed. However, the outcome of these studies was generally disappointing, with either a lack of, or only modest clinical benefit or poor tolerability, precluding further development.

Sorafenib was explored in two phase 2 studies as a single agent. Sorafenib is an oral drug and a potent inhibitor of the RAS/RAF/MEK pathway and also targets VEGFR and c-Kit. In the CALCB 30307 [14] patients with mesothelioma who had received 0–1 prior chemotherapy regimens were treated with sorafenib 400 mg orally twice daily continuously. The primary endpoint was a partial response. Fifty-one patients were enrolled, 50 were evaluable and included in the analysis. Three patients had a partial response (6%), which lasted 3, 6, and 6 months, respectively. Two of the three patients who demonstrated a response had not received prior chemotherapy. Twenty-seven patients (54%) had stable disease. The second study included 53 patients after first-line therapy with platinum pemetrexed using the same dosing as in the CALGB trial [15]. A partial response was seen in 6% of patients. Median PFS was 5.1 months, with 36% of patients being progression-free at 6 months. This outcome was considered a moderate clinical activity.

Sunitinib was studied in two phase 2 studies as a single agent. Sunitinib is a multitargeted tyrosine kinase inhibitor, which targets VEGF receptors, PDGF receptors, and c-Kit among others. The primary endpoint was in both studies the partial response rate. Sunitinib was given at 50 mg daily orally for 4 weeks, followed by a 2-week rest. The first study included 35 patients, of which 18 were treatment naïve. Only one partial response with a duration of 3 months was observed in a previously untreated patient [16]. The second study did meet its primary endpoint and enrolled 53 progressive pretreated patients of which 51 were assessable for response [17]. Six patients (12%) had a partial response. These patients received a median of 4 cycles of sunitinib, with two patients receiving 8 and 12 cycles respectively. The conclusion of the authors was that sunitinib had modest activity in mesothelioma and due to the toxicity of the drug, the dose of 50 mg daily was considered too high. Sunitinib was combined with pemetrexed and cisplatin in a phase I study with an expanded cohort in 10 NSCLC and 1 mesothelioma patients [18]. It was concluded that sunitinib was not tolerated at 37.5 mg continuous daily dosing with standard pemetrexed and cisplatin doses. The one patient with mesothelioma had a partial response more than 18 weeks, the degree to which sunitinib was implicated in the

partial response remains unclear, since pemetrexed and cisplatin are standard treatments for mesothelioma.

Cediranib was studied in two single-arm phase 2 studies after first-line chemotherapy. Cediranib is an oral TKI of VEGFR-1,-2, and -3 as well as c-Kit and PDGFR-β and was given in a dose of 45 mg/day until progression. The SWOG S0509 included 54 patients, 47 evaluable patients showed a PR of 9% (4 patients), 34% had stable disease [19]. Remarkably, two patients with bulky disease showed tumor shrinkage of 91% and 56%, but the median progression free survival for the whole group was short, only 2.6 months. The drug was not well tolerated (fatigue, hypertension, and diarrhea) and the majority of patients needed a dose reduction. The University of Chicago phase II consortium showed the results of 51 patients with cediranib single agent. This trial showed similar results, with a PR of 10% and a PFS of 1.8 months in 50 evaluable patients [20]. Recently the results of a randomized phase 2 study were published [21]. Patients were treated in first-line with platinum-pemetrexed and randomly assigned to cediranib or placebo, followed by maintenance cediranib or placebo. Ninety-two eligible patients were enrolled. Cediranib improved PFS slightly (HR 0.71; $p = 0.62$, 7.2 vs 5.6 months) and increased modified RECIST v1.1 response (50% vs 20%; $p = 0.006$). Unfortunately, no significant difference in overall survival was observed. The cediranib toxicity profile and small incremental PFS benefit precluded additional development in MPM.

Vatalanib is an inhibitor of VEGF receptors, PDGF receptor, and c-KIT. It was studied in a phase 2 trial (CALGB 30107) in a dose of 1250 mg/day continuously after first-line chemotherapy [22]. Forty-seven patients were enrolled and 46 patients were evaluable. The drug was well-tolerated, but PR was only 6%. Median PFS was 4.1 months. Further development of vatalanib as a single agent for patients with MPM was not warranted.

Dovitinib is an inhibitor of VEGF receptors and FGF receptors; it was studied in a phase 2 trial in which 12 patients were enrolled, who had previously received platinum–antifolate combination therapy [23]. Dovitinib was administered orally at 500 mg/day for 5 days on, 2 days off in 28-day cycles. One unconfirmed partial response was observed in the first part of the study. The trial was halted due to a combination of minimal activity with several early progression events and poor tolerability.

3 Inhibition in Angiogenesis: Combination Therapy

In the first-line setting, antiangiogenic agents have been combined with the standard of care cisplatin pemetrexed. The main agents are bevacizumab, nintedanib, and axitinib.

Bevacizumab is a monoclonal antibody that binds to VEGF-A, thereby disrupting the VEGF pathway. The first randomized, double-blind placebo-controlled trial combining cisplatin-gemcitabine with bevacizumab or placebo, was a phase 2 study [24]. One hundred and eight eligible patients were treated with gemcitabine and

cisplatin in the standard dose, 53 patients were assigned to bevacizumab and 55 patients to placebo. Bevacizumab 15 mg/kg or placebo was administered intravenously on day 1 of each cycle. After 6 cycles bevacizumab or placebo was continued every 21 days. The outcome was disappointing. The response rates were similar in both groups (24.5% and 21.8% in the placebo arm ($p = 0.74$)). Stable disease occurred in 51% and 60% of patients, respectively. The median PFS and OS were not significantly different: 6.9 vs 6.0 months and 15.6 vs 14.7 months, respectively. The value of serum VEGF levels at baseline in 56 patients was examined in this study. The median plasma VEGF levels of 144 pg/mL were indeed significantly higher than those observed in phase 3 trials in non-small-cell lung cancer (38.7 pg/mL) [25] and colorectal cancer (44 pg/mL), confirming the importance of angiogenesis in this tumor [26]. Neither baseline VEGF level or mean log VEGF values could discriminate responders from non-responders. Higher baseline log VEGF levels, however, were prognostic for a worse PFS and OS. For OS the death rate increased by a factor 1.37 for each doubling of the VEGF level. In patients with baseline VEGF levels at or below the median, PFS ($p = 0.043$) and OS ($p = 0.028$) were significantly better for bevacizumab than for the placebo arm.

Two phase 2 studies first explored the effect of adding bevacizumab to cisplatin and pemetrexed in chemo naïve patients [27, 28]. They were both single-arm trials with respectively 76 and 53 patients. The studies failed to achieve their primary endpoint of improving PFS compared to historical controls of chemotherapy alone. However, the large French open-label, randomized phase 2/3 study that added bevacizumab to cisplatin and pemetrexed in chemo naïve patients did show a beneficial effect [29]. A total of 448 patients were treated with up to 6 cycles of standard treatment pemetrexed and cisplatin and were randomized between bevacizumab 15 mg/kg or chemotherapy alone. Subsequent maintenance bevacizumab was permitted. Not only PFS but also OS was statistically increased in the bevacizumab arm: median 18.8 months versus 16.1 months (HR 0.77, 95% CI 0.62–0.95). The positive effect of the bevacizumab arm could not be explained by post-study treatment. This was even given less frequently in the bevacizumab arm 62% versus 72% in the standard chemotherapy arm. There was no crossover to bevacizumab. Serum VEGF baseline concentrations were assayed in 372 (83%) patients, representative for the whole study population. Again, high VEGF concentrations were associated with worse PFS and OS. The interaction between the treatment group and VEGF serum concentration was not significant.

The reason why the outcome in the MAPS study was positive in contrast to the study with gemcitabine may lay in the backbone of the treatment. Subsequent studies have shown that adding bevacizumab to a gemcitabine backbone does not improve survival in either pancreatic or lung cancer [25, 30] and preclinical data suggest a negative interaction between bevacizumab and gemcitabine [31]. Some cytotoxic agents, but not gemcitabine, stimulate angiogenesis and tumor regrowth by mobilizing circulating progenitors from bone marrow. VEGF inhibitors may augment chemotherapy by blunting this effect. According to this hypothesis, for optimal activity, bevacizumab should be combined with agents that can rapidly induce these pro-angiogenic cells.

Nintedanib is a multitargeted angiokinase inhibitor, with activity against VEGF 1, 2, and 3, PDGFR and FGF receptors, among others. It was hypothesized that, in contrast to bevacizumab that only inhibits VEGF, this multitargeted approach could enhance efficacy. Nintedanib was studied in the phase 2/3 LUME-Meso trial in patients with epithelioid or biphasic MPM in a first-line setting [32]. Patients were randomized to nintedanib, 200 mg twice daily, or placebo in combination with cisplatin pemetrexed for up to six cycles, followed by nintedanib or placebo maintenance. A total of 87 patients were enrolled, and the outcome was positive. The addition of nintedanib to pemetrexed cisplatin improved PFS (median 9.4 vs 5.7 months; HR 0.54; 95% CI 0.33–0.87; $p = 0.010$) and was associated with a trend toward improved OS (median 18.3 vs 14.2 months; HR 0.77; 95% CI 0.46–1.29; $p = 0.319$) compared to placebo. The positive effect was not clearly seen in the subgroup analysis in the patients with biphasic histology. Therefore, the phase 3 trial was continued with only patients with epithelial subtype MPM [33]. In this trial, 458 patients were treated under the same conditions. Unfortunately, these encouraging findings could not be confirmed. The primary endpoint PFS was not met, median PFS for nintedanib versus placebo was 6.8 versus 7.0 months (HR [95% CI] 1.01[0.79–1.30]; $p = 0.914$). Median OS at the interim analysis for nintedanib versus placebo was 14.4 versus 16.1 months (HR [95% CI] 1.12 [0.79–1.58]; $p = 0.538$). The study has been discontinued as per the study protocol.

Axitinib is an inhibitor of VEGFR-1, -2 and -3, PDGFR, and c-Kit and was tested in an open-label, randomized phase 2 study in combination with cisplatin pemetrexed in treatment naïve mesothelioma patients [34]. In total, 20 patients received chemotherapy and axitinib in a dose of 5 mg tablets twice daily from day 2 until day 19. Eleven patients received chemotherapy only. This imbalance occurred because the first six consecutive patients all received chemotherapy and axitinib, being part of the lead-in cohort. The remaining 26 patients were randomized. Adding axitinib to standard chemotherapy did not improve results. There was no difference in the number of responders between the groups ($p = 0.85$). Complete responses were not observed. The rates of partial response (PR) and stable disease (SD) in the two arms were respectively 36% and 43% in the axitinib arm and 18% and 73% in the chemotherapy-only arm. Although the sample size was too small to draw clear conclusions, a median PFS of 5.8 months (95% CI 4.6–24) in the axitinib group and 8.3 months (95% CI 6-NA) in the chemotherapy-only group ($p = 0.86$) was not promising. In this study, all patients received a thoracoscopy before the start of treatment. After 3 cycles of therapy, a second thoracoscopy was carried out for a palliative pleurectomy and biopsies in all 11 patients treated with chemotherapy only and in 16 patients in the axitinib group. The design of the study was to correlate a possible clinical effect of axitinib to a biomarker. Therefore, intra-tumor changes in vascularization were explored. The lack of positive result in response rate and PFS did not allow identifying such a biomarker. However, in the group of patients receiving only pemetrexed and cisplatin, there was a significant increase in microvessel density in the tumor biopsies ($p < 0.001$). In addition, the number of mature blood vessels ($p = 0.01$) increased after therapy. In the axitinib group, the amount of microvessel density and mature blood vessels remained at the same level during the

treatment. In the axitinib group, serum VEGFR2 levels decreased during treatment, due to the binding of the axitinib to the receptor. Instead, VEGF levels increased during treatment, probably resembling a rebound effect.

3.1 Inhibition in Angiogenesis: Switch Maintenance Therapy

Thalidomide is an oral drug that inhibits the release of VEGF and basic FGF production. It has shown activity in a single-arm phase 2 study, where 40 patients were treated with pemetrexed and a platinum combination and if they had a partial response or stable disease after 4–6 course of chemotherapy, they could switch to thalidomide until progression or intolerable toxicity [35]. Twenty-five percent of patients had more than 6 months stabilization on the drug. This was reason to continue to an open-label, randomized phase 3 study in which 222 patients were treated, 111 in the thalidomide arm, with a dose of 200 mg per day and 111 in the active supportive care arm [36]. The primary endpoint was to determine a more than 50% increase in time to progression, but unfortunately, this was not met. The median time to progression in the thalidomide arm was 3.6 months (95% CI 3.2–4.1) compared with 3.5 months (2.3–4.8) in the active supportive care group (HR 0.95, 95% CI 0.73–1.20, $p = 0.72$). There was also no difference in median overall survival. This was 10.6 months (95% CI 8.1–13.6) in the thalidomide group and 12.9 months (10.4–16.4) in the active supportive care group (HR 1.2, 95% CI 0.9–1.6, $p = 0.21$).

3.2 Conclusions and Future Directions

Since the development of inhibitors of angiogenesis, a substantial amount of studies has been performed in the hope that this new strategy would ameliorate the prognosis of patients with MPM. Many multitargeted agents, that all had in common that the VEGF receptor was blocked, were used in second or further lines, but they usually showed no or limited activity and sometimes even substantial toxicity. The available clinical evidence even seemed to call into question the actual in vivo importance of these targets for MPM and the ability of the current agents to effectively disrupt these targets and turn this effect into a clinical benefit for the patient. However, in first-line studies, positive results have been alternated with negative results. With bevacizumab in combination with pemetrexed and cisplatin, a positive result for PFS as well as OS was achieved for the first time in a large randomized study, but not with gemcitabine and cisplatin. Promising phase 2 data with nintedanib could not be confirmed in the subsequent phase 3 trial that added nintedanib to pemetrexed and cisplatin. To maximize the effect of adding bevacizumab to pemetrexed and cisplatin the key may be in finding validated predictive biomarkers, but until now such biomarkers are not identified. The next step may be combining antiangiogenic therapy with immunotherapy. Angiogenic factors have roles in both

blood vessel formation and regulation of the immune system. High levels of VEGF can inhibit dendritic cell functions and VEGF has been shown to directly modulate T-cell proliferation, migration, and activation in preclinical studies [37]. It has been suggested that combining antiangiogenic agents with immunotherapy may produce synergistic effects. As an illustration, in the randomized phase 3 study in patients with first-line advanced NSCLC, the addition of bevacizumab and the PD-L1 inhibitor atezolizumab to chemotherapy was more effective than the addition of either agent alone [38]. This hypothesis is now being examined in mesothelioma patients in several studies. In a phase 1 study nintedanib is combined with the PD-1 inhibitor pembrolizumab (NCT02856425) also including MPM patients and a phase 2 study is underway evaluating bevacizumab and atezolizumab in MPM patients (NCT03074513). A randomized phase 3 trial comparing atezolizumab plus bevacizumab and standard chemotherapy (carboplatin and pemetrexed) versus bevacizumab and standard chemotherapy as first-line treatment for advanced malignant pleural mesothelioma (NCT03762018) is now recruiting.

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Chapter 22
Systemic Chemotherapy for Unresectable Pleural Mesothelioma from Front Line to Salvage Treatment: How Can We Treat the Patients Failed to PD-1/PD-L1 Inhibitors?

Nobukazu Fujimoto

Abstract There are a limited number of randomized clinical trials on systemic chemotherapy for malignant pleural mesothelioma (MPM). The combination of platinum/pemetrexed is considered a standard front-line treatment. There is no established treatment option for those who progressed after initial treatment with platinum/pemetrexed. In recent years, immune checkpoint inhibitors (ICIs), such as pembrolizumab and nivolumab, demonstrated a favorable response, disease control, and survival in phase II trials. In 2018, nivolumab was approved for advanced or metastatic MPM patients who were resistant or intolerant to previous chemotherapy in Japan. Combinations of ICIs or an ICI and conventional chemotherapy are under investigation to further improve response and survival. If these combination regimens that include anti-program death-1 (PD1)/PD-ligand1 (PD-L1) antibodies demonstrate high enough activity, safety, and tolerability as front-line treatments, the standard regimen with platinum/pemetrexed might be replaced. The best treatment regimen to use for patients who failed PD-1/PD-L1 inhibitors has not yet been elucidated. Based on the possible immunotherapy-induced chemosensitization effect that was recently reported, cytotoxic agents, such as pemetrexed, vinorelbine, or gemcitabine, would be the ideal choice. For patients who experienced a certain time to progression after first-line chemotherapy that included pemetrexed, a pemetrexed-based rechallenge might be administered. Combination treatment with immunotherapy and antiangiogenic agents with/without chemotherapy may offer hope, though there are only preclinical studies to support this strategy so far.

Keywords Pemetrexed · Pembrolizumab · Nivolumab · Immune checkpoint · PD-1

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1 Introduction

Malignant pleural mesothelioma (MPM) is a highly aggressive neoplasm with poor prognosis and a median overall survival (OS) of only approximately 12 months. In particular, patients with unresectable, advanced disease are characterized as having a worse prognosis than other patients. This poor outcome is principally due to a lack of effective systemic therapy [1, 2]. There are some guidelines for MPM treatment, including systemic chemotherapy; however, these recommendations are based on very limited evidence. In fact, there are a limited number of randomized clinical trials on systemic chemotherapy for MPM. The current standards for systemic chemotherapy including recent reports of immune checkpoint inhibitors (ICIs) for MPM, are summarized in this chapter, followed by some future perspectives.

2 Front-Line Chemotherapy

2.1 Platinum/Pemetrexed

Systemic chemotherapy consisting of a platinum plus pemetrexed is a recommended first-line systemic therapy for patients with MPM with a good performance status (PS). A single-blind, placebo-controlled, randomized phase III trial compared cisplatin (75 mg/m^2) alone and cisplatin plus pemetrexed (500 mg/m^2) in 456 previously untreated patients with MPM [3]. The combination of cisplatin and pemetrexed achieved a higher response rate (RR) (41.3 vs 16.7%; $P < 0.001$), superior median OS (12.1 vs 9.3 months; $P = 0.020$), and progression-free survival (PFS) (5.7 vs 3.7 months; $P = 0.001$) than single-agent cisplatin (Fig. 22.1). The toxicity was greater with the combination, producing grade 3/4 neutropenia, leukopenia, and nausea in 27.9%, 17.7%, and 14.6% of patients, respectively. Vitamin supplementation was instituted after the first 117 patients were enrolled, resulting in a significant reduction in toxicity. The combination chemotherapy also improved the quality of life (QoL) of the patients. Using the Lung Cancer Symptom Scale instrument to evaluate QoL, the combination of cisplatin plus pemetrexed demonstrated statistically significant improvements in dyspnea and pain. Another phase III trial that compared the antifolate raltitrexed (80 mg/m^2) plus cisplatin (80 mg/m^2) to cisplatin alone in 250 patients similarly demonstrated a higher RR (23.6 vs 13.6%) and a superior median OS (11.4 vs 8.8 months) and 1-year survival (46 vs 40%) for the raltitrexed/platinum combination than for cisplatin alone [4].

Pemetrexed can be administered in combination with carboplatin, with efficacy comparable to that of cisplatin/pemetrexed [5]. Although no randomized study has directly compared carboplatin to cisplatin in MPM, data from multiple phase II series and an expanded access program suggest that they are likely equivalent [6–8]. In a retrospective pooled analysis, patients over 70 years of age who were treated

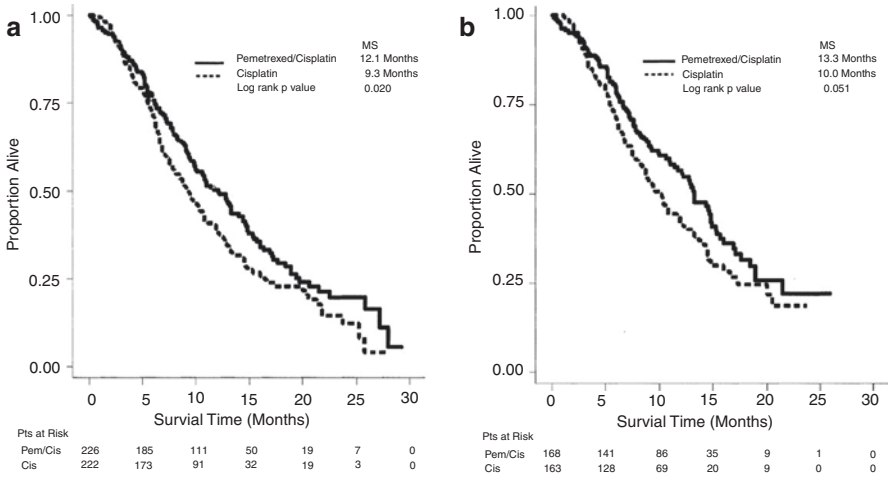

Fig. 22.1 Kaplan-Meier estimates of the overall survival time for all patients (Pts) (**a**) and for fully supplemented patients (**b**) in a phase III study that compared pemetrexed/cisplatin (Pem/Cis) to cisplatin alone (Cis) [3]. *MS* median survival

with pemetrexed and carboplatin achieved similar outcomes to their younger counterparts, though the older patients experienced more frequent hematologic toxicities [9]. MPM usually develops after a long latency period from past asbestos exposure, so there are many older patients who have some comorbidities. The combination of carboplatin/pemetrexed could be a reasonable treatment option for patients who are not candidates for a cisplatin-based regimen [10].

2.2 Beyond Platinum/Pemetrexed

There have been some clinical trials that examined the utility of new agents to further improve the results of platinum/pemetrexed combination chemotherapy. Representative examples are antiangiogenic agents. The Mesothelioma Avastin Cisplatin Pemetrexed Study (MAPS), an open-label, randomized, phase III trial, compared cisplatin/pemetrexed with or without the addition of bevacizumab, which targets vascular endothelial growth factor (VEGF) [11]. The three-drug combination demonstrated a longer OS than cisplatin/pemetrexed (18.8 vs 16.1 months; $P = 0.0167$; hazard ratio [HR], 0.77). PFS was also superior to the triplet treatment (9.2 vs 7.3 months; $P < 0.001$; HR, 0.61). Although the addition of bevacizumab increased the rate of grade 3/4 toxicities (71 vs 62%), especially hypertension (25 vs 0%) and thrombosis (6 vs 1%), there was no detriment to the QoL with the addition of bevacizumab [11]. The cisplatin/pemetrexed/bevacizumab regimen is recommended as one of the first-line treatment options in the National Comprehensive Cancer Network (NCCN) guidelines but is still not considered a standard treatment

in most countries. Bevacizumab is not approved in Japan, and there is no plan for its future approval.

Nintedanib targets VEGF receptors 1–3, platelet-derived growth factor (PDGF) receptors α and β, fibroblast growth factor (FGF) receptors 1–3, and the Src and Abl kinases, which are all implicated in MPM pathogenesis. Based on the favorable findings of the phase II LUME-Meso study [12], a double-blind, randomized, placebo-controlled phase III trial was conducted at 120 institutions in 27 countries [13]. Chemotherapy-naive patients with unresectable epithelioid MPM and an Eastern Cooperative Oncology Group (ECOG) PS 0-1 were randomly assigned to receive cisplatin/pemetrexed with or without nintedanib. PFS was not different between the nintedanib group (median 6.8 months [95% confidence interval (C.I.): 6.1–7.0]) and the placebo group (7.0 months [6.7–7.2]; HR 1.01 [95% C.I.: 0.79–1.30], $P = 0.91$).

2.3 Current Standards

Based on these discouraging situations, the platinum/pemetrexed combination is still considered a standard front-line treatment. In a pivotal study on cisplatin/pemetrexed, patients received a median of six cycles of chemotherapy. The percentage of patients who completed at least four, six, or eight cycles was 71%, 53%, and 5%, respectively [3]. A nonrandomized feasibility study with 27 patients suggested that continuous maintenance treatment with pemetrexed was safe and that responses could be achieved after 6 cycles of induction chemotherapy [14]. However, due to the limitations of the study, such as the heterogeneous patient population, the different induction regimens (pemetrexed/carboplatin or pemetrexed alone), and the small number of patients who received maintenance chemotherapy, maintenance treatment with pemetrexed is not recommended. A break from chemotherapy after four to six cycles of platinum/pemetrexed is currently recommended [15].

3 Salvage Chemotherapy

3.1 Pemetrexed

There was no recommended treatment option for MPM that had progressed after first-line treatment with platinum/pemetrexed. A phase III trial in 243 patients who had not previously received pemetrexed demonstrated a higher RR (18.7 vs 1.7%; $P < 0.001$), superior disease control (59.3 vs 19.2%; $P < 0.0001$), and longer PFS (3.6 vs 1.5 months; $P = 0.0148$) in those who received single-agent pemetrexed than in those with best supportive care [16]. Even for patients who had received a first-line treatment containing pemetrexed, retreatment with pemetrexed-based

chemotherapy is a reasonable option for patients who achieved durable disease control with first-line chemotherapy. A single-center retrospective review reported an overall RR of 19% and a disease control rate (DCR) of 48% among 31 patients who achieved disease control with first-line pemetrexed-based chemotherapy for at least 3 months [17]. A multi-institution retrospective analysis of 30 patients documented a 66% DCR and decreased pain for patients who had at least 6 months of disease control with first-line platinum/pemetrexed and were rechallenged with a pemetrexed-based regimen [18]. A multicenter retrospective analysis showed that patients with MPM who experienced a time to progression of at least 12 months after first-line chemotherapy had a greater likelihood of disease control with retreatment with pemetrexed [19].

3.2 Other Cytotoxic Agents

Other treatment options for salvage chemotherapy in MPM include vinorelbine or gemcitabine; however, the median OS with these agents ranges from 5 to 10 months [20, 21]. Vinorelbine is widely used as a second-line therapy, though there are limited data to support its efficacy. A single-center phase II trial of vinorelbine in 63 patients achieved an RR of 16% and a median OS of 9.6 months. Similarly, a single-center retrospective review of 59 patients reported an RR of 15% and a DCR of 49% [22]. In contrast, a retrospective review of 60 patients who received either vinorelbine or gemcitabine in a second- or third-line setting reported no RR for vinorelbine and an RR of 2% for gemcitabine. The median PFS was 1.7 and 1.6 months for vinorelbine and gemcitabine, respectively [23].

3.3 Other Novel Agents

Vorinostat, an oral histone deacetylase inhibitor, showed some evidence of activity in an initial study [24]. However, the efficacy was not confirmed in a phase III trial, in which 661 previously treated patients were randomly assigned to receive either vorinostat or placebo [25]. Other experimental agents, such as angiogenesis inhibitors [26] or tyrosine kinase inhibitors [27], have also not demonstrated efficacy. Recently, YS110, a humanized IgG1 monoclonal antibody with a high affinity for the CD26 antigen, demonstrated preclinical antitumor effects for CD26-expressing solid tumors. The recommended dose was defined, and encouraging prolonged disease stabilization was observed in a first-in human study for patients with CD26-expressing solid tumors, including MPM [28]. A subsequent phase II study is ongoing.

Given the paucity of active agents in this setting, enrollment in some clinical trials is highly recommended for those with a good PS.

4 Immune Checkpoint Blockade

4.1 Anti-Cytotoxic T Lymphocyte–Associated Antigen 4 (CTLA-4) Antibody

Targeting immune checkpoints with immunomodulatory monoclonal antibodies has been proven to be an effective antitumor strategy across a variety of cancers [29]. The immunosuppressive tumor microenvironment in MPM suggests that MPM might benefit from these kinds of immunotherapy [30, 31]. An anti-CTLA-4 antibody was the first ICI reported in MPM. Phase II studies demonstrated that the anti-CTLA-4 monoclonal antibody tremelimumab had favorable activity as a second-line treatment for MPM [32, 33]. However, in the subsequent phase III DETERMINE study, second- or third-line tremelimumab did not improve OS compared with placebo [34].

4.2 Anti-Programmed Death-1 (PD-1) Antibody

4.2.1 Pembrolizumab

Next, pembrolizumab, an anti-PD-1 antibody, paved the way. In a preliminary report of a nonrandomized, phase Ib trial of pembrolizumab in previously treated patients with PD-1-positive MPM, 20% of patients had an objective response, 72% had disease control, and the median OS was 18 months (95% C.I.: 9.4 to not reached) [35]. Then, a phase II trial assessed the activity of pembrolizumab in a nonselected group of 65 MPM patients [36]. The objective RR (ORR) was 19%, and the DCR was 66%. The median PFS was 4.5 months (95% C.I.: 2.3–6.2), and the median OS was 11.5 months (95% C.I.: 7.6–14).

Based on these promising results, pembrolizumab was used off-label in Switzerland and Australia [37]. Pembrolizumab was administered as a second-line treatment in 48 of 93 patients (52%). In the full cohort, the overall RR was 18%, the median PFS was 3.1 months, and the median OS was 7.2 months. The non-epithelioid histological subtype showed an improved ORR (24 vs 16% [$P = 0.54$) and median PFS (5.6 vs 2.8 months [$P = 0.02$]). The toxicities were as expected.

4.2.2 Nivolumab

Another anti-PD-1 antibody, nivolumab, was first tested in recurrent MPM in the Netherlands [38]. In a single-center trial, patients with MPM received nivolumab (3 mg/kg) intravenously every 2 weeks. Of the 34 patients included, 8 patients (24%) had a partial response, and another 8 had stable disease, resulting in a DCR

of 47%. The Japanese investigators also evaluated the efficacy and safety of nivolumab for advanced MPM patients who were resistant or intolerant to prior chemotherapy [39]. Thirty-four patients were enrolled, 10 patients showed a centrally assessed objective response, and the ORR was 29.4% (95% C.I.: 16.8–46.2). Concerning the histological subtypes, the ORRs were 25.9%, 66.7%, and 25.0% for the epithelioid, sarcomatous, and biphasic subtypes, respectively. The median OS and PFS were 17.3 and 6.1 months, respectively (Fig. 22.2). Based on these findings, nivolumab was approved in Japan for advanced or metastatic MPM patients who were resistant or intolerant to previous chemotherapy.

The toxicity of these ICIs was acceptable in MPM. The grade 3 or 4 toxicities included adrenal insufficiency (3%), pneumonitis (3%), skin rash (3%), colitis (1.6%), confusion (1.6%), hepatitis (1.6%), hyperglycemia (1.6%), and grade 5 hepatitis (1.6%) in a study of pembrolizumab [36]. Adverse events of any grade, such as fatigue (29%) and pruritus (15%), occurred in 26 patients (76%) in a study of nivolumab. Grades 3 and 4 treatment-related adverse events were reported in 9 patients (26%), and pneumonitis, gastrointestinal disorders, and abnormal laboratory results were most commonly seen. One treatment-related death occurred due to pneumonitis and was probably initiated by concurrent amiodarone therapy. These toxicity profiles resemble those in other malignancies, such as melanoma and non-small cell lung cancer (NSCLC), and seem manageable.

Although the effect of these ICIs requires confirmation in larger clinical trials, nivolumab and pembrolizumab would offer hope for patients with MPM. Reported studies with an anti-PD-1 antibody in MPM are summarized in Table 22.1.

5 Future Perspectives

5.1 Combination of ICIs

There are still a number of challenges in systemic chemotherapy for MPM. Immune checkpoint blockade could play the main role in addressing these challenges, at least for the time being. An important issue is the combination of an ICI and other agents, including other ICIs. The combination of antibodies targeting PD-1 or PD-ligand1 (PD-L1) and CTLA-4 warrants investigation given the synergistic roles of the PD-1/PD-L1 and CTLA-4 pathways in T-cell activation [40]. The NIBIT-MESO-1 study investigated the efficacy and safety of first- or second-line tremelimumab combined with durvalumab, an anti-PD-L1 monoclonal antibody [41]. In a phase II study, patients with unresectable pleural or peritoneal mesothelioma received intravenous tremelimumab and durvalumab every 4 weeks for four doses, followed by maintenance therapy with intravenous durvalumab. Eleven (28%) of 40 patients had an objective response. The median PFS was 5.7 months (95% C.I.: 1.7–9.7), and the median OS was 16.6 months (95% C.I.: 13.1–20.1). The treatment-related toxicities were generally manageable and reversible.

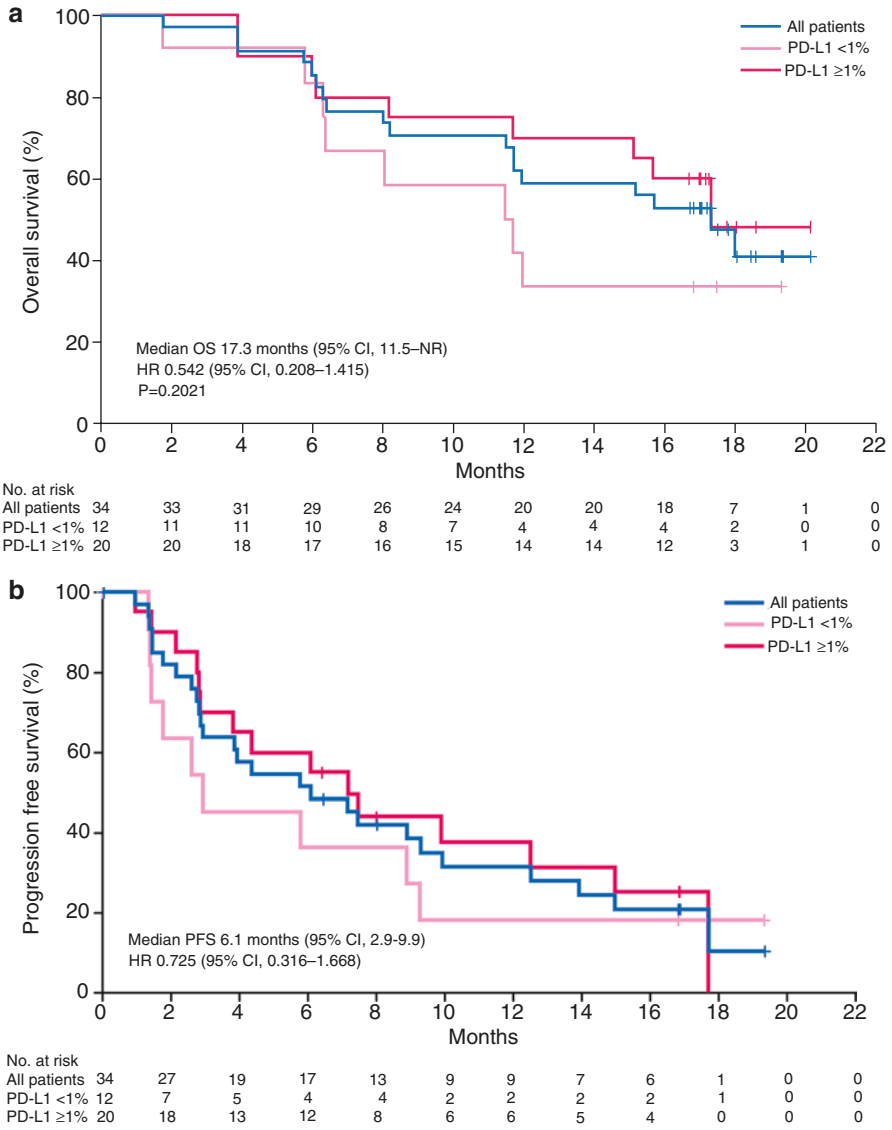

Fig. 22.2 Kaplan-Meier curves for overall survival (**a**) and progression-free survival (**b**) for all patients and according to the PD-L1 expression status in the MERIT study [39]. *HR* hazard ratio, *NR* not reached

Table 22.1 Reported studies with anti-PD-1 antibodies in malignant pleural mesothelioma

Agent	*N*	ORR (%)	DCR (%)	mPFS (m)	mOS (m)	Author	References
Pembrolizumab	25	20	72	5.4	18	Alley et al.	[35]
Pembrolizumab	64	22	61	4.1	10.1	Desai et al.	[36]
Pembrolizumab	93	18	48	3.1	7.0	Metaxas et al.	[37]
Nivolumab	34	26	47	2.6	11.8	Quispel-Janssen et al.	[38]
Nivolumab	34	29	67.6	6.1	17.3	Okada et al.	[39]

DCR disease control rate, *mOS* median overall survival, *mPFS* median progression-free survival, *N* number of cases, *ORR* objective response rate, *PD* programmed death

Another multicenter, randomized, phase II study was performed in France [42]. In that study, patients were randomly allocated to receive nivolumab or nivolumab plus ipilimumab and were treated until progression or an unacceptable toxicity. In the intention-to-treat population, 12-week disease control was achieved by 25 (40%; 95% C.I.: 28–52) of 63 patients in the nivolumab group and by 32 (52%; 95% C.I.: 39–64) of 62 patients in the combination group. The most frequent grade 3 adverse events were asthenia (1 [2%] in the nivolumab group vs 3 [5%] in the combination group), asymptomatic increases in aspartate aminotransferase or alanine aminotransferase (none vs four [7%] of each), and asymptomatic increases in lipase (two [3%] vs one [2%]). These findings indicate that the combination of anti-CTLA-4 and anti-PD1/PD-L1 antibodies appears effective, with a good safety profile in patients with MPM. A phase III, randomized, open-label trial of nivolumab in combination with ipilimumab vs pemetrexed with cisplatin or carboplatin as first-line therapy in unresectable MPM is ongoing. The primary end point of the study, OS, will be reported soon.

5.2 Combination of ICI and Chemotherapy

The advantage of the combination of ICI and chemotherapy has already been demonstrated in NSCLC [43]. The combination of an anti-PD-1/PD-L1 antibody and conventional chemotherapy is also under investigation in MPM. Nowak et al. presented results from a phase II trial of durvalumab with first-line cisplatin/pemetrexed in MPM [44]. The primary end point was PFS at 6 months (PFS6). The proportion of PFS6 was 57% (95% C.I.: 45–68%), and the median PFS was 6.9 months (95% C.I.: 5.5–9.0). The ORR was 48% (95% C.I.: 35–61%). Grade 3–5

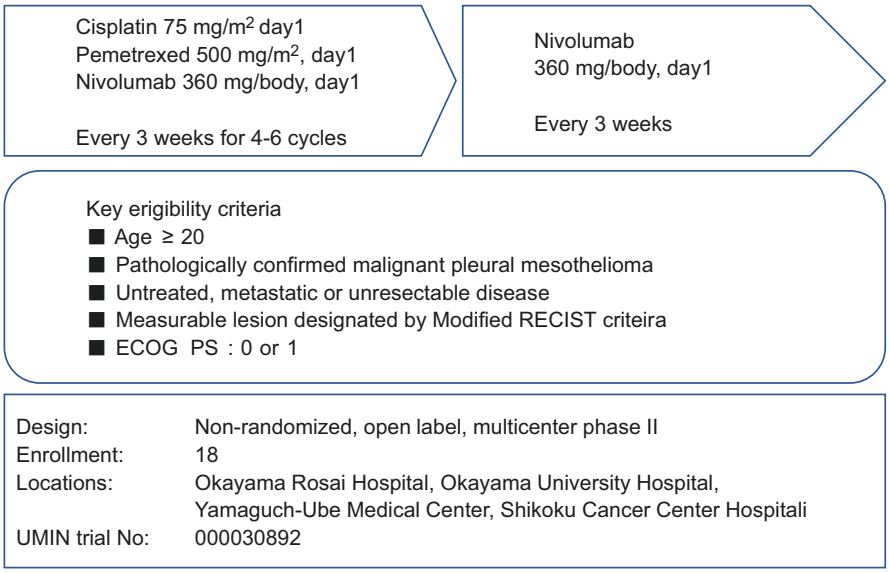

Fig. 22.3 Overview of a phase II trial testing first-line combination chemotherapy with cisplatin/pemetrexed and nivolumab for the treatment of unresectable malignant pleural mesothelioma [45]. *ECOG* Eastern Cooperative Oncology Group, *PS* performance status, *RECIST* Response Evaluation Criteria in Solid Tumors

adverse events occurred in 36 participants, including neutropenia in 13%, nausea in 11%, anemia in 7%, fatigue in 6%, and any grade of peripheral neuropathy in 35%. Another phase II study of the combination of cisplatin/pemetrexed and nivolumab is currently in progress [45] (Fig. 22.3), and the combination of cisplatin/pemetrexed and pembrolizumab is also being evaluated in a large-scale randomized study.

If these combination regimens, including those with an anti-PD1/PD-L1 antibody demonstrate enough activity, safety, and tolerability as a first-line treatment, the standard regimen of cisplatin/pemetrexed might be replaced.

6 How Can We Treat the Patients Failed to PD-1/PD-L1 Inhibitors?

The best treatment regimen to use for patients who failed to PD-1/PD-L1 inhibitors has not yet been elucidated. We could not find any small studies or case series addressing this topic. Based on our experience, currently, cytotoxic agents, such as pemetrexed, vinorelbine, or gemcitabine, would be the best choice. As mentioned in the previous section on "salvage chemotherapy," for patients who experienced a certain time to progression after first-line chemotherapy containing pemetrexed, a pemetrexed-based rechallenge might be administered.

Recently, Schvartman et al. reported that the RR to chemotherapy after exposure to ICIs was higher in advanced NSCLC [46]. Park et al. also reported that ICIs could improve the RR of salvage chemotherapy administered after immunotherapy in patients with NSCLC [47]. The immunomodulatory effects of chemotherapy are considered possible mechanisms of the immunotherapy-induced chemosensitization effect, although the detailed mechanism remains unknown. A prospective study is warranted to examine the effect of rechallenge with pemetrexed or monotherapy with gemcitabine or vinorelbine after the failure of ICI treatment.

Another interesting future prospect is a combination of immunotherapy and other drugs, such as antiangiogenic agents. A recent basic study showed that simultaneous treatment with a PD-1 inhibitor and an anti-VEGFR2 antibody synergistically inhibited tumor growth in vivo [48]. Allen et al. also showed that anti-PD-L1 therapy can sensitize tumors to antiangiogenic treatment and can prolong its efficacy, although this was demonstrated in preclinical models [49]. Combination treatment with immunotherapy and antiangiogenic agents with/without chemotherapy may offer hope.

7 Conclusion

We still have a number of challenges to address in systemic chemotherapy for MPM. Immune checkpoint blockade may play the main role in addressing these challenges. The combination of an ICI and other ICIs and the combination of an ICI and conventional chemotherapy are under investigation. Further study is warranted to investigate whether ICIs could improve the response to salvage chemotherapy administered after immunotherapy.

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Chapter 23
Biomarkers for Immune Checkpoint Inhibitors in Mesothelioma: What Are the Roles of Biomarkers for Optimal Immune Therapy?

Toshiyuki Minami and Takashi Kijima

Abstract Immunotherapy with immune checkpoint inhibitors (ICIs) has dramatically improved prognosis in various types of solid tumors. Several clinical trials have proven that ICIs have a potential to exhibit promising antitumor effects in malignant pleural mesothelioma (MPM) also. In 2018, anti-programmed cell death-1 (PD-1) antibody nivolumab was approved as a second-line treatment regimen for patients with MPM in Japan. Although ICIs have not been approved for the regulatory use in MPM in other countries to date, they are likely to be the key drugs to bring about drastic breakthrough improvement in the outcome of patients with MPM. On the other hand, ICIs do not necessarily exert favorable clinical effects. The overall response rate of ICIs is about 20%. Therefore, it is essential to select appropriate candidates for the use of ICIs. In this chapter, our discussion will focus on molecules that make reliable biomarkers that can be used to effectively select suitable candidates for ICI immunotherapy.

Keywords Malignant pleural mesothelioma · ICIs · Biomarker

1 Introduction

Malignant pleural mesothelioma (MPM) arises from neoplastic transformation of mesothelial cells of the pleura. Occupational or environmental exposure to asbestos is strongly involved in the development of MPM and the incidence of MPM has

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been increasing throughout the world [1]. Multimodal therapy including surgical treatments is applicable only to patients with early-stage disease and good performance status [2]. The standard treatment of inoperable patients is systemic chemotherapy with cisplatin and pemetrexed, which is the only approved regimen with the evidence to prolong overall survival (OS) of those patients. However, the median OS of the patients treated with this regimen is 12 months after diagnosis [3, 4]. Thus, development of a novel therapeutic strategy has been desired over the past 15 years.

2 Immunotherapy in MPM

Cancer immunotherapy has dramatically advanced in various types of cancer, especially since immune checkpoint inhibitors (ICIs) emerged. Currently, clinically available ICIs directed three immune inhibitory molecules, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death-1 (PD-1), and PD-1 ligand-1 (PD-L1) [5]. The ratio for PD-L1-positive MPM, defined as a tumor with which ≥1% of tumor cells express PD-L1, is reported to be 20–40% in MPM [6, 7]. Several clinical trials have been conducted to evaluate the antitumor activity of ICIs. While most of them were performed for patients with relapsed-MPM after standard chemotherapy, ICIs exerted promising results as salvage therapy [8]. For example, in a phase Ib trial (KEYNOTE-028), anti-PD-1 antibody pembrolizumab was administered to patients with PD-L1-positive MPM and exhibited objective response rate (ORR) and disease control rate (DCR) of 20% and 72%, respectively [9]. Another anti-PD-1 antibody nivolumab also showed promising antitumor activity in phase II studies. In a single-arm phase II study performed in the Netherlands (NivoMes trial), the ORR and DCR of the patients with relapsed-MPM were 24% and 50%, respectively [10]. Similarly, in a Japanese phase II study (MERIT), 29% of ORR and 68% of DCR were achieved by nivolumab monotherapy in patients who had received one or two prior chemotherapeutic regimens [11]. Based on the results of MERIT, nivolumab was approved for the regulatory use in patients with recurrent-MPM after prior chemotherapy. On the other hand, antitumor effect of anti-CTLA-4 antibody ipilimumab or tremelimumab was assessed in combination therapy with anti-PD-1 antibody nivolumab or anti-PD-L1 antibody durvalumab. Results from three clinical trials NIBIT-MESO (tremelimumab and durvalumab combination), MAPS-2 (ipilimumab and nivolumab combination), and INITIATE (ipilimumab and nivolumab combination) are currently available. In these studies, combination therapy of anti-CTLA-4 antibody and anti-PD-1/PD-L1 antibody exhibited 26–29% of ORR and 50–68% of DCR in patients with relapsed MPM [12–14]. Although combination therapy with anti-CTLA-4 antibody and anti-PD-1/PD-L1 antibody seems to be active as salvage chemotherapy, the additional antitumor effect of anti-CTLA-4 antibody to anti-PD-1/PD-L1 antibody remains inconclusive and needs to be validated by further studies.

Table 23.1 Major ICI trials in MPM

Study name (NCT #)	ICIs	Phase Line	pts' #	ORR %	DCR %	mPFS Months	mOS Months
KEYNOTE-028 (02054806)	P	Ib ≥1st	25	20	72	5.4	18.0
NivoMes trial (02497508)	N	II 2nd	34	24	47	2.6	11.8
MERIT (ONO-4538)[a]	N	II 2nd or 3rd	34	29	68	6.1	17.3
IFCT MAPS-2 (02716272)	N ± I	II 2nd or 3rd	N: 63 N + I: 62	N: 17 N + I: 31	N: 40 N + I: 52	N: 4.0 N + I: 5.6	N: 11.9 N + I: 15.9
INITIATE (03048474)	N + I	II ≥ 2nd	34	29	68	6.2	NR
NIBIT-MESO-1 (02588131)	D + T	II 1st or 2nd	40	25	63	5.7	16.6

Abbreviations: # number, *D* Durvalumab, *DCR* disease control rate, *I* Ipilimumab, *ICI* immune checkpoint inhibitor, *mOS* median overall survival, *mPFS* median progression free survival, *N* Nivolumab, *NCT* national clinical trial, *NR* not reached, *ORR* objective response rate, *P* Pembrolizumab, *pts* patients, *T* Tremelimumab

[a]Not NCI-assigned #

Thus, immunotherapy with ICIs opened the door to a new era in the treatment of MPM (Table 23.1). Meanwhile, ICIs do not necessarily bring about the expected antitumor effect. Therefore, it is indispensable to investigate reliable predictive biomarkers to select suitable candidates who will receive the meaningful clinical benefit from the treatment using ICI.

3 Predictive Biomarkers for Antitumor Effect of ICIs in MPM

Based on the results from numbers of clinical trials performed in various types of solid tumors, multiple parameters such as PD-L1 expression, DNA mismatch repair-deficiency (MMR-D) or microsatellite instability-high (MSI-H), tumor mutation burden (TMB), tumor-infiltrating lymphocytes (TIL), neutrophil-to-lymphocyte ratio (NLR) are considered to be potential biomarkers which can predict antitumor effect of ICIs [15]. In MPM, several clinical and experimental researches were also carried out to identify predictive biomarkers for ICI response. Here, we will provide an overview of the promising candidates for the biomarkers.

3.1 PD-L1 Expression

Several clinical studies suggested that high PD-L1 expression was associated with poorer outcomes in patients with MPM. Notably, PD-L1 expression was higher in sarcomatoid and biphasic subtypes than in epithelioid subtype [16, 17]. It has been reported that sarcomatoid subtype is refractory to standard systemic chemotherapy and has the poorest outcome with the medium survival time of 7.5 months [18, 19]. These studies indicated that PD-L1 expression played a huge role in the aggressiveness of MPM.

The predictive values of PD-L1 expression in ICIs evaluated in each clinical trial are listed in Table 23.1. In all the listed clinical trials, PD-L1 positivity was commonly defined as ≥1% of tumor cells staining for PD-L1 by immunohistochemistry (IHC). Regarding nivolumab monotherapy, PD-L1 positivity was not correlated with outcome in NivoMes trial [10]. On the other hand, increased ORR (40% for PD-L1-positive vs. 8% for PD-L1-negative) and prolonged survival was observed in patients with PD-L1-positive MPM (hazard ratios of PD-L1-positive to PD-L1-negative were 0.725 for PFS and 0.542 for OS, respectively) in MERIT study [11]. Although the differences in those parameters did not reach statistical significance, the tendency seems to be credible and meaningful. In a phase Ib KEYNOTE-028 trial, pembrolizumab exerted remarkable clinical benefit for patients with PD-L1-positive MPM (ORR was 20%, median PFS was 5.4 months and median OS was 18.0 months) [9]. As for the combination of ICIs, two phase II studies, IFCT MAPS-2 trial, and INITIATE trial, demonstrated the significance of PD-L1 expression as a predictive biomarker in the treatment with nivolumab plus ipilimumab. In IFCT MAPS-2 trial, nivolumab plus ipilimuab combination therapy exhibited higher ORR in patients with PD-L1-positive MPM compared with that in patients with PD-L1-negative MPM (ORR was 39% for PD-L1-positive vs. 12% for PD-L1-negative). Especially, in patients with MPM in which ≥25% of tumor cells express PD-L1, the ORR increased to 71% [13]. Moreover, INITIATE trial revealed that PD-L1 expression was not only associated with the increase of ORR (47% for PD-L1-positive vs. 16% for PD-L1-negative) but also associated with the improvement in PFS and OS (hazard ratios of PD-L1-positive to PD-L1-negative were 0.39 for PFS and 0.16 for OS, respectively) when treated with a combination of nivolumab plus ipilimumab [14]. Thus, despite the limitation due to small sample size in these studies, the presented results indicate that PD-L1 expression could be a reliable biomarker for ICI response (Table 23.2).

Recent studies demonstrated that yes-associated protein (YAP), a potent transcriptional coactivator, was involved in PD-L1 expression [20, 21]. When dephosphorylated, YAP translocates into the nucleus and induces expression of cell-proliferative and anti-apoptotic genes through the interaction with transcription factors. To avoid the overgrowth of cells, nuclear translocation of YAP is strictly regulated by several serine-threonine kinase cascades called Hippo pathway [22]. Whole-exome sequencing analyses have identified frequent genetic alterations of *neurofibromin 2 (NF2)* in MPM [23]. *NF2* gene encodes the tumor suppressor

Table 23.2 ORR by PD-L1 status

Agent (Study name)	PD-L1 positivity[a] (%)	PD-L1 detection antibody clone	ORR (%) in PD-L1 (+)/PD-L1 (−)
Pembrolizumab (KEYNOTE-028)	100[b]	22C3	20
Nivolumab (NivoMes trial)	27	28-8	44/16
Nivolumab (MERIT)	59	28-8	40/8
Nivolumab ± Ipilimumab (IFCT MAPS-2)	41	28-8 SP-263	39/12 32/14
Nivolumab + Ipilimumab (INITIATE)	43	22C3	47/16
Durvalumab + Tremelimumab (NIBIT-MESO-1)	53	SP-263	35/22

[a]PD-L1 positive MPM was defined as the tumor containing more than 1% of PD-L1-expressing tumor cells
[b]In KEYNOTE-028 trial, only patients with PD-L1-positive MPM were enrolled

protein moesin-ezrin-radixin-like protein (merlin) which activates Hippo pathway and inhibits nuclear translocation of YAP. However, *NF2* gene was mutated and inactivated in 40–50% of patients with MPM, which resulted in the disruption of Hippo pathway and allowed nuclear translocation of YAP [24]. This mechanism may imply not only the pathogenesis of MPM but also increased expression of PD-L1 in MPM tumor cells. Therefore, it is worth exploring the possibility of *NF2* gene alteration to be a predictive biomarker of ICI-based immunotherapy.

3.2 MMR-D/MSI-H

MMR is an essential mechanism that corrects DNA replication errors occurring during normal cell division. Therefore, MMR-D causes an increased rate of mismatch errors, which can lead to the development of tumor. These mismatch errors are markedly observed in MS, also called short tandem repeats, consisting of repeated sequences of 1–6 nucleotides. Accumulation of DNA mismatch brings about MSI which can be used to clinically detect MMR-D [15, 25]. Tumor cells with MSI-H produce proteins containing mutation-associated neoantigens, which are responsible for the immune response. Moreover, previous studies showed that the expression of immune checkpoint proteins including PD-1 and PD-L1 was upregulated in MSI-H tumor [26, 27]. Based on these observations, a phase II study to evaluate the clinical activity of pembrolizumab in refractory tumors with MMR-D was conducted, and as expected, patients with MSI-H tumor remarkably responded to pembrolizumab monotherapy. Food and drug administration approved pembrolizumab for the treatment of patients with solid tumor with MMR-D/MSI-H [28, 29].

Therefore, pembrolizumab is also currently available in patients with MPM if tumor cells have MMR-D/MSI-H.

Previous comprehensive analysis using next-generation sequencing (NGS) determined that the prevalence of MSI-H in mesothelioma was 2.4% [30]. Then, several studies were performed to identify MMR-D in MPM mostly by IHC for the direct detection of protein loss of MMR. Unfortunately, the positive ratio of MMR-D in MPM was proven to be extremely low. Arulananda et al. examined by IHC about four common MMR proteins in 335 MPM cases and reported that negative staining of at least one MMR protein was observed only in 6 cases (1.8%). They further analyzed MSI in these 6 cases by multiplex polymerase chain reaction and confirmed to be negative for MSI in all of them [31]. Consistent with the above results, Cedrés et al. recently reported that MMR-D was not identified in 158 MPM samples at all [32]. These studies indicate that MMR-D/MSI-H may not be a candidate as a general biomarker for ICI-response in MPM. However, examination of MMR-D/MSI-H in MPM is still important to make a decision about the use of pembrolizumab.

3.3 TMB

TMB is defined as total number of non-synonymous somatic mutations in tumor cells, and can be quantitatively measured by using NGS. Similar to MMR-D, TMB is associated with neoantigen burden [33]. Recently, the predictive value of TMB on antitumor efficacy of ICI therapy has been confirmed across multiple types of tumor including non-small cell lung cancer (NSCLC) [34, 35].

Tumors related to carcinogen exposure typically have a high TMB because of accumulation of DNA damage [36]. Since carcinogenicity of asbestos is obviously involved in the development of MPM, TMB in MPM was expected to be high. However, a previous study revealed that MPM unexpectedly had quite low TMB [37]. Mansfield et al. recently suggested that the neoantigen expression in MPM was driven by chromosomal rearrangements, which might not be detected by conventional sequencing techniques [38]. Thus, further studies are necessary for the identification of the source of neoantigen in MPM.

3.4 Tumor-Infiltrating Lymphocytes

TILs are absolutely necessary for ICIs to restore antitumor immunity of host and eliminate tumor cells [39]. According to the status of TIL and PD-L1 expressions, tumor microenvironments (TME) can be divided into the following four subtypes: Type I (PD-L1$^+$/TIL$^+$), type II (PD-L1$^-$/TIL$^-$), type III (PD-L1$^+$/TIL$^-$), and type IV (PD-L1$^-$/TIL$^+$). In type I TME regarded as adaptive immune resistance, PD-L1 expression in tumor cells is upregulated by interferon-gamma released from TILs,

which causes suppression of the function of local effector T cells. ICIs have the potential to reverse T cell exhaustion and restore antitumor immunity in patients with this type I TME. In type II TME, detectable immune reaction lacked, and accordingly this situation is recognized as immune ignorance. Attracting T cell infiltrates into tumors and inducing PD-L1 expression on tumor cells will be essential for ICIs to exert antitumor activity. In type III TME categorized as intrinsic resistance, PD-L1 is constitutively expressed on tumor cells without TIL probably due to oncogenic signaling. It will be necessary to recruit lymphocytes into tumors for the practical use of ICIs in patients with this type III TME. Type IV TME represents immune tolerant state, which means that other immune-suppressive pathways rather than PD-1/PD-L1 signal may exist [40]. Collectively, tumors having type I TME are thought to be the most sensitive to ICI therapy [40, 41]. Among TILs in the tumor, $CD8^+$ TILs are considered to play a key role in killing tumor cells [42]. Therefore, quantitative analysis of TIL and PD-L1 is very important to predict the antitumor efficacy of ICIs-based therapy.

Several studies have indicated that the presence of $CD8^+$ TILs as a positive prognostic factor in MPM [43, 44]. However, these $CD8^+$ TILs are suggested to be hypofunctional due to co-expression of inhibitory receptors such as T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) and T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) [45, 46]. Interestingly, Awad et al. reported that $TIM\text{-}3^+/CD8^+$ TILs ratio was significantly higher in PD-L1-positive MPM ($\geq$1% of tumor cells staining for PD-L1) than in PD-L1-negative MPM (<1% of tumor cells staining for PD-L1). They also reported that nonepithelioid (sarcomatoid or biphasic) MPM tumor had higher infiltration of T cells than epithelioid MPM tumor [45]. Actually, MERIT study suggested that sarcomatoid MPM might be more sensitive to nivolumab than epithelioid MPM [11]. Although there have been no reports demonstrating the correlation between TILs and ICI-based immunotherapy in MPM, detailed examination about TILs are crucial for ICIs to exert maximum antitumor effect.

3.5 Neutrophil-to-Lymphocyte Ratio

Chronic inflammation is regarded to be involved in the development and progression of various types of tumor. During this process, the phenotype of neutrophil unfavorably alters by tumor cell-derived cytokines such as transforming growth factor-β, which means that this alteration in neutrophil can cause suppression of cytolytic activity of T cells [47, 48]. A recent study demonstrated that the peripheral blood NLR was directly correlated with tumor-infiltrating neutrophils [49]. Accordingly, high NLR represents dysfunction of T cells. A meta-analysis revealed that high NLR was associated with worse PFS and OS in patients treated with ICIs across different types of malignancies including melanoma, NSCLC, and genitourinary cancers [47].

There are some clinical studies demonstrating that high NLR is associated with poor prognosis also in MPM [50, 51]. Since MPM is also developed through chronic inflammation caused by asbestos exposure, intratumor neutrophil is supposed to acquire unfavorable phenotype. Janssen et al. analyzed the data from NivoMes trial and reported that an increase in NLR of more than 25% from baseline correlated with nonresponse, and Cedrés et al. also suggested that ICI therapy might bring about better outcome in patients with low NLR [10, 32]. However, to date, the predictive value of NLR for ICI-response in MPM remains undetermined because of insufficient scientific evidence due to small sample size. Moreover, the consensus of cut-off value of NLR has been lacking. Therefore, it is currently uncertain whether NLR can be a biomarker in ICI-based immunotherapy in MPM.

3.6 BRCA1-Associated Protein 1

BRCA1-associated protein 1 (BAP1) works as a tumor suppressor through chromatin modulation by deubiquitinating the transcriptional regulator host cell factor 1. Accordingly, nuclear localization is necessary for BAP1 to exert tumor suppressor activity. *BAP1* somatic mutation is considered as driver mutation and is found in 23–36% and 32% of patients with MPM and malignant peritoneal mesothelioma (MPeM), respectively [23, 37, 52]. Therefore, loss of nuclear BAP1 expression detected by IHC is a reliable marker for the diagnosis of malignant mesothelioma [23, 53, 54].

The predictive value of *BAP1* mutation as a biomarker in ICI therapy is mainly examined in MPeM prior to MPM. Shrestha et al. analyzed MPeM clinical samples and found that TME in *BAP1* mutation-positive MPeM was more inflammatory compared with that in *BAP1*-intact MPeM. They also demonstrated that expression of immune checkpoint-related genes including PD-1, PD-L1, CD80, CTLA4, lymphocyte activation gene-3, and inducible T-cell costimulator precursor were upregulated in *BAP1*-mutated MPeM [55]. Additionally, it was reported that more than half of mutations in *BAP1* in MPeM were frameshift indels which resulted in neoantigen formation [56]. On the contrary, as for MPM, there have been few reports demonstrating a significant correlation between *BAP1* inactivation and PD-L1 expression [45, 55, 57]. These observations indicate that *BAP1* mutation can be a promising biomarker for ICI-response, especially in MPeM.

4 Conclusion

Immunotherapy with ICIs is generally tolerable and is expected to bring about a drastic improvement in the outcome of patients with MPM. On the other hand, ICI-based therapy sometimes induces severe immune-related adverse events and does not necessarily provide clinical benefits. Therefore, investigations for biomarkers in

order to select eligible candidates for ICI therapy are essential and urgently needed. Although previous clinical studies suggest that PD-L1 expression seems to be the most promising biomarker for ICI therapy in MPM, it is not the definite biomarker. Since MPM is characterized as inflammatory tumor, further studies focusing on not only MPM cells but also TME would be important to identify a reliable biomarker.

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Chapter 24
Promising Investigational New Drugs for Mesothelioma: What Is the Next Stage of the Treatment for Advanced Mesothelioma?

Dean A. Fennell

Abstract Inter-patient heterogeneity is a major barrier to achieving the effective therapy of mesothelioma. Recent advances in genomics have illuminated the extent of genomic inter-patient heterogeneity, which has led to the development of new strategies to treat this lethal cancer. Targeting of epigenetic, and genomic aberrations involving critical tumour suppressors are now beginning to be translated in the clinic. New synthetic lethal approaches, in parallel with development of new trial designs such as master protocols, have the potential to accelerate the implementation of much-needed improvements in treatment for patients with this deadly cancer.

Keywords Mesothelioma · Relapsed · BAP1 · NF2 · DNA repair · ASS1 CDKN2A · CDK4/6 · MDM2 · MTAP · PRMT5 · MAT2A · Master protocol Stratified therapy

1 Introduction

Our understanding of mesothelioma biology and inter-patient heterogeneity has increased substantially over the last decade and a half since the approval of first-line chemotherapy. The opportunity now exists to leverage this emerging knowledge, in order to advance therapy and substantially improve survival rates. Precision medicine has transformed outcomes in aggressive, metastatic cancers such as non-small cell lung cancer. The development of molecularly stratified approaches, although in its infancy, is emerging as a promising route to achieving the goal of better therapy, and will be the focus of this chapter.

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Inter-patient heterogeneity in mesothelioma represents a key barrier to the success of new therapy for mesothelioma. The histological classification of mesothelioma, represents one of the key determinants of prognosis, ranging from the sarcomatoid subtype that exhibits the most aggressive, often invasive and sometimes metastatic phenotype, to the most indolent, mesotheliomas associated with the longest survival outcomes. Small clinical trials aimed at enrolled unselected patients may therefore be at high risk of sample bias, highlighting the need for randomized clinical trials in the rigorous testing of new, promising investigational agents. In the absence of an approved standard of care, active symptom control or placebo may be considered appropriate options, reflecting the development pathway two decades ago that advanced drug approvals in non-small cell lung cancer. To date, where placebo has been employed in large phase III trials to date, results have been negative [1–4]. However, large well-powered randomized trials of both anti-PD1 immunotherapy [5] and vinorelbine, where clinically useful signals have been seen previously, have employed active symptom control or placebo control, to provide essential data on the magnitude of efficacy, risk versus benefit and value for money.

Given the large number of reported negative trials, illustrates the pressing need to understand better, how we can enrich patients for effective therapy, in the precision medicine era. More recently, genomic interrogation of the somatic mutational landscape of mesothelioma as revealed molecular determinants of poor prognosis that may also serve as drug targets. Cancer gene inactivation is the dominant mode underpinning transformation to mesothelioma [6, 7]. The loss of any gene function may rely on a salvage mechanism to ensure viability, and therefore, may constitute a potential vulnerability, which if targeted effectively, could achieve clinical *synthetic lethality* as has been established for PARP inhibitors in the context of BRCA mutations [8].

2 Targeting the Biphasic/Sarcomatoid Phenotype

Although representing only a fraction of mesotheliomas, the sarcomatoid phenotype exhibits a dismally short prognosis. Despite this, molecular stratification may be feasible and is currently under evaluation in the clinic. Sarcomatoid (and biphasic mesotheliomas) has been shown to frequently exhibit metabolic rewiring associated with frequent epigenetic suppression of argininosuccinate synthetase. This enzyme is required for the synthesis of the amino acid arginine, a conditionally essential amino acid that plays a vital role in tumour physiology, enabling the synthesis of polyamines (via the nitric oxide pathway) for promotes a proliferative and metastatic phenotype. In addition, arginine plays a role in the regulation of both adaptive and innate immune responses critical for tumour suppression [9, 10]. Arginine can be synthesised from the amino acid citrulline, however, under conditions where argininosuccinate synthetase (ASS1) expression is lost, cells exhibit a dependence on exogenous arginine, a condition known as arginine auxotrophy.

Denying ASS1 deficient mesothelioma cells arginine leads to induction of apoptosis, highlighting a potentially exploitable vulnerability [11, 12]. The enzyme arginine deiminase is a hydrolase that catalyses the conversion of arginine to citrulline and can be "weaponised" to combat cancer ASS1 deficient cancers in its pegylated form (ADI-PEG20) [13].

Based on preclinical studies that revealed a synthetic lethal relationship between ASS1 loss and arginine deprivation in mesothelioma, an open label randomised phase II window study (ADAM) was conducted to determine the impact of ADI-PEG20 in mesotheliomas. This study was the first-ever molecularly stratified randomised study for the treatment of mesothelioma [14], and compared ADI-PEG20 administered as a weekly intramuscular dose versus best supportive care in a 2:1 randomisation. ASS1 loss was seen in 48% of tumours. The primary end point was progression-free survival, which was superior for ADI-PEG20 with evidence of metabolic response [15], and an improvement from 2 months to 3.2 months, hazard ratio 0.56 (95% confidence limits 0.33–0.96, $p = 0.03$). Greater benefit was seen for patients with tumours with >75% ASS1 deficiency (Hazard ratio 0.25). This study, therefore, provided the first bench-to-beside proof of concept, demonstrating a signal of efficacy of arginine deprivation in mesothelioma.

ASS1 has been reported to correlate with platinum and antifolate sensitivity in preclinical models [16, 17]. Combining ADI-PEG20 with standard pemetrexed and cisplatin chemotherapy was explored in a phase 1 dose-escalation study [18]. No dose-limiting toxicities were observed and partial responses were observed in 7 of 9 patients (78%) including three with biphasic/sarcomatoid histology. Accordingly, this triplet combination was taken forward into a global randomised phase III trial, called ATOMIC. In the ADAM trial, it was found that sarcomatoid/biphasic mesotheliomas enriched for ASS1 loss, accordingly, ATOMIC has been designed to include only patients with these histological subtypes, rather than molecular prescreening.

Resistance to ADI-PEG20 has been explored and implicates potential treatment strategies to overcome this problem [19]. One mechanism is the re-expression of ASS1 by demethylation [20]. Metabolic reprogramming associated with glutamine addiction and glucose dependence has been reported in melanoma [21]. In mesothelioma, acquired resistance to ADI-PEG20 leads to a compensatory increase in polyamine biosynthetic enzymes, in response to a reduction in polyamine metabolites [19], implicating inhibition of polyamines as a possible approach to overcoming resistance to ADI-PEG20.

3 Targeting 9p21.3 Deletion

Recently, large-scale genomic studies have revealed distinct differences in the somatic mutational landscape of different mesotheliomas that may serve to enable molecular stratification for therapy. It has been known for several years that the long arm of chromosome 9p is deleted in a significant proportion of mesotheliomas,

carrying with it, tumour suppressors. This region carries the locus 9p21.3, which harbours the genes CDKN2A and MTAP. Loss of CDKN2A occurs at a frequency of around 49% [6] has been identified as a poor prognostic biomarker, and maybe a target for therapy. CDKN2A is a bone fide driver of mesothelioma in conditional knockout mice [22]. It encodes two key tumour suppressors, p16ink4a, an endogenous inhibitor of cyclin-dependent kinases 4 and 6; p14ARF is a deubiquitinase of MDM2, an endogenous inhibitor of p53.

Loss of p16ink4a can occur due to deletion or epigenetic suppression resulting in retinoblastoma driven cellular proliferation. Adenoviral studies involving the re-expression of p16ink4a demonstrated inducible tumour suppression in preclinical models, suggesting that either restoration or phenocopying of p16ink4a might be a therapeutic strategy [23]. Recently, the targets of p16ink4a, CDK4/6 have become successful drug targets in the treatment of breast cancer [24, 25] suggesting that in mesothelioma, it might be possible to phenocopy p16ink4a restoration by inhibiting its downstream targets. This strategy is unlikely to be successful in cancers harbouring retinoblastoma mutation due to the bypass of CDK4/6 control, however, this is rarely seen in mesothelioma. In large-scale drug–gene interaction studies, CDKN2A has been shown to promote sensitivity to CDK4/6 inhibition [26, 27]. Preclinical studies of CDK4/6 inhibitors in mesothelioma have been reported and show evidence of activity in the nanomolar concentration range (palbociclib), and lead to a significant decrease in cellular proliferation involving G1 cell cycle arrest in the G1 phase with a low rate of cell death (only 1–5%). Reduction in tumour growth was seen in mesothelioma xenografts in response to palbociblib (from 1335 mm^3 for vehicle to 479 mm^3 for palbociclib alone at 4 weeks) [28]. A phase IIA study has been developed (MIST2) to test the hypothesis that CDK4/6 inhibition has tumour controlling efficacy in patients harbouring p16ink4a negative mesothelioma.

The endogenous MDM2 inhibitor p14ARF is co-deleted in mesotheliomas harbouring 9p21.3 deletion, and results in elevated MDM2, which in turn is negatively prognostic [29]. Adenoviral mediated p14ARF gene transfection was reported to induce G1 cell cycle arrest and apoptosis, which was dependent upon the expression of p53 [30]. Accordingly, inhibition of MDM2 may be an attractive strategy for targeting p14ARF negative mesothelioma, particularly as wild type p53 is found in over 90% of mesotheliomas [7, 31]. Currently, several agents are in development, e.g., ALRN 6924, which has recently been evaluated in combination with a CDK4/6 inhibitor and demonstrated tolerability.

Co-deletion of the gene MTAP (S-methyl-5′-thioadenosine phosphorylase) is frequent in mesothelioma. High-throughput functional genomic screens have recently identified loss of MTAP as a vulnerability to inhibition of the epigenetic regulator and type II protein arginine methyltransferase, PRMT5 [32–35]. MTAP plays a critical role in polyamine metabolism related to salvage of both methionine and adenine. Histone methylation plays a critical role in regulating gene expression. S-adenosylmethionine, generated from the essential amino acid methionine, is used as a critical substrate for histone methylation by PRMT5. MTAP can regenerate methionine or adenine via MTAP mediated biotransformation of the polyamine biosynthesis intermediate Methylthioadenosine (MTA). Loss of MTAP leads to build

up of the MTAP substrate MTA, which directly interacts with and inhibits PRMT5, leading to an allosteric inhibition involving a change in conformation of Glu435 in the MTA bound form of PRMT5, reducing its enzymatic activity and creating a vulnerability to further inhibition. PRMT5 is an essential protein and its loss of function in MTAP negative cells results in a reduction in downstream histone methylation with global consequences for genome regulation, including arrest of cell growth. Inhibition of SAM biosynthesis via inhibition of MAT2A, phenocopies PRMT5 inhibition [33]. SAM mimetic PRMT5 inhibitors are now entering the clinic and due to the relatively higher levels of SAM compared with MTA even in MTAP negative cells, PRMT5 small molecule inhibitors (such as EPZ015666) which are entering the clinic, are not affected by MTAP status [34]. However, combination of novel PRMT5 inhibitors such as GSK3368715 with a PRMT5 are synergistic, and increase selectivity of PRMT5 suggesting a novel combinatorial clinical strategy [32].

4 Targeting BAP1 Inactivation

Inactivation of BAP1 represents one of the most common tumour suppressor aberrations in mesothelioma that can occur through several mechanisms including mutation, copy number loss, or translocations [7]. New approaches are being translated into the clinic that might exploit vulnerabilities associated with this somatic alteration. BAP1 loss has been reported to cause an epigenetic dysregulation involving increased trimethylation of histone H3 lysine K27 (H3K27me3) secondary to upregulation of the polycomb repressive complex 2 (PRC2) which comprises a catalytic subunit, EZH2 (enhancer of zeste 2) [36]. In parallel loss of BAP1 also causes a decrease in H4K20me1, which is reversed by the methyltransferase SETD8 leading to the abrogation of cell proliferation. This is phenocopied by inhibition of EZH2 using a small molecule inhibitor EPZ011989 in vitro and in vivo, implicating EZH2 as a potential therapeutic target in BAP1 mutant mesothelioma.

The EZH2 inhibitor tazemetostat has been subsequently evaluated in a phase II clinical trial, in patients with relapsed pleural mesothelioma [37]. In this clinical trial patients tazemetostat, an EZH2 inhibitor administered as an oral dose of 800 mg po twice a day was evaluated in patients preselected for BAP1 inactivation by immunohistochemistry. A total of 61 patients were enrolled and 12-week disease control (partial response + stable disease) was measured as the primary endpoint. The primary endpoint of the clinical trial was met with 51% demonstrating disease control at 12 weeks, with 25% continuing to 24 weeks. Interestingly, two patients had a confirmed partial response. Together this data provided proof of concept relating to the efficacy of EZH2 inhibition in mesothelioma.

Other approaches to achieve synthetic lethality in BAP1 mutant mesothelioma are being explored in the clinic. BAP1 is an essential factor required for double-strand DNA repair involving assembly of the BRCA1/RAD51 foci following ionising radiation. Recruitment to DNA double-strand break sites is mediated via

phosphorylation of BAP1, and the role of BAP in DNA damage response involves its catalytic activity [38]. Furthermore, BAP1 mutation has been shown to alter the stability of BRCA1 through its deubiquitination [39]. Accordingly, BAP1 may alter homologous recombination efficiency and as such, mediate sensitivity to inhibition of poly-ADP ribose polymerase (PARP) [40]. The use of PARP inhibitors for the treatment of ovarian cancer, which harbours inactivating BRCA1 mutations, is now the standard of care based on a well-established synthetic lethal drug–gene relationship [41–43]. BAP1 may confer a so-called BRCAness phenotype [44], which could be exploitable via PARP inhibition. Clinical trials have now enrolled, involving PARP inhibition in mesothelioma with results expected to be reported in 2020. These studies include evaluation of Rucaparib in BRCA1/BAP1 inactivated mesothelioma, trials.gov identifier NCT03654833. Of note, BRCA1 loss of expression by histochemistry is seen in around 38% of mesotheliomas [45]. In addition, olaparib NCT03531840 and niraparib NCT03207347 are also being evaluated.

Preclinical studies are revealing possible additional strategies for targeting BAP1. In a small molecule screen of 94 drugs, BAP1 was shown to confer sensitivity to inhibition of tumour necrosis factor-related apoptosis-inducing ligand. This interaction was observed in cell lines, primary mesothelioma explants, and using xenografts [46]. To date, TRAIL has demonstrated tolerability in the clinic but activity has been lacking across other cancers; targeting BAP1 mutant mesothelioma may therefore represent a new opportunity for the development of this class of agent. High-throughput drug screens in genomically annotated models may provide an efficient means of rapidly identifying agents with potential to induce synthetic lethality [47]. This approach has also demonstrated potential sensitisation of BAP1 mutation to fibroblast growth factor receptor inhibition [47, 48].

5 Hippo Pathway as a Target for Mesothelioma

Mutations that lead to activation of the Hippo pathway and dysregulated apoptosis and proliferation, may present therapeutic vulnerabilities [49–51]. Genetic inactivation of NF2 (which encodes the Hippo regulator Merlin), LATS1 and LATS2 are common in mesothelioma, and lead to nuclear translocation of YAP and its orthologue TAZ, leading to deregulated, constitutive, oncogenic activation of the TEAD transcription factor [52, 53]. One of the first attempts to target Hippo inactivation involved exploitation of upregulated focal adhesion kinase (FAK) in cells harbouring mutation of NF2 [51, 54, 55]. This observation led to initiation of a merlin stratified, randomised, placebo-controlled phase II trial called COMMAND. This trial compared switch maintenance defactinib, a small molecule FAK inhibitor, to placebo after evidence of disease control following standard first line chemotherapy. This study failed to show any evidence of superior disease control [4]. Interestingly, defactinib has shown limited single agent activity in mesothelioma with a response rate of 13% and stable disease rate of 67% (disease control rate of 80%) [56]. It was shown that that defactinib induced a reduction in immunosuppressive T regulatory

cells and exhausted T cells (CD69+/PD1+), with a contrasting increase in cytotoxic CD8 T cells, and a switch of histology from biphasic to epithelioid in 13%. Immune modulation leading to reduction in T regulatory cells has been reported preclinically [57]. Based on this observation, FAK inhibition is being evaluated in mesothelioma in combination with the anti-PDL1 inhibitor pembrolizumab (clinical trials.gov identifier NCT02758587).

TEAD-driven transcription leads to the expression of oncogenic addiction, which might be disrupted pharmacologically. YAP/TAZ promotes the association of bromodomain-containing protein 4 (BRD4) to their regulated promoters leading to the expression of pro-tumour genes, and this transcription is inhibited by BRD4 inhibitors [58]. This class of small molecule inhibitor has shown preclinical activity in mesothelioma, is currently in development in early phase clinical trials and may represent a strategy to target YAP/TAZ [59].

YAP/TAZ-driven transcription leads to increased susceptibility to iron-dependent cell death process called *ferroptosis* through the upregulation of ACSL4 and TFRC. E-cadherin mediates a non-cell autonomous suppression of ferroptosis via activation of NF2 and the Hippo pathway [60, 61]; this inhibition is removed upon mutation of the Hippo pathway. In contrast, ferroptosis is tumour suppressive and activated by BAP1 mediated SLC7A11 suppression [62, 63]. The small molecule sorafenib is a known activator of ferroptosis, and has demonstrated limited activity in unselected patients with mesothelioma [64–68]. Based on these new findings regarding potential sensitivity in Hippo pathway mutant mesotheliomas, a question remains as to whether or not stratified treatment in this subgroup might exhibit clinically useful efficacy.

Loss of NF2 has been shown to promote RAS induced thyroid cancers via YAP-driven transactivation of RAS, leading to sensitivity to MEK inhibition [69], suggesting that this target could represent a therapeutic opportunity in this molecular subgroup.

Disruption of YAP/TEAD interactions may be feasible pharmacologically. Verteporfin, a clinically used photosensitiser, disrupts this interaction in preclinical models [70–72]. This agent provides proof of concept that disruption of oncogenic signalling via YAP/TAZ can be potentially disrupted by inhibitors. This has led to the development of candidate YAP/TEAD inhibitors capable of blocking the interface between these proteins, which although at an early stage, have potential to target hippo mutant mesothelioma [73, 74].

6 Precision Medicine Platforms for Mesothelioma

Dealing with the extensive inter-patient heterogeneity exhibited by mesothelioma presents a formidable challenge, and a critical barrier to effective therapy of that may be tackled through prospective molecular stratification. In this chapter, some key examples have been provided whereby rational selection of patients similar genotypic or phenotypic characteristics may benefit from targeted therapies.

Umbrella studies provide a new opportunity to stratify patient cohorts into subsets likely to benefit from specific treatments. In the United Kingdom, we have developed and piloted such a platform known as Mesothelioma Stratified Therapy (MiST) comprising an initial molecular panel screening using immunohistochemistry-based assessment of BAP1, p16INK4a, PDL1, and BRCA1. Based on a patient's molecular profile, patients receive a targeted therapy, for example BAP1/BRCA1 subgroup receives the PARP inhibitor rucaparib in MiST1, and if p16ink4a negative the CDK4/6 inhibitor abemaciclib in MiST2 etc. This platform is modular and adaptable, providing a rapid bedside to bench translational engine to test novel drug–gene interaction hypotheses. Understanding signals of efficacy through omic interrogation is fundamental to MiST, as is the inclusion of tissue re-sampling at the time of disease progression in prior responders, in order to understand mechanisms of acquired resistance. MiST aims to drive bedside to bench research in order to model drug sensitivity and so predict either novel combinations or better refinement of biomarkers.

7 Conclusions

There remains an unmet need for treatment in the relapsed setting. Stratified medicine for mesothelioma has arrived but is at a very early stage. New insights into biology have highlighted potentially actionable vulnerabilities for which new agents are only now being tested in the clinic; some for the first time in humans. Umbrella platforms for stratified therapy may provide the best hope for testing new hypotheses and accelerating the identification of effective therapy.

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Chapter 25
Viral Immune Therapy and Other Virotherapies for Advanced Mesothelioma: Are We Ready for Clinical Trials of Viral Immune Therapy?

Kazuma Sakura, Yasushi Shintani, and Meinoshin Okumura

Abstract Mesothelioma is an aggressive tumor that occurs in the pleura, peritoneum, pericardium, and rarely tunica vaginalis. The management of mesothelioma is complex, and the outcomes are not satisfactory, although combined-modality treatment including surgery, chemotherapy, and radiation has been performed. Immune checkpoint inhibitors are expected to improve the outcomes of mesothelioma. However, their efficacy is limited in some patients, in comparison to other cancer outcomes. Therefore, novel or combined therapies for mesothelioma are eagerly awaited. Virotherapy may be a useful tool for mesothelioma treatment. Viral immune therapy involves treating tumors by activating antitumor immunity using viruses. Virotherapy can be divided by the mechanism into viral immune therapy, antitumor oncolytic viral therapy, and gene therapy using viruses as vectors. Viral immune therapy is typically used with oncolytic viruses and activates the antitumor immunity via viral infection or oncolysis. Since viral immune therapy activates the antitumor immunity, virotherapy has received attention concerning its combination with immune checkpoint inhibitors or other immune therapeutic agents. Oncolytic viral therapy involves the induction of direct tumor cell death by oncolytic viruses, while gene therapy uses viruses as vectors to encode tumor suppressor genes or

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other genes. A large number of viruses used for virotherapy have an enhanced antitumor effect due to either genetic or other modifications. We herein review the clinical trials of virotherapy against mesothelioma and describe the non-replicating oncolytic virus (hemagglutinating virus of Japan [Sendai virus] envelope) used for our clinical trials of viral immune therapy against mesothelioma.

Keywords Non-replicating oncolytic virus · Hemagglutinating virus of Japan envelope · Sendai virus · Oncolytic viruses · Virotherapy · Viral immune therapy

1 Introduction

Mesothelioma is an aggressive tumor, and its prognosis is poor even with multimodality treatment. While immune checkpoint inhibitors have been expected to improve the outcomes of mesothelioma, such treatment has been lackluster, as antitumor immunity is complicated. To enhance drug efficacy, immune checkpoint inhibitors are used in combination with chemotherapeutic drugs or radiotherapy, and companion diagnostics are currently being developed in order to identify high-susceptibility patients. However, further novel therapies are eagerly awaited.

In this setting, viral therapy has received attention as therapy for mesothelioma [1]. One reason for this increased attention is that *Talimogene laherparepvec*, a genetically modified herpes simplex virus, was approved as the first viral compound by the US Food and Drug Administration in 2015 [2]. Another reason is that mesothelioma is suitable for virotherapy, as the lesions of pleural mesothelioma develop in the parietal pleura and are localized to the pleural cavity, so there is almost no worry about the clearance of viral compounds by the immune system, and it is easy to administer viral compounds directly to the tumors.

Virotherapy involves the treatment of tumors using viruses that possess oncolytic activity and stimulate antitumor immunity. With the development of genetic engineering, a large number of viruses for virotherapy are prepared via genetic or other modifications in order to enhance the antitumor effect. Specifically, viruses express the genes associated with cytokines or enzymes that metabolize prodrugs to valid drugs in tumor cells. In addition, these developed viruses hardly injure normal cells [2, 3].

We herein report the results of recent clinical trials of virotherapy—mainly oncolytic virotherapy and viral immune therapy—against mesothelioma and explain the application of hemagglutinating virus of Japan envelope as a non-replicating oncolytic virus in our clinical trials of viral immune therapy against mesothelioma and other malignant tumors.

2 Virotherapy

Virotherapy consists of several steps. First, viruses containing a modified viral product infiltrate tumor cells and destroy the cells via oncolysis or the induction of cell death. For this step, artificial viruses are modified so that as few normal

cells as possible sustain collateral damage. Avirulent strains require no such modification. The antitumor immunity is then stimulated by the signals related to the tumor cell destruction. Viruses used for virotherapy, therefore, possess the following characteristics: antitumor efficacy, including oncolysis; tumor selectivity; stimulation of the antitumor immunity; and minimal damage to normal cells [4].

2.1 Antitumor Efficacy

The antitumor efficacy of virotherapy is mostly caused by viral replication, and tumor cell lysis via transgene is known to induce cytotoxic viral proteins, such as the HSV thymidine kinase gene, which metabolizes ganciclovir into a toxic product [5]. Both replication-deficient and replication-competent adenoviral vectors with the HSV thymidine kinase gene have been developed for the treatment of some tumors, including pleural mesothelioma [6].

2.2 Tumor Specificity

The tumor specificity of viruses used for virotherapy is important to enable high-dose administration without worrying over the induction of adverse events due to normal cells being infected. Modification of the viral coat, exploiting abnormal signaling pathways, inserting tumor-specific promoters, and deleting genes (e.g., ICP34.5, E1A, or E1B Protein) have been employed to ensure specificity [7, 8].

2.3 Stimulation of Antitumor Immunity

Sustained antitumor immunity against the tumor response-stimulated viruses is required for successful virotherapy. The immunogenic cell death induced by virotherapy causes tumor-associated antigens to be exposed to antigen-presenting cells, so this phenomenon activates innate and adaptive immunity against the tumor. It has been confirmed that virotherapy by local administration has a systemic antitumor effect against distant metastasis due to antitumor immunity [7]. Beyond simple oncolysis, the cell death associated with oncolytic virotherapy may involve mechanisms that serve to induce an adaptive immune response [9]. Tumor cell oncolysis induced by virotherapy is important for mediating the epitope-spreading phenomenon, although the mechanism has not been fully elucidated [10].

2.4 Safety of Virotherapy

The safety of viruses used for virotherapy is crucial. Concerns about adverse events due to normal cell infection, environmental contamination, and secondary infection must be strictly addressed. Therefore, virotherapy is strictly regulated by the Cartagena protocol on biosafety to prevent contamination. Many replication-competent viruses have been further attenuated through genetic modification in order to enhance their viral replication in tumor cells and improve the safety profile for healthy cells. One common modification is the addition of interferon-beta (IFN-beta) [11]. IFN-beta is the key molecule involved in inhibiting viral replication in healthy human cells. There are often defects in the type I IFN response of tumor cells, allowing for increased viral replication while leaving normal cells unaffected. However, IFN-beta is involved in the stimulation of antitumor immune responses.

3 Virotherapy for Mesothelioma

Many viruses and viral compounds have been involved in early-phase clinical trials for mesothelioma, and they are expected to be useful as novel therapeutic agents. In addition, recent clinical trials have evaluated the efficacy of virotherapy in combination with various drugs to see how these combinations can improve the outcomes for mesothelioma. HSV-1, adenovirus, vaccinia virus, and measles virus are the most extensively studied virotherapy vectors for mesothelioma (Table).

3.1 Herpesvirus Type 1 (HSV-1): HSV1716

A preclinical study by Kucharczuk et al. in 1997 evaluated the replication-competent, neuroattenuated HSV-1716 as oncolytic virotherapy for mesothelioma [12]. Human studies of oncolytic herpesviruses for mesothelioma have been completed. Twelve patients were treated, and an acceptable safety profile of intra-pleural HSV1716 with evidence of viral replication and antitumor immunogenicity was observed [13].

3.2 HSV-1: G47Δ

G47 is genetically engineered HSV-1 with triple mutations that demonstrate augmented viral replication, a strong induction of antitumor immunity and enhanced safety in normal cells. G47 was used in a phase II clinical trial of glioblastoma in Japan. Thirteen patients suffering from either recurrent of residual glioblastoma were registered, and they were treated with the intratumoral administration of G47.

The results showed the 1-year survival rate to be higher than that of the standard treatment regimen as calculated based on the findings of several other clinical trials (92.3 vs 15%).

Recently, a new clinical trial of mesothelioma has just been started in Japan [14].

3.3 Adenovirus: AdV/hIFN-alpha2b

Virotherapy with adenovirus expressing interferon-alpha with intrapleural administration combined with celecoxib and chemotherapy was well tolerated [15]. This was based on the known anti-inflammatory effects of celecoxib, experience in previous clinical trials using celecoxib to reduce cytokine release syndrome with the intrapleural administration of adenoviral vectors [16], and preclinical research showing that cyclooxegenase-2 (COX-2) inhibition augmented the effects of immunotherapy using AdV/IFN-beta in mesothelioma [16]. Based on these results, a randomized phase III trial is ongoing (NCT03710876).

3.4 Adenovirus: ONCOS-102

ONCOS-102 is a granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing oncolytic virus. GM-CSF expression within the tumor recruits antigen-presenting cells and natural killer cells and activates and induces the maturation of antigen-presenting cells, which induce antitumor immunity [17]. Compared with pre- and posttreatment biopsies, ONCOS-102 resulted in a fourfold increase in CD8+ T cells and an almost sixfold increase in the expression of CD3 [18]. Furthermore, a subset of mesothelioma patients demonstrated an increase in the tumor PD-L1 expression following injection with the virus [18]. These observations have led to an interest in combining ONCOS-102 with Durvalumab, and an ongoing clinical trial is using this combination for patients with advanced melanoma (ClinicalTrials.gov: NCT02963831).

3.5 Vaccinia Virus: GL-ONC1

Vaccinia has been shown to have an oncolytic effect on malignant pleural mesothelioma (MPM) cells in vitro and to be effective for treating MPM when delivered intrapleurally in a preclinical mouse model [19]. Eleven pleural mesothelioma patients were treated, and a single dose via intrapleural administration of GL-ONC1 was deemed safe but was best suited to patients with MPM whose disease was limited to the pleura [20].

3.6 *Measles Virus*

The measles virus is a negative-strand RNA paramyxovirus that is highly fusogenic and induces extensive cytopathic effects of syncytial formation [21]. A phase I trial using the attenuated Edmonston strain with insertion of the NIS gene (MV–NIS) was conducted, and 12 patients received MV-NIS therapy. The best therapeutic response was stable disease. The intrapleural administration of MV-NIS is safe, resulting in stable disease in 67% of patients, and may be associated with a favorable overall survival in malignant mesothelioma [22].

4 Non-replicating Oncolytic Virus (Hemagglutinating Virus of Japan Envelope): GEN0101

We use a non-replicating oncolytic virus (hemagglutinating virus of Japan envelope [HVJ-E]) for clinical trials of aggressive tumors, such as mesothelioma, melanoma, and castration-resistant prostate cancer. HVJ-E is derived from HVJ, also known as Sendai virus, which is a paramyovirus with a negative-strand RNA genome (Fig. 25.1). HVJ-E and HVJ have no pathogenicity in humans [23]. HVJ-E is called a non-replicating oncolytic virus and is unlike other viruses, as HVJ-E was developed from the fragmentation of HVJ RNA. As HVJ-E cannot replicate, it is called a "NOT virus," or "modified product from a virus" in a strict sense.

This lack of an ability to replicate is the main point in differentiating HVJ-E from the viruses used in current virotherapies [23]. Therefore, HVJ-E is not regulated by

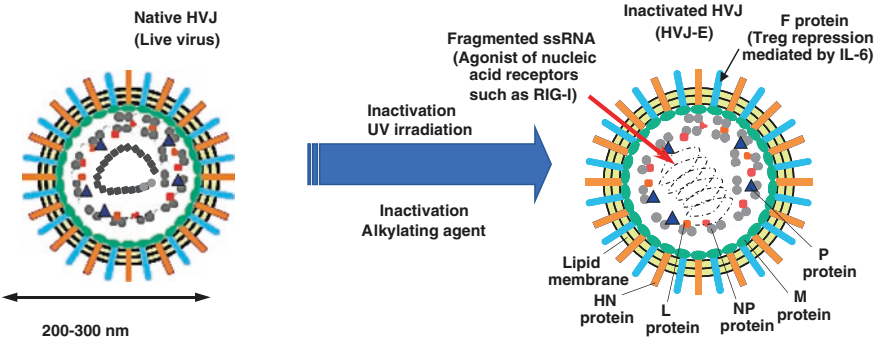
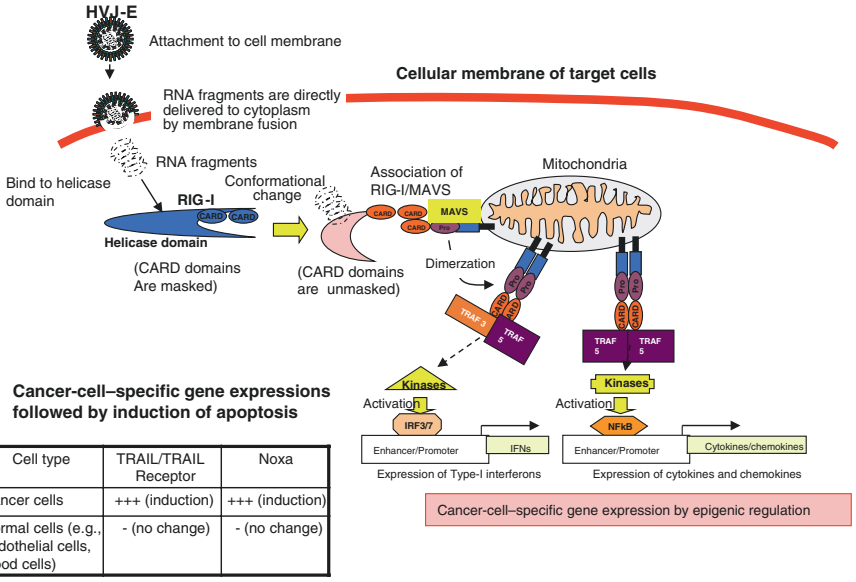


Fig. 25.1 Schematic structure of HVJ-E. The structure of HVJ-E. The fragmented single-stranded viral RNA genome by ultraviolet radiation or an alkylating agent, and nucleocapsid protein (NP), as well as polymerases P and L, is located inside, and the F and N proteins penetrating the envelope are associated with cell fusion. HVJ-E lost the ability of viral genome replication and protein synthesis, but maintain membrane fusion activity



Cancer-cell–specific gene expressions followed by induction of apoptosis

Cell type	TRAIL/TRAIL Receptor	Noxa
Cancer cells	+++ (induction)	+++ (induction)
Normal cells (e.g., endothelial cells, blood cells)	- (no change)	- (no change)

Fig. 25.2 Cancer cell–specific gene expression by epigenic regulation. The mechanism of HVJ-E-induced cancer cell killing involves the introduction of viral genome RNA fragments into the cytoplasm via membrane fusion and the subsequent activation of a retinoic acid-inducible gene-I (RIG-I)-like receptor signal, which results in the upregulation of apoptotic genes such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Noxa through the phosphorylation of interferon-regulatory factor (IRF)-3 and -7

the Cartagena protocol, so there is no restriction on its clinical treatment compared with other viruses. HVJ-E has similar fusogenic and oncolytic activities to HVJ despite its lack of replication activity. However, HVJ-E actually has a greater oncolytic activity than HVJ [24]. The mechanism of oncolytic activity differs from that of all other viruses. Namely, it induces tumor cell-specific apoptosis, except in normal cells, through the activation of the RIG-I/MAVS pathway in the tumor cell nucleus. This unique characteristic is due to differences in the expressions of apoptotic genes, such as Noxa, TRAIL, and TRAIL receptors in tumor cells and normal cells [25] (Fig. 25.2). HVJ-E also exerts antitumor activities, such as enhancing multiple antitumor immunities like the activation of dendritic cells, induction of natural killer cells and cytotoxic T lymphocytes, and suppression of regulatory T cells [24] (Fig. 25.3). In addition, HVJ-E induces innate immunity, promoting the infiltration and activation of natural killer cells via the induction of chemokines and type I interferons [26]. In this way, HVJ-E is able to induce both innate and adaptive immunities.

We conducted the first-in-human study for advanced melanoma patients, and the tolerability was confirmed. HVJ-E reduced not only the size of the treated lesion but also the untreated lesion in the same area. An examination of resected tumor tissue

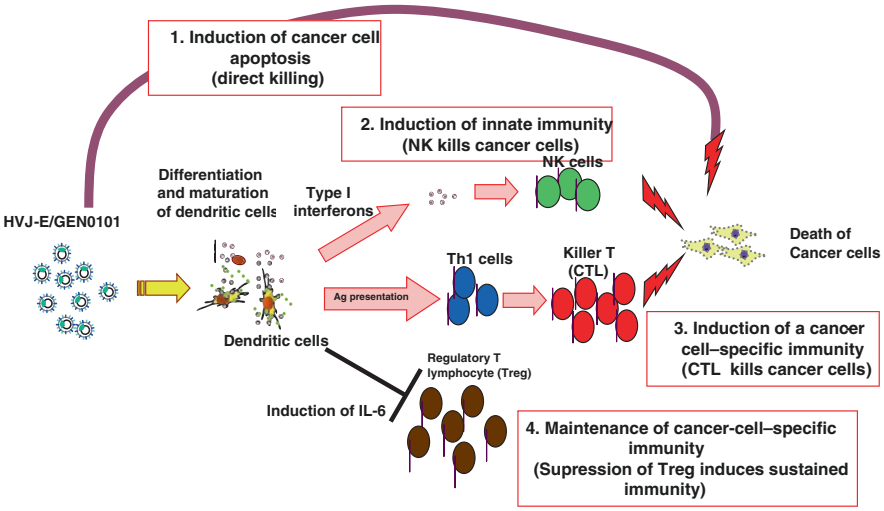


Fig. 25.3 Induction of cancer cell apoptosis, activation of NK cells and CTL, and repression of Treg. HVJ-E elicits antitumor immunity by recruiting immune cells to the tumor microenvironment, facilitating the maturation of dendritic cells, enhancing natural killer (NK) cell activity, and ultimately activating killer T cells targeting cancers. Moreover, the fusion protein of HVJ-E acts directly on dendritic cells and macrophages to produce interleukin (IL)-6, which attenuates the function of regulatory T cells. Thus, HVJ-E provides a multimodal strategy for cancer therapy

showed that administration of HVJ-E had induced the infiltration of cytotoxic T lymphocytes, M1 type macrophages, and natural killer cells into the tumor tissue [27]. These findings suggest that HVJ-E has direct antitumor activity and induces antitumor immunity. We are now conducting a phase II study in advanced melanoma patients in combination with Pembrolizmab. HVJ-E changes the tumor microenvironment from a "cold" to "hot" condition as in other viruses (Fig. 25.4).

We also conducted a clinical study for patients suffering from castration-resistant prostate cancer. HVJ-E was administered into the prostate cancer through the wall of the rectum guided by ultrasonography under general anesthesia. As the tolerability was confirmed [28], we next conducted a phase I study in chemotherapy-resistant mesothelioma patients with the intratumoral administration of HVJ-E guided by ultrasonography. The tolerability of the intratumoral administration into mesothelioma was observed, and a subset of treated tumors shrank, just as they had in the phase I study of melanoma. We are now performing a phase II clinical study in naïve mesothelioma patients in combination with Cisplatin and Pemetrexed. Chemotherapeutic agents and HVJ-E induce tumor cell death through different mechanisms. Chemotherapeutic agents kill tumor cells directly, while HVJ-E induces apoptosis, and thereby stimulates antitumor immunity. As a result, synergistic efficacy is expected.

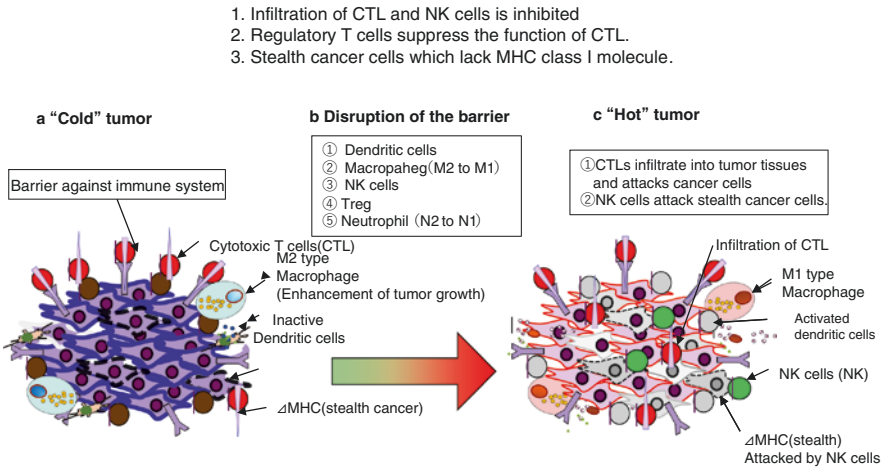


Fig. 25.4 HVJ-E is able to disrupt the barrier of tumor tissues. HVJ-E have the capability of transforming a "cold" tumor microenvironment with M2 macrophages, N2 neutrophils, regulatory T cells, and few immune effector cells into a "hot" environment with M1 macrophages, N1 neutrophils, and increased immune cell and cytokine infiltration

5 Conclusion

While virotherapies for various tumors are being performed, most of these therapies use oncolytic viruses, and virotherapy for mesothelioma is no exception. To expand the indications of virotherapy, it will be necessary to ensure safety, tumor selectivity, and genetic stability. This will likely be resolved with the development of genetic engineering. Virotherapy is expected to become a common therapeutic method due to the fact that it demonstrates strong antitumor activity and it also strongly induces antitumor immunity. In the short term, virotherapy combined with chemotherapy, immune therapy (such as immune checkpoint inhibitors), and radiation therapy will be useful for enhancing therapeutic outcomes, as each therapy induces complementary synergistic effects through immunogenic cell death, the activation of neoantigens, and other immunological activations [9]. The results of combination studies are paramount for understanding the optimal timing and method of administration as well as potential side effects. When exploring these therapies for mesothelioma, it is important to investigate the changes in the tumor microenvironment before and after treatment. Obtaining tissues is more difficult with mesothelioma than melanoma. Therefore, the development of a novel tool to investigate the immunological status and companion diagnostics is desired.

HVJ-E are not viruses in a strict sense, so it is not necessary to ensure their safety, tumor selectivity, or genetic stability. HVJ-E is already known to have similar tumor selectivity and oncolytic activity to other viruses used for virotherapy

[24–26]. Therefore, we are conducting clinical trials in combination with chemotherapy and immunotherapy, as with other viruses. From an immunological perspective, we believe that oncolysis associated with the intracellular growth of viruses is not always necessary. Given their safety, virotherapy in the future may use products from viruses, such as HVJ-E, instead of actual viruses. Viral immune therapy may thus diverge from virotherapy and become distinguished as a safe approach to immune therapy using modified products from viruses, even though viral immune therapy is regarded as a kind of virotherapy at present. However, HVJ-E is the only product for viral immune therapy being used in clinical trials at present. We are now ready to incorporate viral immune therapy into clinical trials for advanced mesothelioma.

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Part VIII
Radiotherapy

Chapter 26
Impact of Radiation Therapy on Malignant Mesothelioma: Are We Ready for Use in Clinical Practice, Combined with Surgery or Alone?

Keiko Shibuya

Abstract Technology for providing radiation therapy (RT) has advanced significantly over the last decade, allowing for more accurate delivery and safer radiation doses. However, malignant mesothelioma (MPM) can rapidly expand in the chest, and full hemithoracic radiation can cause serious lung complications. Traditionally, the main roles of RT included prophylactic irradiation of intervention tracts (PIT), adjuvant therapy after radical surgery (as multimodality therapy), and symptomatic treatment. Of these options, recent findings indicate that PIT is not recommended for routine use. The role of RT as an adjuvant therapy is also changing with changes in surgical methods. Although RT after extrapleural pneumonectomy has shown some effect as a part of tri-modal therapy, the role of RT after lung-sparing surgery is still uncertain. Recently, intensity-modulated pleural RT has been shown to be promising in several studies, but currently, this therapy is only recommended if provided by a team of highly trained experts and when subject to strict dose constraints and aggressive toxicity management. The role of RT as symptomatic treatment is still important. Because MPM has a high infiltrating tendency, symptoms progress at a very fast rate. However, better quality of life may be maintained by using RT, if properly timed with systemic treatment.

MPM is an aggressive tumor with a poor prognosis, but the number of cases per institution is small. It is essential to continue to build a consensus regarding treatment across facilities worldwide.

Keywords Hemithoracic radiation · 3D-CRT · IMRT · Dose constraint

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1 Introduction

Radiation therapy (RT) is one of the standard treatments for lung cancer and several other solid cancers. Malignant pleural mesothelioma (MPM) has a low frequency of early distant metastases, and the first recurrence is often in the ipsilateral thorax. Thus, the value of local treatment for MPM may be greater than that for lung cancer. In addition, from a biological perspective, the radiosensitivity of MPM is at least as high as that of lung cancer [1]. However, because MPM is associated with rapid expansion over a wide area in the thorax, RT for MPM is associated with more challenges. If the dose equivalent to curative dose for lung cancer (>60 Gy) is administered to the entire affected thorax, serious lung complications are almost inevitable [2]. For these reasons, RT has been considered a curative treatment for MPM only in combination with surgery over the last 40 years.

On the other hand, the surgical procedure for MPM changed from pleurectomy/decortication (P/D) to extrapleural pneumonectomy (EPP), and currently the benefits of P/D for MPM, which enables preservation of the lungs, are being reevaluated.

Radical therapy for MPM is still in the exploratory field, and the role of RT has been changing with changes in surgical procedures. However, with the recent advances in RT technology, there is a good possibility that RT will continue to play a role as a part of a multi-modality therapeutic approach for MPM.

MPM has a high infiltrating tendency, so it is likely to cause pain associated with its extension into the chest wall. Invasion to the mediastinum also requires relief of symptoms caused by stenosis/obstruction of mediastinal organs. Palliative treatment to address these symptoms will continue to be an important role of RT.

This chapter explores RT as a symptomatic and prophylactic therapy for MPM, its role as a part of a radical multidisciplinary treatment approach, standard methods of RT, and future prospects.

2 Progression in RT Techniques

RT technology has significantly progressed over the past decade. The most innovative advance was the transition from a two-dimensional (2D) to a three-dimensional (3D) treatment plan based on computed tomography (CT) images. In addition, adoption of a multi-leaf collimator (MLC) has proved useful in making radiation fields fit the shape of the tumors. Using these techniques, RT methods, which are referred to as 3D conformal RT (3D-CRT), were developed and widely used. In fact, 3D-CRT is one of the most popular RT systems.

In addition, in the 2000s, a new technology called high-precision RT, which includes intensity-modulated radiation therapy (IMRT), was developed. IMRT allows the radiation dose intensity of each beam to be intentionally non-uniform by making the MLC move during irradiation. Using IMRT techniques, the dose can be increased gradually or selectively within the tumor without increasing the dose to the normal organs close to the tumor.

3 Role of RT for MPM

MPM has a poor prognosis, and the need for a multidisciplinary treatment approach along with complete surgical resection has been widely investigated as the only way to prolong survival. However, there is insufficient evidence regarding the extent to which the addition of highly invasive local RT treatment to surgery contributes to survival. In addition, there is no definitive consensus on the methods of surgery, the regimen and timing of combined chemotherapy, or the required dose of RT.

Currently, the possible roles of RT for MPM are as follows: (1) prophylactic treatment for dissemination of the puncture/drainage tracts, (2) adjuvant therapy after radical surgery (as multidisciplinary therapy), and (3) symptomatic treatment for mediastinal or chest wall tumor invasion.

3.1 Prophylactic Irradiation of Intervention Tracts (PIT)

As a prophylactic treatment, the significance of chest wall irradiation to the intervention site has been discussed. One of the characteristics of MPM is that it often leads to the development of disseminated proliferation and forms a tumor at the chest wall along the path of drainage of pleural effusion, thoracoscopy, or biopsy. This often causes pain and impairs the patient's quality of life. A biopsy is recommended to obtain the final pathological diagnosis of MPM. In many cases, pleural puncture is also performed for drainage and symptom relief. The frequency of seeding along the puncture site after such treatments is 5–20% [3]. If MPM is suspected, the intervention route should be unified, and if surgery is performed, resection including the skin at the intervention site is performed as much as possible.

Patients who do not undergo surgery were generally treated with PIT. The rationale for this treatment was a randomized controlled trial (RCT) reported in 1995 by Boutin et al. Results of that study showed that the frequency of chest wall dissemination was significantly reduced from 40% to 0% by 21 Gy in 3 fractions of chest wall irradiation (12.5–15 MeV electron beam, 1 cm bolus was used). However, in actual clinical practice, patients have experienced disseminated lesions in the radiation field within a few months after treatment. Thus, the preventive effect of PIT has been questioned. Two small RCTs were conducted to confirm the results of Boutin et al., but these trials did not find any significant differences between the PIT group and non-PIT group [4, 5]. In addition, no clear benefit of PIT was found in a subsequent meta-analysis [6–8].

The reasons for the discrepancies in these results may have something to do with the study type, but may also be due to differences in tissue type or the follow-up period due to differences in prognosis of patient groups. Moreover, the previous 3 RCTs did not include patients receiving systemic chemotherapy, because these studies were conducted before palliative chemotherapy was widely available for MPM. Based on the lack of consensus between studies, a multicenter, open-label,

phase 3 RCT (surgical and large-bore pleural procedures in MPM trial: SMART trial) was conducted. This study compared prophylactic RT with deferred RT given only when a procedure-tract metastasis (PTM) developed [9]. Patients were well matched at baseline, and in both treatment arms, RT of 21 Gy in 3 fractions over 3 days was given. No significant differences were seen between the 2 treatment arms in any endpoint, including symptoms and survival. The proportion of patients developing a PTM was 9% in the prophylactic RT group and 16% in the deferred RT group (odds ratio, 0.51; 95% confidence interval, 0.19–1.32; $p = 0.14$). In conclusion, the findings in this trial did not support the benefit of routine use of PIT in MPM.

However, a predefined subgroup analysis suggested that PIT might improve survival for patients with epithelioid-only histological tumors. In addition, in the subgroup of patients who did not receive chemotherapy, the rate of PTM was lower in the PIT arm.

Based on the above findings, PIT is not routinely recommended, while it may be beneficial in specific patient groups. In any case, skin lesions not only cause symptoms such as pain, but also cause mental distress to patients, so careful attention should be paid to the drainage site.

3.2 Definitive RT in Multidisciplinary Treatments

3.2.1 Surgical Choices and RT

As described above, MPM spreads widely in the thoracic cavity from the early stage, so in principle, the entire hemithorax should be considered the target when radical RT is considered, even though the lesion seems localized radiologically.

Until the early 1980s, when P/D was performed for MPM, most radiation treatments were provided using a 2D treatment plan. It was not possible to deliver a sufficient dose due to adverse events, and RT did not sufficiently contribute to an improvement of prognosis [10, 11]. Gupta et al. analyzed 123 patients who underwent P/D and postoperative RT in 1974–2003, and the median survival time (MST) was only 13.5 months [12]. Although these results were not considered satisfactory, the study found that one of the prognostic factors was the dose of RT. The prognosis was especially poor at doses less than 42 Gy ($p = 0.001$), which suggested the significance of using additional local RT doses.

Since the 1980s, the surgical procedure for MPM changed from lung-sparing P/D to non-lung sparing EPP, and RT changed from 2D to 3D. With these changes, postoperative chest wall irradiation with a dose greater than 50 Gy became safer. Analyses of these treatment experiences supported the idea that postoperative RT contributed to the reduction of local recurrence of MPM. Since the beginning of the 2000s, "tri-modality therapy," which consists of EPP combined with IMRT and chemotherapy, has been widely considered a highly curative treatment method for MPM (Table 26.1).

However, as data about EPP accumulated in Europe and the United States, many reports on serious complications and treatment-related deaths appeared. As a result, discussions on the risks and effects of EPP began again. In particular, multidisciplinary therapy that combined less invasive P/D with systemic chemotherapy was also re-examined.

Currently, there is still no consensus on the role of RT in MPM. Although studies on P/D that can preserve the lungs are underway, postoperative radiation by IMRT has also been attempted after P/D. Further discussion on its significance and safety will be necessary after these studies are completed.

3.2.2 RT after EPP in Tri-modality Therapy

Many studies have been conducted on hemithoracic radiation after EPP as part of a multi-modality approach (Table 26.1). In a propensity score matching study of 2166 patients from the 2000 to 2013 SEER database, postoperative RT was performed in

Table 26.1 Changes in surgery and radiation therapy for malignant pleural mesothelioma over 4 decades

References	Treatments	Study period	No. of patients who received RT	Local failure rate, %	Median survival time, months	Two-year survival rate, %
Hilaris et al., 1984 MSKCC [10]	P/D EBRT (2D-RT) 45 Gy I-125, Ir-192 implantation	1976–1982	41	71	21	40
Baldini et al., 1997 Harvard Univ. [13]	EPP CT EBRT (2D-RT) 30.6 Gy + boost <20 Gy	1987–1993	49	35	22	NA (3y-OS: 34)
Rusch et al., 2001 Phase II study MSKCC [14]	EPP EBRT (3D-CRT) 54 Gy (range, 20–64 Gy)	1995–1998	54	13	Stage I/II: 34 Stage III/IV: 10	NA
Yajnik et al., 2003 MSKCC [15]	EPP EBRT (3D-CRT) 54 Gy (range, 45–54 Gy)	1990–2001	35	37	NA	NA
Rice et al., 2007 MDACC [16]	EPP EBRT (IMRT) 45 Gy (+SIB, 10 Gy)	1999–2005	63	5	14	32

(continued)

Table 26.1 (continued)

References	Treatments	Study period	No. of patients who received RT	Local failure rate, %	Median survival time, months	Two-year survival rate, %
Hasegawa et al., 2016 JMIG [17]	CT EPP EBRT (3D-CRT) 54 Gy	2008–2010	17	41	39.4	NA
Matsuo et al., 2017 Kyoto Univ. [17]	EPP CT EBRT (IMRT) 50.4 Gy (+SIB, 5.4–11.2 Gy)	2006–2013	21	3-year incidence 12.3 (95% CI, 3.2–41.2)	27	47.6
Rimner et al., 2016 Phase II study MSKCC and MDACC [18]	CT P/D EBRT (IMRT) 46.8 Gy (range, 28.8–50.4 Gy)	2008–2014	27	59	23.7	59

MSKCC: Memorial Sloan Kettering Cancer Center, MDACC: MD Anderson Cancer Center, JMIG: Japan Mesothelioma Interest Group, P/D: pleurectomy/decortication, EBRT: external beam radiation therapy, 2DRT: 2-dimensional radiation therapy, EPP: extrapleural pneumonectomy, CT: chemotherapy, 3DCRT: 3-dimensional conformal radiation therapy, IMRT: intensity-modulated radiation therapy, SIB: simultaneous integrated boost, CI: confidence interval, NA: not available, OS: overall survival

469 patients. In the matched population, survival was significantly prolonged in the RT group compared with the non-RT group ($p = 0.012$). However, in patients who survived the first 3 months, no improvement in survival rate could be confirmed. Multivariate analyses of prognostic factors for overall survival (OS) in the matched population did not indicate that the non-RT group had worse survival (hazard ratio: 1.175; $p = 0.12$). Sarcomatoid histology was one of the prognostic factors ($p < 0.0001$) [20]. On the other hand, in a propensity score matching study of 24,914 patients from the National Cancer Database from 2004 to 2013, 454 patients in the surgery alone group and 454 in the postoperative radiation group were compared. Survival time was significantly longer in the postoperative radiation group. In the multivariate analysis, tissue type (epithelial type) and use of chemotherapy, in addition to postoperative radiation, were also significant factors improving OS [21]. Information about the RT technique was not included in either study.

During almost the same period, from 2005 to 2012, a randomized prospective phase II study (SAKK 17/04) was conducted on 151 patients with different histological subtypes of MPM treated with induction chemotherapy plus EPP with or without hemithoracic radiation. However, the efficacy of RT could not be confirmed here [22].

In the 2000s, several studies indicated that the local control rate or OS might have been improved by introducing IMRT instead of 3D-CRT (Table 26.1).

However, serious adverse events were also seen in early reports. In 2006, 6 of 13 patients who received IMRT and chemotherapy after EPP died of radiation pneumonitis. Subsequent reports showed that the volume of the lungs irradiated 5 Gy and more (V_{5Gy}) was 98.6%, indicating that almost the entire contralateral normal lung was exposed to radiation [23]. Currently, adherence to strict dose constraints (described later) is recommended [24].

3.2.3 RT after P/D in Tri-modality Therapy

Even in the 2D-RT era, high-dose hemithoracic radiation was attempted after P/D, but this treatment resulted in total loss of lung function on the irradiated side [2].

In 2011, the feasibility and toxicity of pleural IMRT (median dose, 46.8 Gy; range, 41.4–50.4) to the hemithorax of 36 patients with MPM and intact lungs was evaluated at Memorial Sloan-Kettering Cancer Center (MSKCC) [25]. In that study, 56% of patients underwent P/D before IMRT, and 44% had no resection. Results showed that 7 (20%) of 36 patients had Grade 3 (the Common Terminology Criteria for Adverse Events version 3.0: CTCAE v3.0) or worse pneumonitis (1 had Grade 5), and 5 of 30 patients assessable for late toxicity had continuing Grade 3 pneumonitis [25].

In 2015, an initial report on IMRT after P/D was made by the MD Anderson Cancer Center (MDACC) [26]. In 24 patients with MPM, IMRT after P/D was performed at a dose of 45 Gy in 25 fractions with an optional simultaneous integrated boost (SIB) up to 60 Gy to gross disease, positive margins, fluorodeoxyglucose (FDG)-avid areas on positron emission tomography (PET)/CT, or high-risk areas defined in discussion with the surgeon. Radiation pneumonitis was rare after P/D and IMRT, and was Grade 3 (CTCAE v4.0) in only 2 (8%) patients, but treatment led to progressive significant declines in pulmonary function such as forced vital capacity, 88% to 57%; forced expiratory volume at 1 s, 83% to 58%; and diffusion of carbon monoxide, 87 to 56%. In a matched control analysis, progression-free survival (PFS) and OS were significantly better in patients treated with IMRT after P/D than in patients previously treated with IMRT after EPP in the same institution.

In 2017, results of a retrospective analysis of 209 patients who underwent RT after P/D at MSKCC were reported [27]. This study included 131 patients who underwent hemithoracic IMRT (IMPRINT: intensity-modulated pleural RT) and 78 patients who underwent conventional RT (CONV). In a multivariate analysis, IMPRINT was significantly associated with longer OS ($p = 0.02$). MST and 2-year survival rates were 20.2 months and 42% in the IMPRINT group and 12.3 months and 20% in the CONV group, respectively.

Based on these results, a phase II trial was conducted by MDACC and MSKCC in 2016 to evaluate the safety of IMPRINT [19]. Forty-five patients, including some with stage III ($n = 12$) and stage IV ($n = 15$) disease, were enrolled. A total of 27

patients including a subset of patients undergoing lung-sparing surgery ($n = 16$) and patients with cancer already deemed unresectable ($n = 11$) received hemithoracic pleural IMRT. Grade 2/3 radiation pneumonitis was seen in 8 of 27 patients, and Grade 4/5 radiation pneumonitis did not occur. It was concluded that IMPRINT after P/D was feasible and safe. Although the PFS of 12.4 months was not the primary endpoint, the survival rate result also suggested that hemithoracic pleural IMRT was promising for patients with locally advanced disease.

However, it is important to remember that this study was conducted in 2 highly experienced institutions. Strict lung dose constraints using a normal tissue complication probability of 25% or less were used, and aggressive toxicity management was conducted to prevent severe long-term toxicities. To suppress local recurrence rates, target delineation is also important. Thus, although hemithoracic IMRT is promising as part of a multimodality lung-sparing treatment, currently it has not been widely recommended, because a high skill level and experience are needed to maintain low adverse events and high local control rates. It is recommended that IMPRINT be conducted by a team of experts who have been well trained in using the technology, or as part of a clinical trial conducted in experienced institutions.

3.3 Palliative RT

MPM progresses rapidly and has a strong tendency to infiltrate, so all symptomatic treatment methods should be used when the diagnosis is confirmed, unless radical treatments can be performed. Conventionally, RT alone for MPM has been considered ineffective in prolonging survival, but it has been shown to have a palliative effect. In particular, when the chest wall or vertebral body is infiltrated, the speed of pain progression is rapid, and early consideration of RT is recommended. There is no established recommendation regarding the dose of RT, but some retrospective analyses indicate that pain relief may be dose-dependent. de-Graaf-Strukowska et al. reported that good results were obtained by using ≥4 Gy in a single fraction, and pain relief was achieved in 50% or more patients at a median total dose of 36 Gy [28]. Thus, if the RT field is not too large, the patient's performance status score is relatively good, and the prognosis is expected to be 6 months or longer, high-dose RT of 40 Gy or so may be considered. During the course of RT, it is often necessary to treat several lesions in the chest, and the chest wall always moves due to respiratory motion. Sufficient attention needs to be paid to avoid overlapping irradiation fields. In addition, some sort of proactive treatment plan, such as the irradiation range and beam angle, is required. Finally, the total volume of irradiation and dose to the lungs should be kept in mind, especially when chemotherapy is used in combination.

4 How to Deliver RT to the Entire Pleural Surface?

We are currently conducting dose assessments in CT simulations according to the definitions of both Reports 50 [29] and 62 [30]. First, gross tumor volume (GTV) is defined as a tumor that is visible either directly or on images. The clinical target volume (CTV) is the volume that includes GTV and the pathologic extent of the microscopic tumor growth. Once the CTV is determined, additional margins need to be added to account for expected physiological movements and deformations. Currently, the margin added to the CTV is defined as the internal margin (IM). In addition, a set-up margin (SM) is established to compensate for the uncertainty of reproducing the exact patient position throughout the treatment period, which is usually 3–6 weeks. The planning target volume (PTV) is obtained by adding the IM and SM to the CTV. This is considered to be the volume of the prescribed dose to be administered.

4.1 RT after EPP

As mentioned above, there is no established protocol for RT as a standard therapy after EPP, and evidence is still not sufficient, but previous studies and analyses of past recurrence patterns suggest that the target volume and dose of RT is approximately as described below.

4.1.1 Target Volume in RT

GTV: GTV does not exist after resection. However, evaluating residual lesions by FDG-PET scan before CT simulation is recommended. If an FDG-avid region is found, nuclear medicine physicians and surgeons should be consulted to determine whether this region should be included in the GTV.

CTV: The entire hemithorax (Fig. 26.1) from approximately the thoracic inlet to L1 or L2, and specifically up to about 0.5 cm outside the inner edge of the thorax (pleural bed) should be included. CTV also includes the lower perimeter of the reconstructed diaphragm, its crus, drain sites, and nodal stations of the ipsilateral mediastinum if they were involved at the time of resection. The significance of elective nodal irradiation is controversial, but including the entire mediastinum and supraclavicular nodal regions is not recommended. Intraoperative findings are important for target delineation. Discussions with the surgeon should occur as soon as possible after surgery.

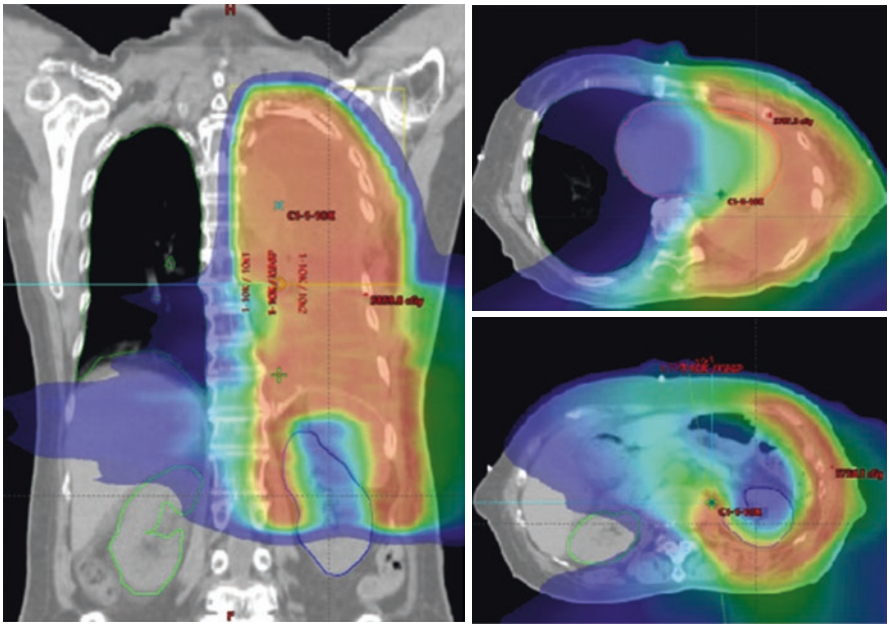

Fig. 26.1 The dose distribution for intensity-modulated radiation therapy (IMRT) after extrapleural pneumonectomy (EPP). Doses are reduced in dose-limiting normal organs, including the contralateral lung, heart, and kidneys

PTV: PTV is defined as the CTV plus approximately 5-mm SM. PTV will usually be extended laterally outside of the ribs. Respiratory motion does not usually need to be considered after reconstruction of the diaphragm, but confirmation by 4-dimensional CT (4D-CT) is required in advance.

Organs at risk: The contralateral lung, heart, esophagus, bilateral kidneys, liver, and spinal cord should be contoured to be evaluated as organs at risk.

4.1.2 RT Technique

According to the method reported by MSKCC [31], in conventional 3D-CRT, the photon-electron technique was usually performed to reduce the dose to organs at risk such as the spinal cord, heart, liver, and kidneys. Briefly, in that study, anterior-posterior opposing fields with blocks to shield the abdomen were used for daily photon irradiation of 1.8 Gy. For right-sided MPM, a heart block was placed at 19.8 Gy. The blocked abdominal and cardiac regions were treated with electron irradiation at 1.53 Gy daily. If the mediastinal lymph node area was included in the initial plan, the medial field border was moved at 41.4 Gy to the ipsilateral edge of the vertebral bodies to block the spinal cord.

If possible, IMRT is the preferred technique. Examples of dose distributions are shown in Fig. 26.1.

4.1.3 RT Dose

In previous studies, RT dose was typically prescribed from 45 to 54 Gy in 1.8 Gy fractions. If there is a possibility of residual tumor cells, a simultaneous boost to 54–60 Gy has been done using IMRT, although this technique is not yet standard. As mentioned above, to minimize the risk of lung toxicity and to keep strict dose constraints on the contralateral normal lung, V_{5Gy} (% volume receiving more than 5 Gy) <60%, V_{20Gy} < 20%, and mean lung dose (MLD) <8.0–8.5% are recommended [24, 32]. In addition, the residual tumor cells are likely to be present in the pleural bed around the diaphragm, so it is often difficult to satisfy the dose constraint to the kidney on the involved side. Renal function and the relative perfusion in each kidney should be assessed with a nuclear perfusion renal scan prior to RT or surgery, and information should be shared with patients and surgeons.

4.2 RT to the Pleural Surface with an Intact Lung

Generally, the target volume is similar to RT after EPP, but because the lungs are intact, attention should be paid to the respiratory motion of the diaphragm or tumors. Using 4D-CT scanning to determine the respiratory movement, and adding IM should be considered. Using IMRT seems fundamental to protect lung tissue, with more stringent dose constraints for lungs than those for IMRT after EPP. In a 2-center, phase II study, the total MLD was limited to 21 Gy or less; V_{20Gy} of the total lung from 37% to 40% or less, and V_{20Gy} of the contralateral lung to less than 7%. The prescribed dose was 50.4 Gy in 1.8 Gy per fraction to 95% or greater of the PTV, and if needed to protect normal tissue, the dose was reduced. No boost dose was delivered. Results of that study revealed that the median dose was 46.8 Gy (28.8–50.4 Gy) [19].

5 Adverse Events After RT

The most common acute adverse events after RT are nausea, malaise, and loss of appetite, which are almost inevitable. Most of them do not need to be treated, but 5 to 30% of cases require some nutritional supplementation. Bone marrow suppression is also seen in more than 50% of patients, but it is rarely more than Grade 3 or a serious problem. Most cases of pneumonia are thought to be caused by postoperative complications or infections, but radiation pneumonitis should be looked for when performing IMRT. In terms of late complications, it is necessary to continue long-term observation of cardiac function if RT is on the left side, and liver function if RT is on the right side. Surgery has a major effect on cardiac function, and frequent checks are required. In addition, it may not be possible to sustain renal

function on the affected side for a long period, so monitoring renal function on the healthy side is also important, especially if chemotherapy is added to the therapeutic regimen.

6 Conclusion

Distant metastases with MPM occur relatively late in the disease course, so while the tumor remains in the hemithorax, opportunities exist for treatments that prolong survival. It has not been proven whether RT alone can definitively cure MPM. With the rapid progression of technology, the safety of RT can be expected to improve more and more in the future. However, the lung can be greatly affected even at low doses of RT, and the mechanism of radiation pneumonitis has not been completely elucidated. Previous reports have shown that irradiating a dose equivalent to the curative dose for solid cancers to the entire lung may not only lead to complete dysfunction of the lung, but can also be life threatening. So far, combination therapy with cytoreductive surgery is essential for the treatment of MPM with widespread lesions. Whether to use EPP or P/D is still controversial. Even after lung-sparing surgery, there is increasing evidence that RT can be used as an adjuvant therapy if performed carefully with IMRT.

On the other hand, even with symptomatic treatment, better quality of life may be maintained by using RT if properly timed with systemic treatment. For a patient with unresectable or recurrent disease, a medical oncologist, radiation oncologist, and specialist in palliative care should collaborate and use a comprehensive approach to treatment from the time of diagnosis.

The role of RT may change in the future if used in combination with new drugs. For example, low-dose irradiation aimed at the abscopal effect of RT in combination with antibodies against immune checkpoints may become an important treatment strategy.

The number of patients with MPM is increasing, but the number of cases per institution is still small. It is necessary to continue to build consensus across facilities worldwide regarding the best treatment approach for this disease.

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Part IX
Surgical Intervention; Current Status and Future Perspectives

Chapter 27
TNM Classification and the Role of Curative Intent Surgery for Mesothelioma: Is the Debate on Extrapleural Pneumonectomy Versus Lung-sparing Macroscopic Resection Over?

Nobuyuki Kondo and Seiki Hasegawa

Abstract As a cytoreduction is the aim of surgery for malignant pleural mesothelioma, the surgical resection is recognized as a part of multimodality treatment. There are two types of curative intent surgery: extrapleural pneumonectomy (EPP) and lung-sparing macroscopic resection, both of which are a highly invasive and high-risk procedure.

The staging system has been revised based on the analysis of an international large database produced by IASLC. The current eighth edition of the TNM classification since 2017 is estimated that the prediction of prognosis from the clinical stage will be improved.

Among surgery achieving macroscopic complete resection, EPP or lung-sparing macroscopic resection is no longer a simple choice. Postoperative quality of life is also an important factor in adapting surgical procedures. We are moving to the direction of starting from the minimum resection range preserving the lung parenchyma to the maximum resection including the ipsilateral lung and surrounding organs depends on the degree of tumor invasion.

Keywords Curative intent surgery · Extrapleural pneumonectomy · Lung-sparing macroscopic resection · Pleurectomy/decortication · TNM classification

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1 Introduction

As the variability of consensus on the treatment, therapeutic approaches for malignant pleural mesothelioma are not well established yet. The current approach tends to evaluate each case by a multidisciplinary team to perform the best strategy according to patients' character and the stage of this disease.

For MPM, true radical surgery is impossible regardless of the surgical procedure. For this reason, surgery is recognized as a part of multimodality treatment.

There are two types of curative intent surgery: extrapleural pneumonectomy (EPP) and lung-sparing macroscopic resection (pleurectomy/decortication, P/D), both of which are a highly invasive and high-risk procedure.

There has been controversy over the superiority or inferiority of these surgical procedures, but now there is a consensus that most of the surgical indications should be given a lung-sparing macroscopic resection (P/D) [1].

Outcomes of MPM treatment have been improved recently but are not yet satisfactory. There is also a change in the method of clinical diagnosis that had been unreliable. The staging system has been revised to the eighth edition since 2017 [2], and new concepts such as tumor thickness and tumor volume have been introduced for T factor evaluation [3].

2 TNM Classification for Surgical Treatment

In most solid cancers, the tumor stage is one of the most important prognostic factors. Otherwise, in MPM, accurate staging is quite difficult because of the nature of the tumor. As the previous seventh edition of the TNM classification used the data mainly derived from small single-institution retrospective series, many limitations of this system became apparent [4]. (Table 27.1)

The TNM system had been updated based on the analysis of an international large database produced by IASLC. In 2016, the eighth edition of the TNM classification for MPM has introduced important recommendations in several different components of the staging system [2]. (Table 27.2)

2.1 The T-component in the Eighth Edition of the TNM Classification for MPM

Based on the revision of the database, the main change in the eighth edition was that the subclassification of T1a and T1b were grouped into a single T1 category. This means that there is no longer any distinction between tumors invading both of the parietal and visceral pleura and tumors restricted parietal pleura [3].

Table 27.1 7th edition of the TNM classification

7th edition		Definition	
T	T1a	Tumor involving the ipsilateral parietal pleura only	
	T1b	Tumor involving the ipsilateral parietal and partial visceral pleura only	
	T2	Tumor involving ipsilateral Pleura (parietal or visceral) with invasion of:	diaphragmatic muscle pulmonary parenchyma *confluent tumor on visceral pleura
	T3	Tumor involving ipsilateral pleura (parietal or visceral) with the invasion of:	endothoracic fascia mediastinal fat chest wall: solitary, resectable pericardium (non-transmural invasion)
	T4	Tumor involving ipsilateral pleura (parietal or visceral) with invasion of:	peritoneum mediastinal organs (esophagus, trachea, heart, great vessels) chest wall; diffuse or multifocal, unresectable contralateral pleura vertebrae
N	NXR	Lymph nodes cannot be assessed	
	N0	No regional lymph node metastasis	
	N1	Metastasis to:	ipsilateral: hilar, bronchopulmonary
	N2	Metastasis to:	ipsilateral: subcarinal, paratracheal, aortopulmonary, paraesophageal
	N3	Metastasis to:	contralateral supraclavicular
M	M0	No distant metastasis	
	M1	Distant metastasis	

Another contribution of this revision was support for the concept that the tumor thickness or the tumor volume can be a prognostic factor [5]. Recently pleural thickness in MPM after chemotherapy revealed an independent prognostic factor [6].

Future work should address prospective collections of tumor measurement data to further refine the T-component in MPM.

2.2 The N-Component

For the N-component, the survival analysis of the IASLC database reflected the anatomical difference of lymphatic drainage pattern between MPM and lung cancer. In the eighth edition, based on the fact there was no difference between previous pN1 and pN2 patients, they were combined into an N1 category. Then the previous N3 disease was sifted as new N2 disease.

Table 27.2 Eighth edition of the TNM classification

8th edition		Definition	
T	T1	Tumor involving the ipsilateral parietal or visceral pleura only	
	T2	Tumor involving ipsilateral pleura (parietal or visceral) with the invasion of:	diaphragmatic muscle pulmonary parenchyma
	T3	Tumor involving ipsilateral pleura (parietal or visceral) with the invasion of:	endothoracic fascia mediastinal fat chest wall: solitary, resectable pericardium (non-transmural invasion)
	T4	Tumor involving ipsilateral pleura (parietal or visceral) with invasion of:	peritoneum mediastinal organs (esophagus, trachea, heart, great vessels) chest wall; diffuse or multifocal, unresectable contralateral pleura
N	Nx	Lymph nodes cannot be assessed	
	N0	No regional lymph node metastasis	
	N1	Metastasis to:	ipsilateral
	N2	Metastasis to:	contralateral supraclavicular
M	M0	No distant metastasis	
	M1	Distant metastasis	

From these results, the survival of MPM patients was affected by the unique anatomical locations of lymph nodes which were different from lung cancer [2, 7].

2.3 The M-Component

No redefinition was published for the M component. Only M1 involvement disease was considered as stage IV in the new edition, in contrast to the seventh edition which T4 and N3 disease were included in stage IV as well. (Table 27.3)

3 Curative Intent Surgery

3.1 The Role of Surgery

Surgery for MPM has an important role in diagnosis, staging, and therapy. Curative intent surgery for MPM is quite specific compared to other solid cancers.

Table 27.3 Comparison of TNM stage between eighth edition and seventh edition

Eighth stage	T* seventh	N* seventh	M	Seventh stage
I A	1a	0	0	I A
	1b	0	0	I B
	2	0	0	II
I B	2	0	0	
	3	0	0	III
II	1a, 1b, 2	1	0	
III A	3	1	0	
III B	1a ,1b, 2	2	0	
	3	2	0	
	4	0, 1, 2	0	IV
	any T	3	0	
IV	any T	any N	1	

First of all, MPM is a diffusely growing cancer that cannot secure a surgical margin. For this reason, the goal of surgery is macroscopic complete resection (MCR) [8, 9].

Second, the results of surgery alone are poor, and surgery is positioned as part of multidisciplinary treatment. For this reason, in each guideline, surgical treatment is inserted into multidisciplinary treatment combined with chemotherapy or radiation treatment [10].

Third, risk benefits are poor. Since this tumor spreads widely in the unilateral thoracic cavity, it requires a large thoracotomy, extensive pleural resection, and combined resection of surrounding tissue.

Finally, even with current modalities, it is difficult to determine the preoperative clinical stage. Often upstaging at the pathological stage, the clinical stage does not reflect prognosis. This is a factor that makes it difficult to apply high-risk surgery for MPM [4].

3.2 Indication for Curative Intent Surgery

The most recent international guideline is described in Meeting Summary of the International Mesothelioma Interest Group (IMIG) Congress [9].

The consensus of the attendees was as following: Surgery is indicated for patients with histologically identified MPM when MCR is estimated achievable as a multimodality treatment.

Patients with sarcomatoid subtype or with advanced stages is a contraindication for surgery because of significantly poor prognosis after resection [4, 11].

N1 disease in the eighth edition of TNM classification is not an absolute contraindication as demonstrated in IASLC analysis, mediastinal lymph nodes are "local" nodes for MPM [7].

It is still recommended to confirm histological diagnosis until the establishment of reliable evidence of the cytological diagnosis [12].

In summary, surgery for MPM should be dedicated to selected patients with resectable disease, epithelioid histological type, and good performance status.

3.3 Two Surgical Options for MPM

There are two surgical options as a treatment for MPM.

Extrapleural pneumonectomy (EPP) and lung-sparing macroscopic resection. The latter was generally called pleurectomy/decortication (P/D). The name "decortication" mimics that for chronic empyema, but the procedures are completely different from each other.

3.3.1 Extrapleural Pneumonectomy (EPP)

EPP was reported as a curative intent resection of MPM by Butchart in 1976. The surgical procedure involves *en bloc* resection of the parietal and visceral pleura, ipsilateral lung and if necessary, hemidiaphragm and/or pericardium. Although the initial perioperative mortality rate was as high as 31%, both perioperative complications and mortality have improved significantly over the past 40 years [13, 14].

Removing one lung allows more efficient control of local lesions. That is, high-dose radiation therapy can be performed without the risk of radiation pneumonitis.

Sugarbaker DJ et al. reported that in 1999, patients with epithelioid subtype and N0 disease who underwent EPP surgery combined with multidisciplinary treatment had a 5-year survival rate of 46% and a 30-day mortality rate of 3.8%. It was reported that it was significantly lower than before [15].

Advantages of EPP include the fact that surgical procedures are highly standardized, that MCR is relatively easy to achieve, and that postoperative radiotherapy can be performed because there is minimal radiotoxicity because of the absence of the ipsilateral lung. These advantages have been estimated to increase the radicality and improve prognosis as part of multimodality treatment. However, these beliefs did not differ significantly between those who performed each type of surgery and those who did not [16].

Disadvantages are, as this procedure is lung sacrificing surgery, almost 50% of perioperative morbidity, and the inferiority in safety and decrease in quality of life. Complications associated with a diaphragm and pericardial reconstruction cannot be ignored [17].

3.3.2 Lung-Sparing Macroscopic Resection (Pleurectomy/Decortication, P/D)

As there is little ambiguity in the EPP procedure, there is a common understanding among surgeons. On the other hand, lung-preserving surgery, i.e., P/D has various styles, purposes, and nomenclature, and has not yet been unified. The term P/D was first published in 1993 by Rusch, but confusion concerning P/D continued for a time [18].

The consensus report published jointly by IASLC and IMIG in 2011 defined as follows [19].

(1) Extended P/D: parietal and visceral pleurectomy to remove all gross tumor with resection of the diaphragm and/or pericardium.
(2) P/D: parietal and visceral pleurectomy to remove all gross tumors without diaphragm or pericardial resection.
(3) Partial pleurectomy: the partial removal of parietal and/or visceral pleura for diagnostic or palliative purposes.

Here, the following two questions arise from the viewpoint of complete macroscopic resection. (1) Whether or not to remove the visceral pleura without macroscopic lesions. There is a mixture of both the standpoint that the pleura can be left if there is no macroscopic tumor and the theory that the visceral pleura should be completely resected because there should be a tumor microscopically. (2) If there is tumor infiltration into the lung parenchyma, combined resection of the lung is necessary. How is it defined as a surgical procedure when parenchymal resection is performed?

In 2019, the Joint NCI-IASLC-MARF Taskforce has started to form an international consensus of surgery-based treatments for MPM, including these issues [20].

The advantage of P/D is that the ipsilateral lung parenchyma is preserved, so it can be applied to patients with limited cardiopulmonary reserve, and additional postoperative chemotherapy is more feasible. Maintaining postoperative quality of life by preserving the lungs is a major advantage gained by avoiding lung resection [21].

On the other hand, the potential drawback of P/D is that the operation time tends to be longer and the cytoreduction ability is lower than EPP. In particular, the radicality and effectiveness of P/D in MPM patients with advanced disease was one of the major controversies. Besides, the prolonged air leakage from the preserved lung parenchyma is a unique complication of P/D [22, 23].

3.4 The Definition of Macroscopic Complete Resection (MCR)

Here, the definition of MCR should be reconfirmed. In pleural malignancies without resection margin, it is the current consensus that macroscopic resection, in other words, cytoreduction of the tumor is the aim of surgical resection. MCR is a situation where it cannot be said that there are no residual tumor cells.

Therefore, lung parenchyma/diaphragm/pericardium tends to preserve if MCR can be achieved. On the other hand, removing all of them can also make R0 resection only for the ipsilateral lung parenchyma/diaphragm/pericardium. Considering the importance of surgical safety and quality of life after surgery, the direction to take is clear.

4 The Debate on Extra-pleural Pneumonectomy Versus Lung-Sparing Macroscopic Resection (P/D)

There are no randomized controlled trials directly comparing P/D with EPP. Therefore, there is no clear evidence of the superiority or inferiority of these procedures [24].

The procedure should be decided on a case-by-case based on curability, safety, postoperative quality of life, and postoperative outcomes. The efficiency of tumor cytoreduction would be higher for EPP that removes the ipsilateral lung than P/D that preserves the lung parenchyma. However, it is not certain that this difference is parallel to the difference in postoperative survival.

According to the systematic review of surgical risks by Cao, et al., the surgery-related mortality (P/D: 2.9% versus EPP: 6.2%) and the perioperative morbidity (P/D: 27.9% versus EPP: 62.0%) were both significantly higher in EPP [25].

Postoperative quality of life is also an important selection factor for adapting surgical procedures. The postoperative QOL reduction of EPP with total pneumonectomy is clearly inferior to lung-preserving surgery. This is reflected in the difference in the treatment intensity after surgery and at the recurrence. In other words, P/D is feasible to treat adequately after surgery and after recurrence.

Postoperative survival was also mentioned: median overall survival was 13-29 months for P/D, 12-22 months for EPP, and P/D tended to be preferred.

However, the debate of EPP or P/D has decreased in recent years. As data supporting P/D in terms of postoperative survival and quality of life increased, major Western centers have shifted the resectable MPM surgical approach from EPP to P/D [26–29].

Currently, few high-volume centers preferentially use EPP as a surgical technique for obtaining MCR.

5 Conclusion

The MPM staging system has been revised to version 8 from 2017, and it is estimated that the prognosis prediction from the clinical stage will be improved. In the future, it is expected that new concepts such as maximum tumor thickness and tumor volume will be introduced into T factors that are still unreliable.

For surgery as part of multidisciplinary treatment, there are two methods for achieving MCR: EPP and lung-sparing resection (P/D). Since the 2011 IMIG consensus report, it has gone through a debate on the choice of EPP or P/D and is no longer a simple alternative. Intending to achieve MCR, we have started internationally in the direction of starting from the minimum resection range that preserves the lungs and surrounding organs and adding necessary resections depending on the extent of the disease.

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Chapter 28
Surgery and Adjuvant or Neoadjuvant Setting of Radiotherapy: What Is the Role of Radiotherapy in Combination with Lung-Sparing Surgery?

Fumihiro Tanaka and Kazue Yoneda

Abstract Malignant pleural mesothelioma (MPM) is a locally aggressive disease. Whereas curative-intent surgery, non-lung-sparing surgery (extrapleural pneumonectomy [EPP]), or lung-sparing surgery (pleurectomy/decortication [P/D]), may achieve a macroscopic complete resection (MCR), surgery alone is generally insufficient for local disease control. Adjuvant or neoadjuvant radiotherapy (RT) in combination with surgery potentially reduces locoregional recurrence rate, although no definitive evidence showing a robust survival benefit with its use has been reported. Adjuvant hemithoracic RT after non-lung-sparing surgery (EPP) may be offered to selected patients with good performance status. In the neoadjuvant setting before EPP, the delivery of high-dose RT to the entire hemithorax with two intact lungs without significant lung toxicities is technically challenging. However, modern RT techniques such as intensity-modulated RT (IMRT) have enabled neoadjuvant RT following EPP. Lung-sparing surgery has been increasingly employed, as is associated with lower operative morbidity and mortality. Adjuvant hemithoracic IMRT can be performed with acceptable toxicities and may provide a favorable survival in patients who received lung-sparing surgery. Despite these promising results, either neoadjuvant IMRT before EPP or adjuvant IMRT after P/D remains experimental due to its potential risk of fetal lung toxicities, and should only be performed in highly experienced centers, preferably in the context of a clinical trial. Here, we reviewed the current status and future perspectives of adjuvant or neoadjuvant RT in combination with surgery for MPM.

Keywords Malignant pleural mesothelioma (MPM) · Surgery · Extrapleural pneumonectomy (EPP) · Pleurectomy/decortication (P/D) · Radiotherapy

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1 Introduction

The goal of curative-intent surgery for malignant pleural mesothelioma (MPM), extrapleural pneumonectomy (EPP), or lung-sparing surgery such as pleurectomy/decortication (P/D), is to achieve a macroscopic complete resection (MCR). Surgery alone may not provide a favorable prognosis and should be performed as a part of multimodality treatment in combination with chemotherapy and radiotherapy (RT) [1]. MPM is a locally aggressive disease, and frequent local recurrence after surgery is associated with a poor prognosis. While adjuvant or neoadjuvant RT potentially contributes to improvement in local disease control [2], it may be associated with significant toxicities by damaging surrounding organs such as the lung, heart, and esophagus. When the lung is intact, it may be technically challenging to deliver high-dose RT to the entire pleural surface with acceptable toxicities. Accordingly, RT is commonly performed after the entire removal of the ipsilateral lung with EPP. Several retrospective and prospective studies have revealed that postoperative adjuvant hemithoracic RT can reduce local recurrence rate after EPP [3–6], whereas no randomized controlled study showing the efficacy has been reported.

Recently, lung-sparing surgery has been preferably employed to achieve MCR, as it is associated with lower operative morbidity and mortality [7–11]. The increasing number of patients who underwent lung-sparing surgery may indicate the necessity of development and establishment of adjuvant or neoadjuvant RT for patients with two intact lungs. Recent technological advances in RT such as lung-sparing intensity-modulated radiotherapy (IMRT) have enabled the delivery of high-dose RT with acceptable toxicities in patients who underwent lung-sparing surgery [12–15]. Here, we reviewed the current status and future perspectives on adjuvant or neoadjuvant radiotherapy in combination with surgery for resectable MPM.

2 Adjuvant Hemithoracic RT Following EPP

High-dose hemithoracic RT is the delivery of radiotherapy (>40Gy) to the entire ipsilateral hemithorax [16, 17]. A number of retrospective and prospective studies have revealed that adjuvant hemithoracic can reduce locoregional recurrence rate after EPP, whereas no randomized controlled trial showing the efficacy of postoperative RT has been reported [3–6, 11]. For example, a propensity-matched analysis of the National Cancer Database showed that postoperative RT provided a significantly improved survival among patients who underwent surgery for stage I-II (hazard ratio [HR], 0.52; P = 0.035) [2]. In a single-arm phase II study conducted in the Memorial Sloan-Kettering Hospital, postoperative locoregional recurrence was

documented only in 7(13%) of 54 patients who underwent adjuvant hemithoracic RT (54Gy in 30 daily fractions of 1.8Gy) following EPP [6]. In the phase II study, distant metastasis was the most common form of postoperative relapse (distant relapse only, 55%; distant and locoregional relapse, 9%), which indicates that the addition of effective systemic treatment was essential to improve the prognosis [6].

Accordingly, several phase II studies of trimodality treatment consisting of adjuvant hemithoracic RT following neoadjuvant chemotherapy and EPP have been conducted [18–23] (Table 28.1). However, only 40–71% patients actually completed the entire trimodality treatment including postoperative adjuvant RT, whereas each eligible patient had had adequate organ functions and had been enrolled in each clinical trial. These results indicate that adjuvant hemithoracic RT following EPP may be feasible only for selected patients in daily clinical practice. The Mesothelioma and Radical Surgery (MARS) trial is a prospective study to assess the feasibility of randomizing patients to receive EPP or not [24]. After completion of platinum-based chemotherapy, patients were randomly assigned to receive EPP followed by hemithoracic RT or to receive no EPP. While it had not been planned to compare survival between two groups, the EPP group showed a poor prognosis as compared with the non-EPP group (median overall survival time, 14.4 months for the EPP group and 19.5 months for the non-EPP group; HR, 2.75 [95% confidence interval (CI), 1.21–3.93; P = 0.016]). These negative results of the use of EPP are extremely controversial, as the MARS trial was not a randomized study evaluating the survival benefit with EPP [25]. However, since the MARS trial [24] and several retrospective studies showing favorable outcomes achieved with lung-sparing surgery [7–11] were reported, the use of EPP for resectable MPM has gradually declined [12–15].

The SAKK17/04 trial was a two-part phase II study of neoadjuvant chemotherapy and EPP with or without postoperative adjuvant hemithoracic RT [26, 27]. In part 1, patients received 3 cycles of neoadjuvant chemotherapy consisting of cisplatin plus pemetrexed followed by EPP and the feasibility of achieving MCR with EPP after chemotherapy was assessed. Among 96 (64%) patients who achieved MCR in part 1, 54 patients were enrolled in part 2 to assess the efficacy of adjuvant RT and were randomly assigned (1:1) to receive high-dose hemithoracic RT or not. Twenty-five (93%) of 27 patients assigned to the RT-group completed postoperative RT with the median dose of 55.9Gy. The median locoregional relapse-free survival was 7.6 months for the non-RT group and 9.4 months in the RT-group, which showed that postoperative hemithoracic RT radiotherapy provided no significant clinical benefit [26].

Hemithoracic RT is commonly delivered using three-dimensional(3D) conformal technique [5, 11, 28], which may cause significant toxicity such as pneumonitis and may not deliver sufficient RT to the target area due to dose inhomogeneities. Alternatively, the IMRT technique has been developed to overcome these dosimetric constraints [28–31]. In fact, a retrospective study of 63 patients who received postoperative IMRT following EPP showed that only 3 patients (5%) had

Table 28.1 Prospective phase II studies on multimodality treatment of adjuvant hemithoracic radiotherapy (RT) followed by neoadjuvant chemotherapy and extrapleural pneumonectomy (EPP) for resectable malignant pleural mesothelioma (MPM)

Author & Year [reference]	Neoadjuvant chemotherapy	Adjuvant radiotherapy	Completion of EPP	Achievement of MCR with EPP	Completion of entire treatment	MST (months)	Operative mortality
Rea 2007 [18]	CG 3–4 cycles	45Gy (1.8Gy/fr)	81% (17/21)	NS	71% (15/21)	25.5	0%
Weder 2007 (SAKK17/00) [19]	CG 3 cycles	50–60Gy (2Gy/fr)	74% (45/61)	61% (37/61)	59% (36/61)	19.8	2.2% (1/45)
Krug 2009 [20]	CP 4 cycles	54Gy (1.8Gy/fr)	70% (54/77)	NS	52% (40/77)	16.8	3.7% (2/54, 30day)
Van Schil 2010 (EORTC08031) [21]	CP 3 cycles	54Gy (1.8Gy/fr)	72% (42/58)	69% (40/58)	64% (37/58)	18.4	6.5% (3/46, 30day)
Federico 2013 [22]	CP 3 cycles	54Gy (1.8Gy/fr)	76% (41/54)	NS	41% (22/54)	15.5	4.4% (2/45, 30day)
Hasegawa 2016 (JMIG0601) [23]	CP 3 cycles	54Gy (1.8Gy/fr)	71% (30/42)	71% (30/42)	40% (17/42)	19.9	3% (1/33, 30day) 12% (4/33, 90day)

RT radiotherapy; *EPP* extrapleural pneumonectomy; *MPM* malignant pleural mesothelioma; *MCR* macroscopic complete resection; *MST* median overall survival time; *CG* cisplatin plus gemcitabine; *CP* cisplatin plus pemetrexed; *Gy/fr* gray per fraction; *NS* not supplied; *SAKK* Swiss Group for Clinical Cancer Research; *EORTC* European Organisation for Research and Treatment of Cancer; *JMIG* Japan Mesothelioma Interest Group

postoperative recurrence within the irradiated field [32]. In addition, a retrospective study of 38 patients who received 3D-conformal RT (3D-CRT, n = 24) or IMRT (n = 14) after EPP following neoadjuvant chemotherapy showed a lower local recurrence rate with the use of IMRT (14.3% for the IMRT-group versus 41.7% for the 3D-CRT-group) [33]. However, a careful attention should be payed to the potential risk of fatal pneumonitis associated with IMRT, if the RT dose is not carefully controlled [30, 31, 34, 35]. Allen and coworkers reported that 6 (46%) of 13 patients treated with IMRT following EPP developed fatal pneumonitis [34]. Rice and coworkers also reported a high mortality rate (37%) after IMRT following EPP. In the study, fatal pulmonary toxicities were associated with RT dose to the contralateral lung, and the volume of lung receiving 20Gy (V20) was a significant risk-factor for pulmonary-related death [35]. Accordingly, it is highly recommended that the radiation dose to the contralateral lung shall be minimized, preferably with V20 less than 5%, after EPP [31]. With increasing experience and improved technique to reduce radiation dose to the contralateral lung, IMRT following EPP may provide excellent local control with acceptable toxicities, but should be performed only in centers with greater experience.

3 Neoadjuvant Hemithoracic RT Followed by EPP

Hemithoracic RT can be principally performed in the neoadjuvant setting before EPP. In fact, a single-institutional prospective phase I/II study (Surgery for Mesothelioma After Radiation Therapy, SMART) showed that a short accelerated course of high-dose hypo-fractionated hemithoracic RT (25Gy in 5 fractions over 5–7 days) followed by EPP was feasible, as it was associated with a favorable prognosis (the median overall survival, 36 months) and with acceptable toxicities (morbidity, 39% with grade 3–5 complications; overall treatment-related mortality, 4.8%) [36, 37]. The authors also revealed that there was no significant difference in the surgical risks between patients who received neoadjuvant chemotherapy followed by EPP and those who received neoadjuvant accelerated hemithoracic RT followed by EPP (90-day operative mortality, 6.2%, and 3.2%, respectively) [38]. However, as only single-institutional studies have been reported, this potentially high-risk strategy remains experimental and shall be performed in highly experienced centers, preferably within the context of a clinical trial.

4 Adjuvant RT Following Lung-Sparing Surgery

RT after lung-sparing surgery such as P/D is challenging, as it is associated with a significant risk of radiation pneumonitis, especially in the intact ipsilateral lung. The investigators at the Memorial Sloan-Kettering Cancer Center have pioneered

this potentially high-risk treatment strategy. They first employed 3D-CRT in this setting, but failed to show the efficacy of postoperative RT following P/D as residual diseases after P/D cannot be eradicated [16]. Accordingly, they employed IMRT to deliver an optimized dose of RT. In a retrospective study, they showed the feasibility of pleural IMRT to the hemithorax of patients with two intact lungs (incidence of grade 3 or greater pneumonitis, 20%) [39]. Based on the promising results, they initiated a phase II study of hemithoracic intensity-modulated pleural radiation therapy (IMPRINT) as a part of lung-sparing multimodality therapy in patients with MPM [40]. Two (7%) of 27 patients who received IMPRINT experienced grade 3 radiation pneumonitis and no patient experienced grade 4 or 5 toxicity. The median progression-free and overall survival were 12.4 and 23.7 months, respectively. Thereafter, in a retrospective analysis, they also analyzed the therapeutic outcomes of patients who underwent P/D and adjuvant RT with conventional technique or with the IMRT technique [41]. They showed a significant favorable prognosis (median overall survival, 20.2 months for the IMRT-group versus 12.3 months for the conventional RT-group; $P = 0.001$) along with lower esophageal toxicity (grade 2 or greater esophagitis, 23% versus 47%, respectively; $P = 0.0007$) in the IMRT-group. There was no significant difference in lung toxicities (grade 2 or greater pneumonitis, 26% versus 35%, respectively; $P = 0.17$). Accordingly, adjuvant hemithoracic IMRT may be offered to patients who received lung-sparing surgery. However, this potentially toxic regimen shall be performed in highly experienced centers, preferably within the context of a clinical trial.

5 Conclusions

Recommendations of adjuvant or neoadjuvant RT in a variety of guidelines are listed in Table 28.2 [42–45]. To summarize, RT in combination with surgery, EPP, or lung-sparing surgery, have not been established as standard care of treatment, due to lack of definitive evidence in a randomized controlled study showing the safety and efficacy. In daily clinical practice, only adjuvant hemithoracic RT after EPP may be offered to selected patients with good performance status and adequate organ functions. Adjuvant RT after lung-sparing surgery such as P/D should be only performed in experienced centers, preferably in the context of clinical a clinical trial, due to the potential high risk of fatal radiation pneumonitis. Neoadjuvant RT before surgery should not be performed in daily clinical practice, as this high-risk strategy remains experimental [46].

Table 28.2 Recommendation of adjuvant or neoadjuvant radiotherapy (RT) in combination with surgery for malignant pleural mesothelioma (MPM)

Guidelines			
JLCS (2018) [42]	ASCO (2018) [43]	NCCN (2020) [44]	ESMO (2015) [45]
Adjuvant radiotherapy following EPP			
• Hemithoracic adjuvant RT may be offered to patients who undergo EPP.	• Hemithoracic adjuvant RT may be offered to patients who undergo non-lung-sparing surgery (EPP), preferably in centers of excellence with experience.	• For patients with resectable MPM who undergo EPP, adjuvant RT can be recommended for patients with good PS to improve local control.	• RT can be given in an adjuvant setting after surgery or chemo-surgery to reduce local failure rate. However, no evidence is available for its use as a standard treatment.
Adjuvant radiotherapy following lung-sparing surgery			
• No definitive evidence is available for the use of RT following P/D.	• Hemithoracic adjuvant IMRT may be offered to patients who undergo lung-sparing cytoreductive surgery (P/D or extended P/D). • This potentially toxic regimen should only be performed in highly experienced centers, preferably in the context of a clinical trial.	• When there is limited or no resection of disease, delivery of high-dose RT to the entire hemithorax in the setting of an intact lung has not been shown to be associated with significant survival benefit, and the toxicity is significant. • RT under such circumstances after P/D is usually not recommended. • Hemithoracic IMRT after P/D may be considered in centers with experience and experts in these methods.	
Neoadjuvant radiotherapy followed by EPP			
	• Hemithoracic neoadjuvant RT may be offered to patients who undergo non-lung-sparing surgery (EPP). • This potentially toxic regimen remains experimental and should only be performed in highly experienced centers with the context of a clinical trial.		

(continued)

Table 28.2 (continued)

Guidelines			
JLCS (2018) [42]	ASCO (2018) [43]	NCCN (2020) [44]	ESMO (2015) [45]
Neoadjuvant radiotherapy followed by lung-sparing surgery			
	• Due to the potential for severe pulmonary toxicity, neoadjuvant RT is not recommended for patients who undergo lung-sparing surgical cytoreductive surgery		
RT techniques in adjuvant or neoadjuvant setting			
• For adjuvant RT following EPP, 3D-CRT, or IMRT may be offered.	• For adjuvant or neoadjuvant hemithoracic RT, 3D, or IMRT may be offered, respecting guidelines of organs at risk. • Proton therapy may be considered in centers with significant experience, preferably in the context of a clinical trial.	• Recommendations regarding RT should be made by radiation oncologists with experience in managing MPM • CT simulation-guided planning using either IMRT or conventional photon/electron RT is acceptable. • IMRT is a promising technique that allows for a more conformal high-dose RT and improved coverage to the hemithorax. IMRT or other modern technology (such as tomotherapy or protons) should only be used in experienced centers or on protocol. When IMRT is applied, the NCI and ASTRO/ACR IMRT guidelines should be strictly followed.	• When postoperative RT is applied, strict constrains must be ahead to in order to avoid toxicity to neighboring organs, and special, tissue sparing, techniques should be used.

RT radiotherapy; *MPM* malignant pleural mesothelioma; *JLCS* Japan Lung Cancer Society; *ASCO* American Society of Clinical Oncology; *NCCN* National Comprehensive Cancer Network; *ESMO* European Society of Clinical Oncology; *EPP* extrapleural pneumonectomy; *P/D* pleurectomy/decortication; *PS* performance status; *IMRT* intensity-modulated radiotherapy; *3D-CRT* three-dimensional conformal radiotherapy; *NCI* National Cancer Institute; *ASTRO* American Society for Radiation Oncology; *ACR* American College of Radiology

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Chapter 29
Loco-Regional Treatment with Surgical Intervention in Mesothelioma: What Is the Role of Enhancing Local Control Approaches?

Takao Morohoshi

Abstract Intracavitary chemotherapy could deliver drugs to residual tumor cells with less toxicity as compared to systemic chemotherapy. Furthermore, in order to improve the local effect of surgery, additional intraoperative loco-regional treatments have been proposed. These intraoperative adjunctive therapies are heated intraoperative chemotherapy (HITHOC), heated intraoperative povidone-iodine (PVP-I), and photodynamic therapy (PDT).

MesoVATS trial; in the aspects of local pain relief and control of other symptoms caused by pleural effusion, debulking the tumor, and partial pleurectomy by VATS was compared to talc pleurodesis. Investigators concluded that there was no difference in survival between VATS partial pleurectomy (VAT-PP) and talc pleurodesis. VAT-PP resulted in more complications, longer hospital stay and more expensiveness. VAT-PP may only have a role to resolve the situation of trapped lung.

Keywords Intracavitary chemotherapy · Hyperthermic intrathoracic chemotherapy(HITHOC) · Debulking surgery · Miscellaneous additional loco-regional treatment

1 Introduction: Intrathoracic/Intracavitary Therapies

Even though the standard therapy for malignant pleural mesothelioma (MPM) is still chemotherapy with platinum and antifolate agents, the outcomes are not satisfactory, just leads to a median survival of only 12 months.

During the last decade or two, several reports suggested that multimodality therapy, including surgery, may result in significant improvement in the survival of some selected patients [1–5].

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The role of surgery is to remove all gross tumors, achieving a macroscopic complete resection (MCR). To improve the local effect of surgery, additional intraoperative loco-regional/intrapleural cavitary therapies have been proposed, as follows.

2 Intracavitary Chemotherapy

Intracavitary chemotherapy (IC) is a combined modality treatment, to kill residual tumor cells on the surface of the thoracic cavity, consisted of cytoreductive surgery and supplemental chemotherapy. It offers several advantages for local control following EPP or P/D, including improved drug delivery to residual tumor cells and lower toxicity as compared to systemic chemotherapy.

The safety and feasibility to IC were confirmed as an administration of cisplatin to ovarian carcinoma patients in the 1980s [6].

In the 1990s, several reports of the pharmacokinetics of intrapleural chemotherapy, for malignant pleural mesothelioma were published [7–9].

The 28 patients with MPM received a novel treatment by combining surgical resection with immediate postoperative intrapleural chemotherapy with cisplatin and mitomycin, and 23 of 28 patients received subsequent systemic chemotherapy, started 3 to 5 weeks postoperatively [8]. In this group, 23 patients, no grade 3 or 4 toxicities were observed. Rusch et al. concluded that this short but aggressive combined modality regimen was generally well tolerated, overall survival was as good or better than with previously reported multimodality approaches, but other strategies are needed to improve local control.

Lerza et al. studied intrapleural simultaneous administration of CDDP and CBDCA in 10 patients with malignant effusions, investigated the pharmacokinetics of CDDP and CBDCA. Platinum originating from CDDP and intact CBDCA in plasma and in pleural fluid were measured. Intrapleural combined treatment of CDDP and CBDCA increased the extent of their residence time (MRT) compared with single intravenous CDDP administration. The intrapleural treatment with CDDP and CBDCA was well tolerated, from the viewpoint of toxicity and myelosuppression [9].

3 Hyperthermic Intrathoracic Chemotherapy (HITHOC)

Hyperthermia has a key role in increasing drug penetration into the tumor cells and enhances their cytotoxic effects by modifying cells membrane permeability.

Hyperthermic intrathoracic chemotherapy (HITHOC) is carried out as an additional procedure following the surgical cytoreduction of the pleural tumor. Different studies explored the pharmacokinetics of HITHOC using cisplatin alone or in association with other drugs as Anthracyclines [10, 11].

Ratto et al. [10] reported in 1999, the study to investigate the feasibility, safety, and pharmacokinetics of a multimodality therapy including an operation, pleural

space perfusion(60 minutes) with cisplatin (100 mg/m^2), hyperthermia (41.5°C), and postoperative radiotherapy (55 Gy to chest wall incisions). The local tissue/perfusate ratio of platinum concentrations tended to be higher after hyperthermic perfusion rather than normothermic perfusion. They concluded this multimodality approach is feasible, pharmacokinetically advantageous, and safe enough to undergo further clinical investigations.

Cisplatin is the standard chemotherapeutic agent and a concentration of 150–175 mg/m^2 body surface area is recommended. And the premise of the hyperthermic intrapleural perfusion is that the elevated temperatures (41–42°C) enable chemotherapy to penetrate the tumor cells, however the limited cardiotoxicity after extensive thoracic surgery is also an advantage of the HITHOC.

Cardiotoxicity was monitored in 13 MPM patients who underwent HITHOC with doxorubicin (25–54 mg/m^2) and cisplatin (65–120 mg/m^2) following the cytoreductive surgery, no clinical cardiac failure or treatment-related death was observed [11].

Matsuzaki et al. revealed a part of the adjuvant effect of HITHOC as induction of apoptosis [12].

In the early 2000s, a preliminary report of hyperthermic intrathoracic chemotherapy with cisplatin and doxorubicin after cytoreductive surgery for 22 patients with stage I MPM had been published. No operative mortality but significant morbidity was seen in 13 patients (65%), including bronchopleural fistula, diaphragm rupture, wound dehiscence, persistent air leakage, and chylous effusion. The median follow-up was 14 months. The median survival was 11 months, with a 1-year survival of 42%. They concluded survival data were less encouraging [13].

Another phase I to II study of HITHOC after P/D for 44 patients with MPM reported on 2006, resulted in high postoperative mortality and low recurrence-free survival [14].

Sugarbaker and colleagues compared outcomes of the cytoreductive surgical procedure (EPP or P/D), with or without the use of HITHOC immediately after surgery. HITHOC group patients received EPP (n = 53), P/D (n = 19), and non-HITHOC group 21, 10, respectively. Patients treated with HITHOC had a significant longer interval to recurrence (HITHOC group:27.1 months versus non-HITHOC group:12.8 months) and significant better survival 35.3 versus 22.8 months, respectively [15].

As regards surgical procedure, P/D and HITHOC, followed by systemic chemotherapy and/or radiotherapy is seemed to be better than EPP, HITHOC, and following adjuvant therapy [16, 17]. Ishibashi et al. compared DFI after two different surgical approaches (EPP or P/D) both associated HITHOC with cisplatin and noticed a significant better DFI after P/D [16].

10-year experience in the treatment of early-stage MPM with lung and diaphragm sparing approach and HITHOC allows promising long term outcomes with an ideal sparing of pulmonary and diaphragmatic function [17]. Bertoglio and colleagues reported results of a protocol of surgical pleurectomy and partial decortication followed by HITHOC using cisplatin (80 mg/m^2) and doxorubicin(25 mg/m^2) and adjuvant chemotherapy (cisplatin and pemetrexed) for early-stage (I–II) MPM [18].

4 Local Control Procedure and Debulking Surgery for Malignant Pleural Mesothelioma

Huge tumor removal and partial pleurectomy may be effective for symptom control, such as intolerable pain due to tumor invasion to the chest wall, massive pleural effusion, in advanced stage malignant pleural mesothelioma (MPM).

Several investigators [19, 20] reported VATS pleurectomy with debulking the huge tumor is feasible in the majority of cases and independently improves survival for patients with advanced MPM [19]. But Debulking surgery has a beneficial role in symptom control for unresectable MPM, however, this procedure should be reserved for those patients who present with epithelial cell type and before significant loss of weight [20].

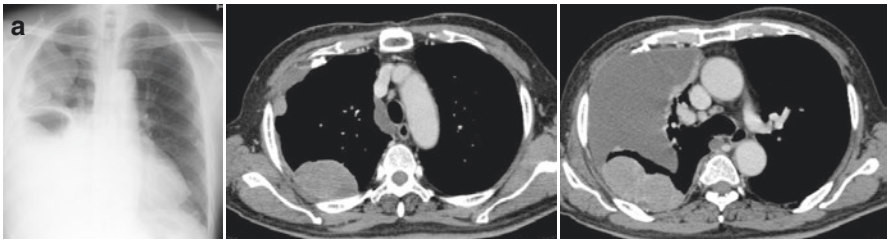
Nakas A.et al. reported that 63 patients over 65 years of age underwent therapeutic surgery for MPM. 13 patients underwent EPP, 8 had a radical P/D and 42 had VATS P/D (pleurectomy/decortication). Even though there was no significant difference in the overall mean survival between the two groups (EPP and VATS P/D), postoperative stay and 30-day mortality were significantly lower for VATS P/D than for EPP. They concluded, "VATS P/D should be considered in the therapeutic strategy for MPM, rather than EPP or radical P/D" [21].

Whereas, the MesoVATS trial, which was a randomized trial of VATS pleurectomy/decortication with debulking the tumor, compared with talc pleurodesis in MPM patients in the United Kingdom, settled the discussion down, as follows. VATS P/D (VAT-PP: partial pleurectomy with debulking the tumor) did not improve survival over talc pleurodesis, but may also have a role in to resolve the trapped lung to be inflated for several months after the surgery. [22] Furthermore, the MesoVATS trial did not report the survival subgroup analysis by stage or the number of post-study systemic therapies, which could have impacted the overall survival outcomes (Fig. 29.1a–c).

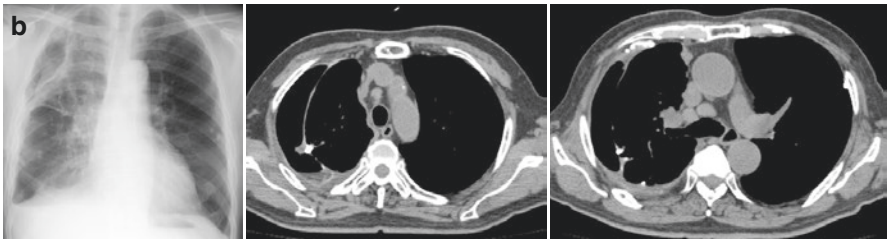
5 Miscellaneous Additional Intraoperative Procedures and Surgery for Recurrent Tumor

5.1 Talc Pleurodesis

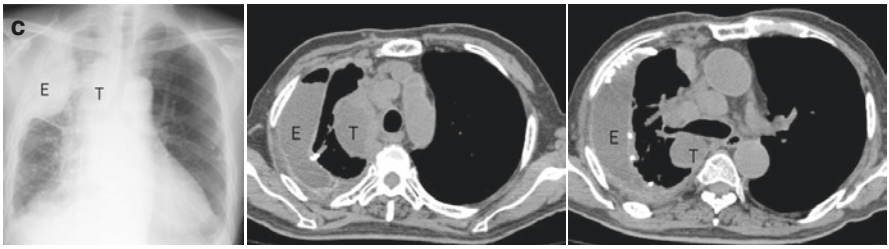
Talc (Mg3Si4O10(OH)2) is administered into the pleural cavity by poudrage or slurry. Success rates (complete and partial response) for talc slurry range from 81% to 100% [23]. Rena et al. investigated prognostic effect of persistent lung expansion after pleural talc poudrage in non-surgically resected MPM patients [24]. 146 of 172 patients demonstrated a complete lung expansion at discharge, persistent lung expansion after talcage and nil fluid recurrence is demonstrated to be a strong factor in predicting survival rather than clinical stage and other clinical variables in not surgically resected MPM patients.



The images show the huge tumor, mesothelioma, and massive pleural effusion in right thorax.



10 days after surgery three months after surgery 1) Three months after surgery 2)



The images, eight months after surgery. E; pleural effusion. T; tumor

Fig. 29.1 Case file; VATS-PP (Video-assisted thoracoscopic partial pleurectomy) for mesothelioma: 74 y. o., male visited a local hospital because of the shortness of breath and right-sided chest pain. The diagnosis, made by the CT-guided needle biopsy, was biphasic mesothelioma (**a**). The patient was referred to the author's hospital, and he underwent video-assisted thoracoscopic tumor removal and partial pleurectomy with pleural poudrage by talc. Within a few months since the surgery, he was free from shortness of breath (**b**). Since 7 to 10 months after surgery, he had been afflicted with the symptom, dyspnea, and chest pain (**c**). He died ten months after surgery because of the recurrence of biphasic mesothelioma

5.2 Povidone-Iodine

Povidone-iodine (PVP-I) has been used as an antiseptic agent over several decades, in addition, it is used to prevent tumor cell seeding following resection of colorectal carcinoma, breast carcinoma, and hepatoma. Opitz et al [25] have shown that PVP-I has a direct cytotoxic effect on mesothelioma cells and induces necrosis of mesothelioma cells in vitro. In vitro studies of PVP-I in MPM cell lines demonstrated that PVP-I cause cell necrosis, through the production of reactive oxygen intermediates, including an inflammatory reaction that may lead to an anti-tumor response.

Lang-Lazdunski et al. [26] used heated PVP-I in vivo, intrapleural space, intraoperatively. Subsequent 102 patients underwent P/D and hyperthermic pleural lavage with PVP-I followed by prophylactic radiotherapy to the chest wall (21Gy), and systemic chemotherapy. They used sterile water mixed 10% PVP-I at 40–41°C for 15 minutes. The overall median survival was 32 months and 5-year survival rate was 23.1%. Median survival and 5-year survival rate were 35.0 months and 30.7% for epithelioid mesothelioma. Median survival was 45.0 months for R0–R1 resection versus 17.4 months for R2 resection (P = .0001). They concluded P/D, hyperthermic pleural lavage with povidone-iodine, prophylactic chest wall radiotherapy, and systemic chemotherapy is a safe and well-tolerated multimodality therapy.

5.3 Photodynamic Therapy (PDT)

PDT is a therapy of non-ionizing radiation therapy that use photosensitizer and light to produce singlet oxygen. Since the 1990s, PDT had become popular in the treatment of thoracic malignancies. PDT is only capable to be used after EPP and radical pleurectomy. Initially, the patient is given a nontoxic photosensitizing agent, usually porfimer sodium Photofrin or meta-tetra hydroxyphenyl chlorin (m-THPC) Foscan, that is subsequently activated in the presence of oxygen by visible light of a specific wavelength. This reaction produces singlet oxygen, a highly reactive form of oxygen, and is thought to be the principle effector of a number of mechanisms by which PDT induces tumorigenic cell death. The ability to change any of these different elements of PDT and therefore modulate its effect makes PDT an interestingly flexible and customizable modality of treatment for MPM. The first phase III trial assessing the benefit of PDT for MPM was performed by Pass and colleagues [27]. Friedberg and colleagues refined the technique of PDT over the years to the point of photosensitivity complications have become practically nonexistent, and morbidity and mortality rates are similar to those of patients without PDT treatment [28]. The depth of penetration associated with PDT is also ideal for intraoperative procedures: PDT penetrates several millimeters below the illuminated surface, a depth that is well suited for the purposes of reaching microscopic tumor left over from cytoreductive surgery but that is also superficial enough to prevent damage to underlying lung parenchyma [29]. Friedberg et al [30] reported the use of PDT in the management of MPM as follows: *From 2005 to 2013, 90 patients underwent extended pleurectomy decortication (EPD) combined with intraoperative PDT and preoperative and/or postoperative chemotherapy. All patients had a preoperative diagnosis of epithelial subtype, of which 17 patients proved to be mixed histology after EPD. The remaining 73 patients with pure epithelial subtype were analyzed. All patients received EPD and PDT; 92% also received chemotherapy. The median follow-up was 5.3 years for living patients. Macroscopic complete resection was achieved in all 73 patients. 30-day mortality was 3% and 90-day mortality was 3%. For all 73 patients (89%:AJCC Stage III/IV, 69% N2 disease, median tumor volume 550 ml), the median overall and disease-free survivals were 3 years and 1.2 years, respectively. For the 19 patients without lymph node*

metastases (74%: AJCC Stage III/IV, median tumor volume 325 ml), the median overall and disease-free survivals were 7.3 years and 2.3 years, respectively. Regarding overall survival, it is approximately triple the disease free survival, perhaps PDT related. The impact of PDT is unclear, but it is hoped that it will be established by an ongoing randomized trial.

Due to the small number of trials and limited centers with experience, intraoperative PDT for MPM is not considered the standard of care and should only be considered for well-designed clinical trials.

6 Conclusion: What is the Role of Enhancing Local Control Approaches?

Despite promising results, no high-quality evidences are currently available, and controlled randomized trials are required to establish the exact role of intracavitary therapies and to standardize the technique.

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Correction to: Malignant Pleural Mesothelioma

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Correction to: Viral Immune Therapy and Other Virotherapies for Advanced Mesothelioma: Are We Ready for Clinical Trials of Viral Immune Therapy?

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In chapter 25, Table 1 - Current clinical trials of virontherapy for pleural mesothelioma has been included.

The online version of this chapter can be found at https://doi.org/10.1007/978-981-15-9158-7_25

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Table 25.1 Current clinical trials of virotherapy for pleural mesothelioma

Virus	Genome	Alias	Phase	Trial condition	CT#	Results	Reference
Herpes Simplex Virus I	ds DNA	**HSV1716** (replication competent) (ICP34.5 deletion to abolish neurovirulence)	**I/II**	Completed	NCT01721018	Twelve Patients were treated. An acceptable safety profile of intrapleural HSV1716 with evidence of viral replication and antitumor immunogenicity.	[12, 13]
		G47Δ (ICP34.5 & ICP47 deletion to maintain MHC class I expression of host cells)	**I**	Recruiting	UMIN000034063	To assess the safety of G47delta administered into the pleural cavity in subjects with progressive malignant pleural mesothelioma.	[14]
Adenovirus	ds DNA	**AdV/HSV-tk** (replication incompetent)	**II**	Not recruiting	NCT01997190	No tumor response, good safety profile, 2/21 patients	[6]
		AdV/hIFN-beta (replication incompetent)	**I**			repeated dosing safe, response by CT scan at 60 days in 2/10 patients	[6]
		AdV/hIFN-alpha2b (replication incompetent)	**I/II**	Completed	NCT01212367	P-I; 55/9 SD or PR at 60 days ref. #93 P-II: PR in 25%, SD in 62.5%, median Survival 13 months, 6/40 patients lived over 2 years ref. #94	[15, 16]
		AdV/hIFN-alpha2b (replication incompetent) in combination w/ Celecoxib and Gemcitabine	**III**	Recruiting	NCT03710876		[18]
		ONCOS-102 (Adv5/3-D24-GM-CSF) insertion of Ad3 fiber knob to Ad5-D24-GM-CSF in combination w/ Cisplatin/Pemetrexed	**I/II**	Not recruiting	NCT02879669	The intrapleural combined w/ chemotherapeutic agents vs chemotherapeutic agents alone. Two patients given, it w/ cyclophosphamide. One patient was recruited.	[17, 18]

		ONCOS-102 in combination w/ Durvalumab	**I/II**	Recruiting	NCT02963831		
Vaccinia	ds DNA	**GL-ONC1**	**I**	Not recruiting	NCT01766739	Eleven MPM patients were treated. Single dose, intrapleural administration of GL-ONC1 is safe, but is best suited for patients with MPM whose disease is limited to the pleura.	[19, 20]
Measles	ss(−) RNA	**MV-NIS** Edmonston strain w/ insertion of NIS gene (replication competent)	**I**	Not recruiting	NCT01503177 NCT01119664	The bestresponse was SD. The intrapleural administration of MV-NIS is safe, resulting in SD in 67% of patients.	[21, 22]
Inactivated Sendai virus (Non-replicating)	ss(−) RNA	**GEN0101**	**I**	Completed	UMIN000019345	Safety and tolerability of GEN0101 intratumoral adaministration for six chemotherapy-resisitant MPM patients were confirmed.	[25, 26]
	ss(−) RNA	**GEN0101** in combination w/ Cisplatin/Pemetrexed	**II**	Recruiting	JapicCTI-194617 ref. NCT 03818893		