

## SHORT COMMUNICATION

# Frequent genomic rearrangements of BRCA1 associated protein-1 (*BAP1*) gene in Japanese malignant mesothelioma—characterization of deletions at exon level

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Malignant mesothelioma (MM) is an asbestos-related malignancy arising from surface serosal cells of pleural and peritoneal cavities. Somatic mutations of BRCA1 associated protein-1 (*BAP1*) gene were recently found in MM as well as in uveal melanoma and kidney cancer among the Caucasian and Japanese people. However, frequency of mutations varies among the reported studies, which might be due to presence of undetected gross rearrangements of *BAP1* gene that might escape detection by sequencing strategy. We investigated the presence and frequency of gross genomic rearrangements in the *BAP1* gene by multiplex ligation-dependent probe amplification (MLPA) in 17 Japanese cases of MM tumors. We found five tumors with partial deletion of *BAP1* gene; each tumors displayed partial deletion of exons 1–4 (MM39), exons 1–5 (MM48), exons 11–17 (MM57), exons 1–15 (MM19) and exons 1–16 (MM21). Two tumors (MM34, MM14) had biallelic deletion and four tumors (MM29, MM35, MM45 and MM56) had monoallelic deletion of entire *BAP1* gene. Therefore, MLPA analysis revealed large gene rearrangements of *BAP1* gene in 65% of MM (11/17). Unusually high frequency of large deletions indicates that the 3p21 chromosomal region surrounding *BAP1* gene is structurally unstable. MLPA was useful in characterizing both monoallelic and biallelic deletion of *BAP1* gene precisely at exon level.

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## INTRODUCTION

Malignant mesothelioma (MM) is highly aggressive neoplasm that arises from the uncontrolled proliferation of surface serosal cells of the pleural, peritoneal and pericardial cavities. MM has been strongly associated with environmental exposure to asbestos.<sup>1,2</sup> However, molecular mechanism of MM remains largely unknown. Somatic mutations in BRCA1 associated protein-1 (*BAP1*) gene were recently found in 84% of metastasizing uveal melanoma,<sup>3</sup> whereas lower frequencies of somatic *BAP1* mutation was found in MM in Caucasian people (22, 23, 20 and 36%).<sup>4–7</sup> In Japanese cases, we previously described multiple sequence-level mutations and biallelic deletions of *BAP1* gene on seven mesothelioma genome.<sup>8</sup> The variable incidence of *BAP1* mutations among studies may reflect difference in ethnic background, difference in procedure of tumor cell isolation or difference in methodology of mutation detection.

Most *BAP1* mutations described to date are nucleotide-level mutations detected by sequencing strategies, thus, partial or complete

gene deletion at exon level might have escaped detection in these strategies. In the present study, we performed multiplex ligation-dependent probe amplification (MLPA) analysis that has been recently used as a highly sensitive method for detection of deletion<sup>8,9</sup> in order to explore frequency and precise nature of genomic rearrangements at exon level affecting *BAP1* gene in MM tumors.

## MATERIALS AND METHODS

MMs were obtained from the patients diagnosed with MM by pathological examinations at the Hospital of Hyogo College of Medicine as described previously.<sup>10</sup> Out of 18 cases studied, sufficient amount of tumor DNA were available in 17 tumors for the present study. ID number of MM tumors classified by histological types are as follows: epitheloid histological type: MM14, MM19, MM21, MM26, MM29, MM34, MM35, MM39, MM45, MM48, MM56, MM57 and MM67; biphasic histological type: MM16, MM30, MM80; and sarcomatoid histological type: MM46. This study was approved by the Ethics Committee of Hyogo College of Medicine and performed in accordance with the Declaration of Helsinki (1995) of the World

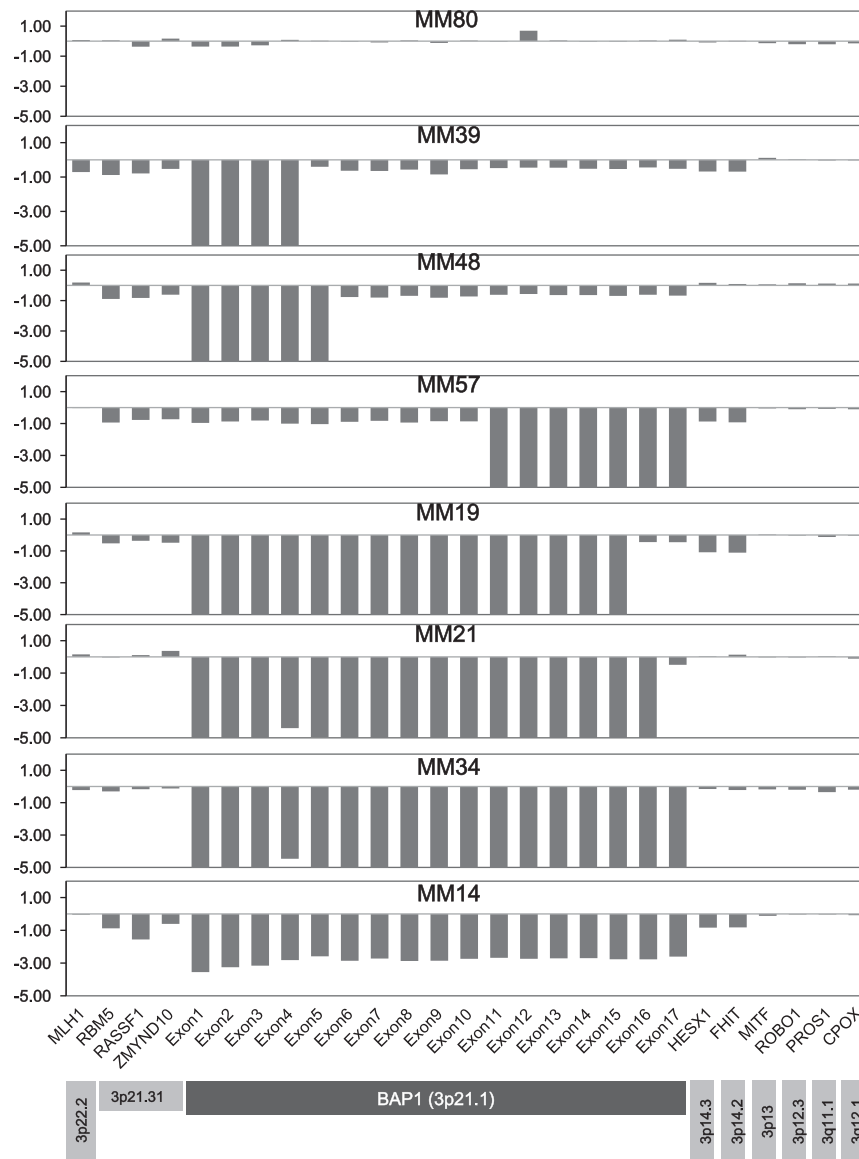
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**Figure 1** MLPA analysis. MLPA data for all 17 exons of *BAP1* gene on 3p21.1, as well as those for 10 control probes on chromosome 3p and chromosome 3q are displayed on x axis. Log<sub>2</sub> ratio of MLPA data for each probe is indicated on y axis. Panel from top to bottom: MM80, MM39, MM48, MM57, MM19, MM21, MM34 and MM14.

Medical Association (as revised in Tokyo 2004). All patients provided written informed consent.

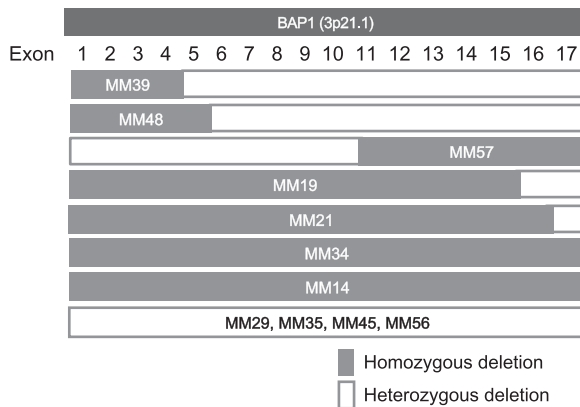
MLPA analysis of genomic DNA for each exons of *BAP1* gene and data analysis was carried out using MLPA system of MRC-Holland (Amsterdam, The Netherlands) for *BAP1* gene. The peak areas achieved using *BAP1* specific probes in each sample was first normalized by the average of peak areas achieved by control probes specific for locations from other chromosome. A corresponding calculation was performed for genomic DNA isolated from peripheral blood of 11 individuals and diploid status was defined as the average value from these control DNA. A final ratio dividing the value from the patient samples by the control average value was scored as monoallelic loss when below 0.7 (log<sub>2</sub> ratio below -0.52) and biallelic deletion when below 0.25 (log<sub>2</sub> ratio below -2).

## RESULTS

MLPA data for all tumors with partial deletion or biallelic deletion are displayed in Figure 1 (MM39, MM48, MM57, MM19, MM21, MM34

and MM14). Alteration in each tumor is schematically summarized in Figure 2.

Five tumors had partial or interstitial deletion of *BAP1* gene; tumor MM39 with partial deletion of exons 1–4, tumor MM48 with partial deletion of exons 1–5, tumor MM57 with partial deletion of exons 11–17, tumor MM19 with partial deletion of exons 1–15 and tumor MM21 with partial deletion of exons 1–16. The remaining allele of *BAP1* for each tumor was entirely lost, respectively. Therefore, we were able to precisely characterize gross gene deletions in these tumors at exon level by the use of MLPA that clarified copy number status of each of the 17 exons of *BAP1* gene. In addition, tumor MM34 and MM14 had biallelic deletion of entire *BAP1* gene. Tumors M29, MM35, MM45 and MM56 had monoallelic deletion of entire *BAP1* gene (data not shown). Sequence-level mutations on the remaining allele were described previously among all four tumors with loss of heterozygosity (LOH).<sup>10,11</sup> Together with sequence-level mutations, MLPA revealed *BAP1* mutations in 22 out of 34 alleles



**Figure 2** Genomic alteration of 11 MM tumors identified at the *BAP1* gene. Region of biallelic deletion, and monoallelic deletion at *BAP1* gene identified in 11 MM tumors with rearrangements are shown schematically for each exon of *BAP1* gene. Each horizontal thick bar represent genome of the *BAP1* gene for MM tumors. ID number of each MM tumors is also indicated along the bar. Top panel shows each exon of *BAP1* gene from left to right. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

(65%) of the 17 MM cases that accounts for biallelic inactivation of *BAP1* gene in 11 of these tumors (65%). Although MM29 apparently displayed exon 9 deletion by MLPA, it was later found to be caused by failure of MLPA ligation because of 3-bp deletion at MLPA probe site of exon 9 (data not shown).

## DISCUSSIONS

We detected high frequency of somatic gross deletion in the *BAP1* gene among 17 Japanese MM tumors and characterized them at exon level. Unusually high frequency of gene rearrangements indicates that the 3p21 chromosomal region surrounding *BAP1* gene is structurally unstable. Region of partial deletions observed in *BAP1* genes are unique to each tumor and involved region are different among MM tumors, thus no common site of genomic break is apparent on the *BAP1* gene.

We previously described association between *BAP1* mutation and epithelioid-type among MM in our previous report.<sup>10</sup> We here sought any relationship between types of *BAP1* mutation and histological types of MM, but no apparent tendency was observed because the number of non-epithelioid-type MM were too small in the present study.

To date, multiple somatic *BAP1* mutations have been described in MM tumors, but most of them are limited to nucleotide-level mutations such as non-sense substitutions or small deletions/insertions: 22% (4/18); 23% (12/53); 20% (24/121); and 33% (7/22) among sporadic Caucasian MM tumors by sequencing strategy.<sup>4-7</sup> Yoshikawa *et al.*<sup>10</sup> divided the *BAP1* gene into 10 PCR-parts and carried out genomic PCR and nucleotide sequencing of each of the 10 parts with detection of sequence-level mutation and biallelic deletion only on the respective PCR-part of the *BAP1* gene. In the present study, we were able to characterize both monoallelic deletion and

biallelic deletion of *BAP1* gene precisely at every one of the 17 exons that constitute the *BAP1* gene by the use of MLPA. Therefore, the significant difference in *BAP1* mutation frequencies among the various reports might be due to differences in methodology for detection of mutation. Nasu *et al.*<sup>12</sup> recently described detection of four partial or biallelic deletions of *BAP1* gene by MLPA among 22 Caucasian MMs, in addition to some point mutations and splicing mutations. Future investigation in larger panel of MM tumors would be warranted by using comprehensive molecular analysis, that is, sequence analysis, MLPA, immunohistochemistry, to fully understand types and nature of *BAP1* gene alteration in MM tumors.<sup>13</sup> It is also necessary in the future to carry out the analysis in a much larger set of MM tumors to verify the usefulness of MLPA as a screening method.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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