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**Prevalence of BRCA-1 associated protein 1 germline
mutation in sporadic malignant pleural mesothelioma
cases**

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Abstract

Objective: 23% of mesothelioma tumor specimens have a mutation in the BRCA1-associated protein 1 (BAP1) gene and germline BAP1 mutations predispose to malignant pleural mesothelioma (MPM). Our aim was to investigate germline BAP1 mutations in sporadic MPM patients.

Materials and Methods: Exonic DNA from peripheral blood leucocytes of 78 MPM patients was screened for germline BAP1 mutation.

Results: One out of 78 patients showed a germline synonymous mutation in exon 11. In all other patients wild-type sequence without any single-nucleotide polymorphisms was detected.

Conclusions: Taking into account previous similar screenings, the prevalence of germline BAP1 mutations in sporadic MPM patients can be estimated around 1-2%, suggesting a minor role of germline BAP1 mutation in the pathogenesis of sporadic MPM.

Keywords: BAP1, Mesothelioma, Tumor predisposition syndrome, Germline mutation, BAP1 cancer syndrome, Germline mutation screening

1. Introduction

Malignant pleural mesothelioma (MPM) is a rare cancer that originates from the pleural lining and that has a strong association to asbestos. The prognosis and the treatment options at the moment are very poor with a patient median survival time of less than 12 months after diagnosis [1]. To develop more effective MPM-specific therapeutics, much effort has been put into the investigation of cancer genes driving MPM oncogenesis. Besides the two most abundant alterations in MPM concerning cyclin-dependent kinase inhibitor 2A (CDKN2A) and neurofibromatosis 2 (NF2) genes, recently genetic alterations in the BAP1 (BRCA1-associated protein 1) gene, which is localized on chromosome 3 (3p21.1), have been identified in 23% of MPM specimens [2].

BAP1 was initially identified in lung cancer cell lines as a protein that binds to BRCA1 [3]. It is a 90 kDa nuclear-localised deubiquitinating enzyme with ubiquitin carboxyl hydrolase (UCH) activity; and it is the only member of the UCH family with two nuclear localisation signal (NLS) motifs [4]. BAP1-mediated tumour suppression requires both deubiquitinating activity and nuclear localisation of BAP1 [4]. BRCA1 does not seem to be necessary for the tumor suppressor activity of BAP1 [4] and it is not a substrate of BAP1 [5]. However, BAP1 is part of essential cell cycle regulators[6] and probably associated with regulation of transcription [7]. BAP1 binds and deubiquitinates the transcriptional regulator host cell factor 1 (HCF-1), which interacts with histone-modifying complexes [8, 9]. Together, these data

indicate a complex mode of action for BAP1 involving different cellular pathways. It is even hypothesized that BAP1 effects can vary in different cell types and/or species [10]. BAP1 was shown to fulfill criteria of a genuine tumor suppressor gene, which presumably becomes apparent after a two-step inactivation according Knudson's two-hit model [4, 9, 11]: one allele of BAP1 being inactivated via inherited mutation (or monosomy of chromosome 3 [12]); and the remaining allele being lost by somatic BAP mutation(s) leading to biallelic inactivation [9, 13].

Sporadic BAP1 mutation have been described in uveal melanoma, cutaneous melanoma and other melanocytic tumors, renal cell carcinoma and other cancers [9]. Besides the common sporadic BAP1 mutations, germline BAP1 mutations have been detected in families with a high incidence of MPM [14]. Individuals with heterozygous BAP1 germline mutations are affected by a newfound tumor predisposition syndrome characterized by very high risk of developing MPM, uveal melanoma (UV), cutaneous melanoma, atypical melanocytic benign neoplasms [15, 16] and different cancer types, such as lung adenocarcinoma, meningioma [17], and renal cell carcinoma [18] (<http://omim.org/entry/614327>).

To date only two studies [13, 14] investigated the frequency of this germline mutation in 26 and 9 sporadic MPM patients, respectively, and found a prevalence of 8% and 0%, respectively. In this study, we present the largest screening to date of germline BAP1 mutation in sporadic MPM patients.

2. Materials and Methods

2.1. Patient blood samples

78 blood samples were obtained from patients of a multicenter clinical study (SAKK 17/04, NCT00334594, unpublished) with pathologically proven diagnosis of MPM and after written informed consent. Blood samples were obtained between 2008 and 2012. Staging was based on the IMIG classification [19] selecting the lowest stage in case of ambiguous results. In SAKK17/04 trial only patients diagnosed with stage I, II or III MPM were included. The study was approved by the Ethics Committee of the University Hospital Zürich. Clinical records were reviewed to document the patient's sex, age and stage and histology of malignant pleural mesothelioma.

Peripheral whole blood was collected by needle venipuncture in serum tubes. Total DNA was purified according to the protocol of Qiagen DNeasy[®] Blood & Tissue Kit.

2.2. PCR and Analysis

PCR of different fragments was performed for unreported samples to define optimal PCR conditions. Products were confirmed by electrophoresis on a 2% agarose gel and excised. After purification according to the Macherey-Nagel NucleoSpin[®] Gel and PCR Clean-up protocol products were send for Sanger sequencing (done by Microsynth AG, Balgach, Switzerland).

Then eleven PCR fragments that encompassed the entire BAP1 coding exons and adjacent intron regions were amplified for all samples including a positive control (cell lines H2452 and H28). Primers and conditions used are listed in Supplementary Table 1. PCR product clean up and high-throughput sequencing was done by Microsynth AG (Balgach, Switzerland).

Sequencing results were analyzed with CLC Main Workbench (Version 6.1.1, CLCbio, Aarhus, Denmark) using NCBI Sequence: NG_031859.1 as reference.

We used variation data observed in 413 exomes from the CoLaus cohort [20], which were sequenced at the Wellcome Trust Sanger Institute (WTSI) as part of a partnership between the Institute, the CoLaus principal investigators and the Quantitative Sciences dept. of GlaxoSmithKline.

3. Results

We screened peripheral blood samples of 78 sporadic MPM patients for germline BAP1 mutation. In these 78 patients there were 72 males and 6 females (*Supplementary Table II*). The median age was 61.2 years (SD \pm 6.1 years) at the date of diagnosis. The histopathology showed 69 tumors (88.5%) being epithelioid, 5 (6.4%) of sarcomatoid and 4 (5.1%) of the biphasic type. The stages at the time of diagnosis distributed as follows: 15 stage I (19.2%), 37 stage II (47.4%) and 26 stage III (33.3%). As positive control we sequenced the DNA of cell lines NCI-H2452 and NCI-H28 and confirmed the already reported missense mutation c.284C>A (p.A95D) in NCI-H2452 [2, 13] and the 23-bp deletion at intron 6/exon7 junction (c.483-19_486del) in NCI-H28 [13].

The sequencing results of germline DNA showed wild-type BAP1 sequence except for patient 76, where we found a known synonymous variant in exon 11 (rs71651686, c.1026C>T, *Fig. 1*), which does not result in a change of the encoded amino acid (p.Ser342=). This patient is still alive 24 months after diagnosis and has a tumor with epithelioid histotype (*Supplementary Table II*). He has a brother who died of leukemia. In all other samples we did not find any other alterations.

4. Discussion

The screening of 78 MPM patients revealed absence of non-synonymous germline BAP1 mutation. This observation is in contrast to the study of Testa et al.[14], where 2 individuals with non-synonymous germline BAP1 mutation out of 26 sporadic MPM patients (8%) have been observed. The two patients carrying BAP1 mutations had been previously treated for uveal melanoma (UM), while none of the remaining 24 individuals had UM in history. None of the 78 patients of our study reported UM history. However, our observations are consistent with the study of Yoshikawa et al.[13]. In that study no germline mutations were detected but the number of matching non tumoral samples available was very small (nine) to draw any definitive conclusion about the frequency of germline mutation in sporadic cancer.

Altogether, 113 MPM patients were screened until to date for germline BAP1 mutation. Collectively, the prevalence of germline BAP1 mutations in sporadic MPM patients can be considered around 1-2%.

Similar screenings have been conducted for sporadic UM, where BAP1 has a predominant role in tumor predisposition syndrome. One study revealed that 2/66 (3%) unselected UM patients harbored a germline BAP1 mutation [21]. Harbour et al. found germline mutations in the BAP1 gene in 1/57 (1.75%) of UM patients [12] and Abdel-Rahman in 1/53 (1.9%) [17]. In another study [22], the prevalence of germline BAP1 mutation was 8% (4/50) in metastatic

UM versus 0% (0/50) in non-metastatic UM. Taking into account all these results, 8/276 of sporadic UM patients had BAP1 cancer syndrome, which corresponds to a prevalence of 2.9%, similar to what is observed in MPM patients.

The prevalence of the silent mutation in the exon 11, which was detected in one patient (0.6%), is consistent with minor allele frequency observed in the Swiss normal population. Since synonymous mutations are able to cause changes in protein expression, conformation and function and are not as “silent” as supposed years ago [23], the relevance of this change for predisposition to tumor development remains to be explored. It is noteworthy that we could not find any nucleotide alterations at the position c.912, which correspond to the most frequent SNP, reported in the NCBI dbSNP database (rs201809705, c.912C>A, p.Ala304=) and again this is in line with the minor allele frequency observed in the Swiss cohort, indicating that wide variation exists, stressing the importance of control population. In previously BAP1 screening reports, SNPs were not mentioned, probably because of the supposed unimportant role of synonymous mutations. Only Aoude et al.[21] reported one synonymous variant in germline BAP1 (rs28997577, on the same exon 24 nucleotides away from the synonymous variant rs71651686) out of 66 UM patients.

5. Conclusion

Taken together, the screenings performed up to the present suggest a minor role of germline BAP1 mutation in the pathogenesis of sporadic MPM. Nevertheless, if a patient reports asbestos exposure and UM in the past, or if there is a familial predisposition syndrome, germline BAP1 testing must be considered.

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Conflict of interest statement

The authors declare no competing financial interests.

7. References

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8. Legends to the figures

Figure 1.

Electropherogram of DNA alteration in exon 11 of BAP1 gene in patient 76. The synonymous variation c.1026C>T does not lead to a change in the encoded amino acid (p.Ser342=) It is reported on Ensembl and dbSNP database (rs71651686).

Figure 1

