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Short communication

Absence of germline mutations in *BAP1* in sporadic cases of malignant mesothelioma

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ABSTRACT

Malignant mesothelioma (MM) is a uniformly fatal tumour caused predominantly by exposure to asbestos. It is not known why some exposed individuals get mesothelioma and others do not. There is some epidemiological evidence of host susceptibility. *BAP1* gene somatic mutations and allelic loss are common in mesothelioma and recently a *BAP1* cancer syndrome was described in which affected individuals and families had an increased risk of cancer of multiple types, including MM. To determine if *BAP1* mutations could underlie any of the sporadic mesothelioma cases in our cohort of patients, we performed targeted deep sequencing of the *BAP1* exome on the IonTorrent Proton sequencer in 115 unrelated MM cases. No exonic germline *BAP1* mutations of known functional significance were observed, further supporting the notion that sporadic germline *BAP1* mutations are not relevant to the genetic susceptibility of MM.

1. Introduction

Malignant mesothelioma (MM) is an aggressive, incurable tumour of serosal surfaces. Along with the principal causative agent asbestos, there is evidence of genetic involvement in MM oncogenesis, with a reported two-fold increased rate of MM in individuals with a first-degree relative with MM, after adjustment for asbestos exposure (de Klerk et al., 2013). Recently, germline mutations in BRCA1-associated protein 1 (*BAP1*) were reported in MM patients (Testa et al., 2011). The majority of MM cases with germline *BAP1* mutations arise in individuals or families with a strong history of malignancy, including uveal melanoma (Testa et al., 2011; Carbone et al., 2012; Ribeiro et al., 2013), though the original study also reported that 7.7% (2/26) of sporadic MM cases carried *BAP1* germline mutations (Testa et al., 2011). Of note these two cases were reported to have no known asbestos exposure. However, subsequent studies of nearly 200 sporadic European MM cases have not identified any relevant germline *BAP1* mutations (Betti et al., 2015; Rusch et al., 2015). The identification of genetic susceptibility markers for MM, that are independent of asbestos exposure, could impact on the screening of asbestos-exposed individuals and has the potential to affect the medico-legal responsibilities of governments and the asbestos manufacturing/production industry. Because this is an important issue we conducted the current study to determine the prevalence of germline *BAP1* mutations in our cohort of patients with MM.

2. Methods

Germline DNA was analysed from 115 unrelated predominantly European-Australian MM patients randomly selected from a previously described cohort (Cadby et al., 2013). A 3584 bp region of *BAP1* including all exons and 3′ and 5′ untranslated regions were sequenced using Ion Ampliseq (Life Technologies, Victoria, Australia) protocols and reagents on the Ion Torrent Proton sequencer using an Ion PIv2 chip (Life Technologies) as described by the manufacturer. Sequenced

Abbreviations: MM, malignant mesothelioma; *BAP1*, BRCA1 associated protein-1; *NF2*, neurofibromatosis type 2; SNP, single nucleotide polymorphism; DNA, deoxyribonucleic acid.

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reads were aligned to the hg19 reference genome using the Torrent Mapping Alignment Program. Germline DNA mutations were identified using the Torrent Suite variant caller plugin with high stringency germline parameters and annotated using Ion Reporter 4.2 (Life Technologies). All variants were assessed visually using Integrative Genomics Viewer (Thorvaldsdottir et al., 2013). In addition, germline variants were independently identified using the Genome Analysis Toolkit (McKenna et al., 2010). Single nucleotide polymorphisms (SNPs) were annotated against the current dbSNP database (dbSNP build 142), SIFT (Kumar et al., 2009) and PolyPhen-2 (Adzhubei et al., 2010) databases using ANNOVAR (Wang et al., 2010). Novel SNPs and small insertions and deletions (indels) were considered significant when located in coding regions and predicted to be deleterious by either SIFT or PolyPhen-2. This study was approved by the Ethics Committee of Sir Charles Gairdner Hospital.

3. Results

There were 115 unrelated MM cases in this study; 12 cases were female and >90% were known to have been exposed to asbestos. Sixty cases had tumours with epithelial histology, 14 biphasic, 6 sarcomatoid and 35 were of unspecified histologies or had a diagnosis made on immunocytological grounds. The overall mean age at diagnosis was 68 years (range 42–94) and median survival for the group was 15 (95%CI 11–19) months. *BAP1* exons were sequenced to a median depth of 1736x (interquartile range 566–1213). No germline *BAP1* mutations of any known functional significance were observed in the 115 MM cases. A total of 8 known SNPs were identified within the *BAP1* locus, with minor allele frequencies (MAF) ranging from 0.1 to 3.4% (Table 1). The three exonic SNPs were synonymous and of no known functional significance. A single base insertion was identified in an intronic region between exons 1 and 2 of 7 samples and was of unknown significance. The frequency of SNPs within *BAP1* (0.089%) was in accordance with the expected rate (0.1%) reported by the International HapMap Consortium (International HapMap, 2003). In addition, three SNPs were identified in the downstream region of the *BAP1* gene. All detected SNPs were in Hardy–Weinberg equilibrium. Assuming a prevalence of germline *BAP1* mutations of 7.7% in MM patients as previously described (Testa et al., 2011), the Poisson distribution probability that there were no mutations in the 115 cases was $p = 0.0001$, or $p = 10^{-10}$ if all three studies on sporadic MM were combined (Testa et al., 2011; Betti et al., 2015; Rusch et al., 2015). Conversely, the upper 95% confidence limit for zero mutations out of 115 cases was 2.6% or 1.0% with all 3 studies combined.

4. Conclusion

Using targeted resequencing, no *BAP1* germline mutations of known functional significance were identified in 115 MM cases. Variation in the sample population was consistent with the expected rate for an outbred population. These data provide support for two recent studies showing no known functionally significant *BAP1* germline mutations in 78 MM patients (Rusch et al., 2015) and 103 MM cases (Betti et al., 2015).

It is of note that the two MM cases reported by Testa and colleagues to harbour germline *BAP1* mutations also had uveal and cutaneous melanoma with no known asbestos exposure. It is possible that these two MM cases were associated with the recently described *BAP1* cancer syndrome (Testa et al., 2011; Carbone et al., 2012; Ascoli et al., 2014). The phenotype of this syndrome often includes uveal and cutaneous melanoma, and has been associated with other cancers such as melanocytic tumours (Wiesner et al., 2011) and renal cell carcinomas (Popova et al., 2013). The absence of uveal melanomas in our large population of MM patients further supports the notion that this familial *BAP1* syndrome is a rarity.

In our study we examined the prevalence of *BAP1* mutations in unrelated MM cases. In some instances DNA was available from one or more first degree relatives who also developed MM. Such families are part of our asbestos surveillance programme (de Klerk et al., 2013) and have varying degrees of asbestos exposure. There was no indication of familial *BAP1* mutations in eight families examined (data not shown), although other as yet unknown genetic susceptibility factors may be present.

Whether genetic factors may play a role in MM is unclear. Whether a germline mutation can predispose an individual to develop MM via a traditional Knudson-like two-hit carcinogenesis model (Knudson, 1971) or whether it can affect the susceptibility of the individual to the carcinogenic properties of asbestos remain to be determined. From genetically engineered mouse models of mesothelioma it is apparent that loss of the neurofibromatosis type 2 (NF2) gene and/or Ink4a/Arf and p53 is sufficient to induce MM in the absence of asbestos, or to accelerate MM development following asbestos exposure, but these genetic losses are not MM-specific and a range of different tumours can develop in these mice (Jongsma et al., 2008). In mice expressing the oncogene SV40 T antigen in the mesothelial cell compartment, MM is only observed following asbestos exposure (Robinson et al., 2006). Recently, *Bap1*$^{+/-}$ knockout mice were found to have increased susceptibility to asbestos induced MM compared to wild type litter mates (Xu et al., 2014). In conclusion, germline *BAP1* mutations are not a major predisposing factor to asbestos-induced MM in Australian population-based studies.

Table 1
SNPs identified in an unselected general population sampling of 115 individuals within *BAP1*.

Chrm 3 location	Location in *BAP1*	Mutation	Heterozygotes (no.)	Homozygotes (no.)	VAF[a]	Reference SNP	MAF[b]
52443786	Intronic (between exon 1 and 2)	Insertion	7	0	0.030		ND[c]
52440827	Intronic (between exon 8 and 9)		1	0	0.004		ND
52440418	Intronic (between exon 8 and 9)		2	0	0.009	rs139414598	0.005
52439240	Exon 11	Synonymous	2	0	0.009	rs28997577	0.005
52439216	Exon 11	Synonymous	1	0	0.004	rs71651686	0.001
52437748	Exon 13	Synonymous	1	0	0.004	rs34736117	0.016
52436917	Intronic (between exon 14 and 15)		2	0	0.009	rs146661777	ND
52435860	UTR3		12	0	0.052	rs123598	0.034

[a] VAF = variant allele frequency in the context of the sample population.
[b] MAF = minor allele frequency as reported by 1000 Genomes.
[c] ND = not described.

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Availability of sequence data

Sequence data is available through the European Nucleotide Archive under accession number PRJEB8372.

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