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Review

Advances in the Molecular Biology of Malignant Mesothelioma

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Malignant mesothelioma (MM) is a highly aggressive tumor with a dismal prognosis. The incidence of MM is increasing as a result of widespread exposure to asbestos. As for the molecular alterations that occur in MM, chromosome alterations including homo-deletion of the *P16* and *P14* genes located in the 9p21 are well known. Mutations are rare in the *P53* and *Ras* genes, which are frequently present in epithelial solid tumors. However, mutations are frequently present in the neurofibromatosis type 2 gene. Epigenetic alterations including DNA methylation have been found in the MM, the profile of which is different from that of lung cancer, although differential diagnosis is sometimes clinically difficult. As in other malignant tumors, genes that are related to immortalization, proliferation, metastasis, angiogenesis, and anti-apoptosis are also overexpressed in MM, contributing to its malignant phenotype. It is of interest that simian virus 40 has been implicated to be one of the causative factors of MM in western countries. Although the causative role of asbestos is well-known in MM, much less information is available for MM than for other malignant tumors regarding the molecular alterations that occur in the disease. In terms of future tasks, it will be necessary to apply the knowledge that is learned about molecular alterations to clinical practice and to further elucidate the pathogenesis of MM with extensive research.

Key words: malignant mesothelioma, P16, methylation

Malignant mesothelioma (MM) is an aggressive tumor that develops from the pleura or other mesothelial surfaces and is strongly associated with exposure to asbestos. Many MMs have already progressed by the time they are discovered and show an extremely poor prognosis [1]. Multimodality therapy centered on surgical resection is indicated for the treatment of MM in the early stage, but so many cases are found at advanced stage. No method of treatment, including chemotherapy or radiotherapy, has yet been established for advanced cases. Thus, new strategies for the diagnosis and treatment of MM are needed. In fact, much less information regarding molecular biological alterations is available for MM than for other solid neoplasms, even though an understanding of their molecular biological characteristics is needed to establish diagnostic, treatment, and prevention methods. This review summarizes the representative molecular biological alterations in MM that have been

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demonstrated thus far.

Molecular Alterations in MM

Effect of asbestos. Asbestos, especially amphiboles fibers with a high length-to-diameter ratio, is a well-established carcinogen of MM [2, 3]. Research on the molecular alterations produced by asbestos in mesothelial cells has been under way since the 1990s. There may be a 35- to 40-year latent period before the development of asbestos-induced MM. The clinical manifestations of MM are thought to arise as a result of a build-up of many molecular alterations. Asbestos fibers show increased expression of the proto-oncogenes *c-fos* and *c-jun* [4]. These proteins are localized in the nucleus, resulting in cell proliferation and transcription, which are thought to be the initial intranuclear alterations caused by asbestos. Asbestos in nature also has a cytotoxic effect on mesothelial cells *in vitro*. On the other hand, asbestos also promotes secretion of the pro-inflammatory cytokine TNF-α in mesothelial cells and macrophages, leading to the activation of NF-κB through promoting the expression of the TNF-α receptor in mesothelial cells [5, 6]. Because NF-κB plays a role in cell proliferation and anti-apoptosis, MM cells are assumed to undergo neoplastic transformation as a result of the activation of the NF-κB pathway [5].

Chromosome alterations. Numerous alterations at the chromosome level have been reported in MM based on the results of comparative genomic hybridization and loss of homozygosity (LOH) analyses. While both chromosome amplifications and deletions have been found, deletions are more frequent than amplifications, with more involved sites. There have been many publications regarding the alterations of chromosome loci. The most frequent deletions and the chromosome locus amplifications that have been published are shown in Table 1 [7–17]. A particularly high frequency of homo-deletions is seen in the 9p21 region in MM, thus causing a high frequency of deletions of the *P16* and *P14* genes that are located on 9p21 and a loss of expression of their proteins [18]. Indeed, an attempt is being made to apply this finding for diagnosis by using the fluorescence *in situ* hybridization method to identify homo-deletion of the *P16* gene in MM cells in pleural fluid [19]. As a potential therapeutic strategy, cell cycle arrest is induced in MM cells to which either p16 or p14 expression constructs have been introduced [20, 21]. The mechanism of the *P16* alterations and malignant transformation has been found to involve the loss of p16 protein, which causes a breakdown of the cell-cycle control mechanism by inhibiting the phosphorylation of retinoblastoma (Rb) protein, which controls the cell cycle. In addition, the loss of p14 protein results in the activation of mdm^2 protein, a p53 ubiquitin ligase, and this activation is thought to be linked to the destabilization of p53 protein, thus causing alterations in cell cycle control [22] (Fig. 1). Deletion of *P16* is significantly more common in MMs that have a sarcomatous component and is considered to be a negative prognostic factor for MM independent of the sarcomatoid type [23].

Differences in chromosome alterations have also been reported in the histological subtypes, which may contribute to the biological and morphological differences among subtypes [13, 24].

Gene mutations. Neurofibromatosis type 2 *(NF2)* gene mutations have been discovered in MM [25]. The *NF2* gene is originally known to be the causative gene in neurofibroma (type II). *NF2* gene mutations have been observed in approximately 50% of MM cases. There has been a report of LOH of 22q12, where the *NF2* gene is located, in almost 100% of mutant cases [26]. Because *NF2* gene mutations are not observed in lung cancer, the presence of these mutations is considered useful for making the

Table 1 Representative chromosomal abnormalities in MM

Chromosomal abnormalities		Related genes
Loss	Gain	
1p21–22		
3p21		*RASSF1A*
4p, 4q		
	5p	
6q		
	8q	
9p21		*P16, P14*
13q13–14		*RB*
14q		
15q		
17p12		*P53*
	17q	
22q12		*NF2*

differential diagnosis from lung cancer, especially from adenocarcinoma of the lung [25, 27, 28]. However, since there are no hot spots at the mutation sites in the *NF2* gene, it is necessary to examine all 17 exons in order to detect mutations, as it is not realistic to use *NF2* gene mutations as a marker for diagnosis at the present time. When a simple method to detect *NF2* gene mutations is developed, the *NF2* gene is likely to become a valuable diagnostic marker. While mutations in the Wilms' tumor 1 *(WT1)* gene have also been found, their frequency is low, and thus it is difficult to use them for molecular diagnosis [29, 30]. Mutations in other genes including the *P53*, *Ras*, and *RB* genes, which have been found at high frequencies in other malignant tumors, are very rare [31–33]. It is assumed that alterations in the *P53* and *RB* genes in particular are unnecessary, because even if the *P53* and *RB* genes are of the wild type, they do not function properly because of the *P14* and *P16* genes deletions as described above. However, the reason that the DNA sequences of *P53* and *RB* are intact in MM is unclear. This may be a crucial key to understanding the pathogenesis of MM.

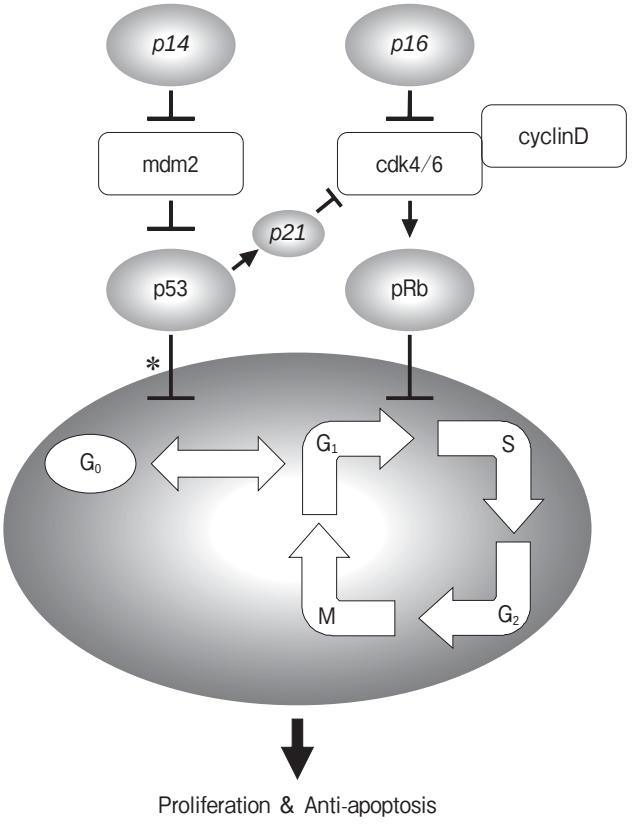

Fig. 1 Schema showing the role of p16 and p14 in the cell cycle. The loss of p16 and p14 function leads to alterations in cell cycle control, resulting in cell proliferation and anti-apoptosis. *G_0 arrest by p53 pathway mainly occurs when DNA is damaged.

DNA methylation. Gene inactivation by epigenetic alteration has been established as a crucial mechanism that satisfies Knudson's hypothesis that both alleles of a tumor suppressor gene (TSG) must be inactivated for carcinogenesis. Promoter methylation and the associated event of histone deacetylation are epigenetic changes in chromatin structure that cause gene silencing without altering the DNA sequence [34, 35]. Methylation of TSGs, including methylation of the *RASSF1A* gene, has been observed in MM as well, strongly suggesting that methylation of the promoter region of TSGs contributes to the neoplastic transformation and progression of MM [36–39]. Since the methylation profile varies with the histological subtype of the MMs, the mechanism of neoplastic transformation may differ according to the histological subtype [37]. The methylation profile of MM is clearly different from those of adenocarcinoma of the lung, and thus it can be used as a diagnostic tool [37]. Because the histological findings of these 2 diseases are sometimes too similar to distinguish them, the methylation profile along with *NF2* mutation status can be useful for performing differential diagnosis.

Most of the studies of methylation in MM have thus far been conducted on specimens in the United States, where SV40 infection is suspected to be one of the causes. Because the methylation profile of some genes differs between SV40-infected and non-infected MMs, the methylation profile of MM in counties like Japan where there is no SV40 involvement may be totally different from that in the United States. Our previous study indicates that methylation of the insulin-like growth factor-binding protein-3 *(IGFBP-3)* gene, which is thought to control IGF function by suppressing the IGF-1 receptor, has been shown to be more frequent in MM in Japan than in the United States. This finding suggests the presence of racial or regional differences in the genes that undergo methylation in MM [40].

Activation of telomerase. Telomerase activation, which is thought to be one of the causes of immortalization, is increased in malignant tumor cells.

It is also known to be high in MM [41]. The expression of hTERT, which is closely associated with telomerase activity, has been found in 90% of MM cases. Since its expression is not observed in mesothelial cells in non-tumorous portions, it may be capable of serving as a marker for the malignant transformation of mesothelial cells [41].

Cell proliferation. An increase in cell proliferation is seen in MM as in other malignant tumors. Cell proliferation increases particularly as a result of the autocrine and paracrine function of growth factors: epidermal growth factor (EGF) [42], hepatocyte growth factor (HGF) [43], platelet-derived growth factor (PDGF) [44], transforming growth factor β[45], IGF-1, and IGF-II [46]. At the same time, high levels of expression of the receptors for these growth factors such as EGFR, c-MET, and IGF-R1 have been found. Moreover, alterations of the Wnt signaling system, which is involved in the proliferation and generation of normal cells, have recently been reported in MM [47].

Invasiveness and angiogenesis. MMs are highly locally invasive tumors but distant metastasis also sometimes occurs in advanced cases. The matrix metalloproteinases (MMPs) MMP-2 and MMP-9, in particular, are known to be related to the invasion and metastasis of MM. MMP-2 is also considered to be a negative prognostic factor [48]. It has also been suggested that the microvessel density of tumors is a prognostic indicator [49], and that the vascular endothelial growth factor that contributes to angiogenesis is overexpressed [50].

Anti-apoptosis. The Bcl-2 family of proteins is known to be involved in apoptosis and strong expression of these protein is seen in many malignant tumors. Whereas the frequency of expression of Bcl-2 protein itself in MM is low, strong expression of Bcl-XL (a member of the Bcl-2 family) and Bax, which have potent anti-apoptotic potential, has been found [51–53]. Strong expression of the inhibitor of apoptosis protein (IAP) and survivin, which are considered resistance factors for chemotherapy, has also been observed [54].

Association with SV40. There is a hypothesis in western countries that infection with SV40, a virus whose original hosts are monkeys, is one of the causative factors of MM [55, 56]. In support of the hypothesis, SV40 large T antigen DNA has been detected in approximately half of MM cases in the United States [57]. The reason why SV40 was detected in human tissue has been presumed to be that the polio vaccine produced between 1955 and 1963 was contaminated with SV40 in the manufacturing process, and that SV40 infected humans through inoculation with the contaminated vaccine. There have been many arguments against this explanation, and this topic continues to invite debate [58]. There is concern that the vaccine that may have been contaminated was used in Japan from 1961 to 1963, but the result of a study in Japan regarding the association between MM and SV40 was negative [59]. Inactivation of p53 and Rb protein by SV40 large T antigen has been demonstrated as the mechanism of carcinogenesis by SV40 [60]. Moreover, in human mesothelial cells that were infected with SV40 and immortalized, an increase in hTERT activity was observed in the early stage, and methylation and a decrease in the expression of the *RASSF1A* gene was observed in the late stage, both of which are assumed to be related to SV40-associated MM [61, 62]. Malignant transformation of mesothelial cells by SV40 is thought to be promoted by its combination with the presence of asbestos [60].

Attempts at comprehensive analysis of expression. The differential display and microarray methods are useful assays for the comprehensive analysis of mRNA expression. Attempts have been made to identify altered gene expression levels through the comprehensive analysis of mRNA expression in MM tissue and in non-tumorous tissue. Alterations in the level of variety gene expression, including the activation of apoptosis-associated genes and gene groups involved in glucose metabolism, mRNA transcription, and cytoskeleton formation, have been occasionally reported [63, 64] but not universally identified. Although some studies have also explored the possibility of identifying prognostic factors by expression profiling, they have not reached the clinical application stage and require further investigation [65, 66].

Concluding Remarks

The worldwide incidence of MM is considered to be increasing [1], especially in Japan and non-western countries, where asbestos continued to be heavily used

after its use had been discontinued in western countries. Thus, the urgent establishment of preventive, diagnostic and treatment strategies for MM is mandatory. Also, the roles of erionite and of genetic factors, as shown by the outbreak in certain areas of Caddadochia, Turkey, can be crucial clues for understanding the pathogenesis of MM [67]. So far, extensive research has made some of the critical molecular alterations clear, but the findings are still not adequately reflected in clinical settings. In terms of future tasks, it will be necessary to apply what has been learned so far to clinical practice and to further elucidate the diseases using a molecular biological approach.

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