CASE REPORT

Stepwise increase of MIB-1 index in frequently relapsed malignant peritoneal mesothelioma

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Abstract We identified the stepwise increase of MIB-1 index in a long-surviving malignant peritoneal mesothelioma (MPM) patient with a history of frequent relapse. A 29-year-old Japanese woman showed upper abdominal induration with adnexal tumor. Imaging study with biochemical analyses strongly suggested peritoneal tumor. On primary surgery, all tumors were resected completely without any residual tumor. Histologically, the tumor was diagnosed as MPM, for which she received adjuvant chemotherapy containing platinum agent. Two years later, the tumor relapsed in her pelvic cavity, but was resected completely with hysterectomy and salpingo-oophorectomy. Histologically, the tumor was diagnosed as MPM relapse. She underwent intraperitoneal chemotherapy with cisplatin that achieved progression-free survival of 5 years. However, relapse was detected again in pelvic cavity without any dissemination in upper abdominal cavity. The tumors were completely removed and were revealed to be compatible with MPM. She received gemcitabine and carboplatin chemotherapy. However, 2 years later, the tumor relapsed again in left upper abdominal cavity, for which she wouldn't receive 4th treatment. To investigate the longevity of this patient in association with the histologic findings, the MIB-1 index was examined in the primary and relapse tumors. The rate of MIB-1 index positive cells was calculated by counting 500 cells. MIB-1 indices were 4.2 ± 1.1

(mean $\pm$ SE), 11.8 ± 2.3 , and 37.3 ± 2.5 in primary, 1st- and 2nd-relapsed tumor, respectively, demonstrating stepwise increase of MIB-1 expression over the surviving time of more than 9 years. Increase in MIB-1 index was not associated with mitotic index but may be indicating drug sensitivity, resulting in >2-year progression-free interval in each relapse.

Keywords Malignant peritoneal mesothelioma · Long-term survival · Drug sensitivity · MIB-1 index

Introduction

Malignant mesothelioma is a highly symptomatic and rapidly progressive malignant tumor [1]. The primary site of the disease is mainly the pleura; whereas the peritoneum is affected with considerably lower frequency. A review on peritoneal malignant mesothelioma incidence and mortality in cohorts exposed to asbestos has shown a consistent relationship with asbestos [2]. Treatment effectiveness is very poor when the disease is inoperable and usually survival after diagnosis has been observed to be particularly poor. Some studies reported a shorter median survival time compared with pleural mesotheliomas [3]. Whereas, Baratti et al. reported combined treatment comprising peritonectomy and hyperthermic intraperitoneal chemotherapy (HIPEC) has resulted in survival benefit for peritoneal surface malignancies, including diffuse malignant peritoneal mesothelioma [4]. Additionally, they also showed lower MIB-1 labeling index was recognized as an independent predictor of better prognosis.

MIB-1 monoclonal antibody is a promising tool for determining cell proliferation on routine histologic material. The widely used Ki-67 has been replaced by MIB-1

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antibody that has similar epitope but recognizes the target in paraffin-embedded tissues [5, 6]. MIB-1 index is often compared with other classic factors measuring cell proliferation rate including thymidine-kinase assay and mitotic index that are important components of histologic grading system for ovarian or peritoneal cancer. However, MIB-1 assay is convenient because of their minimal tissue requirements and suitability to routinely fixed tissues and is widely used along with mitotic index. One study demonstrated the increase in MIB-1 staining from primary to recurrent in identical patient with ovarian clear cell carcinoma and speculating MIB-1 staining may be affected by treatment [7].

In this study, stepwise increase of MIB-1 index was demonstrated in a case of malignant peritoneal mesothelioma. The longevity of this patient might be associated with the low MIB-index in the primary tumor and increased index value in the relapsed tumor substantiating higher drug sensitivity.

Case presentation

The subject is a 29-year-old Japanese woman presenting huge cystic tumor in right adnexal region with left upper abdominal induration. She had no obvious exposure to asbestos in the past. CA 125 level was slightly elevated to 63 μ /ml. Computed axial tomography (CAT) and magnetic resonance imaging (MRI) presented huge multilocular cystic masses with extensive infiltration to the omentum. Partial omentectomy and tumorectomy on the right adnexal region were performed without any residual tumor. Histologically, the neoplastic cells were short-spindled or polygonal in shape and had abundant cytoplasm and oval nucleus, with occasional multinucleated cells. Nuclear atypia was inconspicuous to mild, and mitotic figures were rare (Fig. 1). Immunohistochemically, the tumor cells were positive for calretinin, WT-1, D2-40, and cytokeratin (CAM5.2, CK19, CK20) but negative for epithelial membrane antigen, Ber-EP4, carcinoembryonic antigen, CD10, and alpha-inhibin. These findings confirmed the diagnosis of malignant peritoneal mesothelioma. Cytology of the ascites fluid showed small clusters of round or ovoid cells compatible with malignant peritoneal mesothelioma. The patient received 2 cycles of chemotherapy comprising carboplatin and docetaxel followed by 4-cycle combination of cisplatin, therarubicin, and cyclophosphamide. At the completion of the treatment, the level of CA 125 decreased to 8 U/ml. After a 2-year remission, cystic tumor was detected in upper pelvic cavity on CAT or MRI scanning with CA 125 level elevated to 46 U/ml, suggesting a relapse of the malignant peritoneal mesothelioma. Hysterectomy, salpingo-oophorectomy, and tumorectomy were performed

Fig. 1 H-E staining of the primary tumor. Tumor cells grow diffusely in solid pattern presenting malignant peritoneal mesothelioma

leaving no residual micro-peritoneal dissemination behind. The histologic finding was well compatible with a relapse of malignant mesothelioma with positive ascites cytology. Considering the intraperitoneal residual floating cancer cells, she underwent treatment with a combination of intraperitoneal cisplatin with intravenous therarubicin and cyclophosphamide for 6 cycles, achieving progression-free survival of 5 years with normalization of CA 125 level to 8 U/ml.

However, relapse was detected again in the pelvic cavity on a tri-monthly regular physical examination without elevation of CA 125 level. MRI presented a well-defined solid mass in pelvic cavity, suggesting a relapse of malignant peritoneal mesothelioma. All tumors were again completely resected and were histologically revealed to be well consistent with malignant mesothelioma. She received 6 cycles of chemotherapy with gemcitabine and carboplatin and is alive without apparent tumor for the next 2 years. At that time, she showed the relapsed tumor in left upper abdominal cavity, for which she would not receive 4th treatment.

Immunohistochemical assay

Paraffin-embedded blocks from primary tumor, 1st- and 2nd-relapsed tumor specimens were evaluated independently for Ki-67 expression using the MIB-1 monoclonal antibody (mouse anti-Ki-67, Invitrogen Corp. USA).

MIB-1 index study

The MIB-1 index was determined by semi-quantitative visual method on each of primary tumor, 1st- and 2nd-relapsed tumor specimens. Tumor cells were estimated

positive for MIB-1 when clear nuclear staining was confirmed. The area of highest positivity was probed by scanning the entire tumor cut surface at low power. After this first examination, a quantitative assessment was made at high power magnification ($\times 400$) by counting a total of 500 tumor cells within representative fields. All MIB-1 stained nuclei were counted as positive, regardless of type of staining or staining intensity. The percentage of cells showing definite nuclear immunoreactivity was calculated as MIB-1 labeling index.

Statistical analysis

Statistical analysis was performed using Stata/MP 12.1 (Stata Corporation, USA.). Associations on Ki-67 expression among primary tumor, 1st- and 2nd-relapsed tumor were assessed using Student's *t* test.

Results

Figure 2 shows MIB-1 staining of the primary and relapsed tumor, clearly demonstrating the stepwise increase of MIB-1 positive cells. Figure 3 shows linear increase of MIB-1 index over the 9-year-period and Table 1 summarizes the statistical analyses. In the primary tumor, MIB-1 index was 4.2 ± 1.1 (mean $\pm$ SE)% with a quite rare mitosis. The first relapsed tumor showed slight but significant increase to $11.8 \pm 2.3\%$ and the second relapsed tumor showed significantly further increase of MIB-1 index to $37.3 \pm 2.5\%$, suggesting an aggressive and proliferative potential of the relapsed tumor. It is of note that nuclear atypia was inconspicuous to mild, and mitotic figures were quite rare in all three specimens.

Fig. 2 Representative MIB-1 staining of primary (a), 1st- (b) and 2nd- (c) relapsed tumor

Fig. 3 Chronological change of MIB-1 index on a series of primary, 1st- and 2nd-relapsed tumor. The *x*-axis indicates the date after treatment for each data point corresponding to the time when each of the specimens was collected. Index values showed the lineal increase over the time. Closed circle mean, bars SE

Discussion

Regardless of the treatment modality, lower MIB-1 index ($<10\%$) was documented to be an independent predictor of better prognosis in malignant peritoneal mesothelioma [4]. Our case showed a low-level index (4.2%) in the primary tumor that can be classified as having good prognosis. However, her relapsed tumor showed an extremely much higher value (37%) that can be regarded as having poor prognosis. In this paper, we discuss the stepwise increase of MIB-1 index in identical patient of malignant peritoneal mesothelioma focusing on its possible mechanisms, comparison to other tumors and the clinical significance of MIB-1 in the recurrent tumor associated with longevity of our patient regardless of frequent aggressive treatment.

First, concerning to the biological mechanism of Ki-67 expression on cellular proliferation, a strong correlation is generally observed between MIB-1 index and mitotic index that defines the histologic and nuclear grading. In our case, MIB-1 increase was not associated with the change of mitotic figure. The biological mechanism of increasing MIB-1 with rare or no mitotic figure is, at present, unknown. Ki-67 antigen expression increases during progression of the mitotic cycle, increasing during the latter half of S phase and peaking in the G2 and M phases [8]. Cell cycle analyses were not performed in this study; however, the possible short metaphase and long inter-prophase might contribute to the dissociation of MIB-1 index and mitotic figure in our case.

Second, comparing the level of MIB-1 index to the other tumor, the MIB-1 cutoff value differs depending on

Table 1 xxx

Tumor	Mean	SE	<i>T</i> test	95% CI
Primary	4.21	0.038 ^a	0.038	2.78–13.26
1st relapse	11.83	0.002 ^b	0.002	9.42–39.75
2nd relapse	37.34	0.000 ^c	0.000	1.47–40.08

SE standard error, 95% CI 95% confident interval

^aPrimary vs 1st relapse

^b1st vs 2nd relapse

^cPrimary vs 2nd relapse

the tumor species and is reported to be roughly 10–30% in breast cancer or lymphoma [9, 10]. In malignant peritoneal mesothelioma, Baratti et al. have documented that a MIB-1 cutoff of less than 10% has been acceptably identified in a subset of long survivors [4]. These data is based on MIB-1 index on primary tumor; however, the chronological change has never been reported as of yet whatever the origin of the tumor might be. Insofar as we know, our case is the first English written report. Since few other study reports are available, it is hard to argue whether this is a feature specific to our case or it could be applied to malignant mesothelioma in general or even other tumors.

Finally, we made in-depth arguments on clinical significance of MIB-1 in the recurrent tumor. In relation to the drug sensitivity, studies have suggested that highly proliferative tumors show increased sensitivity to adjuvant chemotherapy in patients with breast cancer [11, 12]. Spyrtos et al. have tried to identify tumor sensitivity to chemotherapy by setting the cutoff value of MIB-1 index >25% in breast cancer patients [9]. When this criterion is assigned to our case, the second relapsed tumor (MIB-1 index of 37) is highly sensitive to chemotherapy. On the other hand, considering Gynecologic Oncology Group (GOG) clinical criteria that >2-year progression-free survival after previous chemotherapy is clinically diagnosed as platinum-sensitive; the maintenance of chemo-sensitivity on relapse might be related to long survival in our case. Although our patient received platinum-containing chemotherapy throughout her whole treatment history, it should be noted that more than 2 years platinum-free interval is sufficient time to recover platinum sensitivity even when the tumor cells acquire resistance transiently whatever the status of MIB-1 index is.

Our data shown here have discerned a number of clinically important concepts on which new treatment modalities might be developed upon relapse. Since there have been only few reports showing the chronological change of MIB-1 index in identical patient, more similar cases should be accumulated and investigated to have a better

understanding of the biological and clinical significance of MIB-1 index in such cases.

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Compliance with ethical standards

The authors report no conflicts of interest. Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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