We discovered that germline BAP1 mutations cause a novel cancer syndrome characterized by a very high incidence of MM. In some BAP1-mutation carrying families, MM accounts for more than 50% of deaths. We hypothesize that this may be due to increased susceptibility to MM from exposure to modest amounts of asbestos that would normally not cause MM in the population at large. In order to study the mechanism(s), we assembled a unique cohort and set of reagents. We have conducted a number of in vitro and in vivo experiments and obtained quite exciting results during this year. We found that BAP1 status regulate NF-kB activity and HMGB1 release, and we also found that monoallelic BAP1 loss increases susceptibility to low doses of asbestos by using a mouse model.
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1. Introduction

Malignant mesothelioma (MM) is an aggressive tumor, which arises from the cells of the mesothelium, the protective lining that covers the lungs and many other internal organs. MM is associated with asbestos exposure. In spite of the stringent regulations that were introduced in the 1970s and 80s to limit asbestos exposure, the incidence of MM has reached 3,200 cases per year in the US in 2003 and it has remained stable since then (1), while it continues to increase worldwide. We recently discovered that germline BAP1 mutations are associated with a novel cancer syndrome characterized by MM, uveal melanoma, cutaneous melanoma, benign “melanocytic BAP1-mutated atypical intradermal tumors” (MBAITs), and possibly by other cancers (2-4). Our data were independently confirmed and expanded by several other researchers (5-9). Close to 100% of BAP1 mutation carriers in these families developed MM or other tumors. Our findings suggested to us the hypothesis that these individuals may be unusually susceptible to a modest amount of asbestos exposure that is not associated with an overall increase of MM within the general population (3, 4). In order to study the mechanism(s) by which mutated BAP1 causes MM and whether mutated BAP1 influences factors critical for MM pathogenesis, we assembled a unique cohort and set of reagents. We have conducted a number of in vitro and in vivo experiments and obtained quite exciting results during this year.

2. Keywords:
mesothelioma, BAP1, asbestos, mechanisms

3. Overall Project Summary:

(1) To assess the impact of BAP1 on the regulation of pathways critical for the response of HM cells to asbestos fibers, as proposed in Aim 1, we performed experiments using human primary mesothelial cells (HM) that are “knocked down” for BAP1 expression using specific BAP1 siRNA. We found that BAP1 status influences NF-κB activity at basal level as well as upon TNF-α treatment (Fig. 1).

![Figure 1. BAP1 silencing in HM increases NF-κB p65 nuclear levels and its translocation after TNF-α treatment.](image)

Primary human mesothelial cells (HM) were seeded in 20% FBS media and then mock- (scrambled) or siRNAs-BAP1 (siBAP1#1 and siBAP1#5) transfected for 24 hours in 1% FBS media. Subsequently, HM cells were treated with 10 ng/ml TNF-α, or vehicle as control, in 1% FBS media for 1 hour. Cell fractionation was performed to isolate nuclear and cytoplasmic fraction. Lamin B1 (nuclear extract) and α-tubulin (cytoplasmic extract) were used as loading controls. Numbers indicate relative densitometry units normalized on Lamin B1 for nuclear extract, and α-tubulin for cytoplasmic extract.
Moreover, we also found that BAP1 status influences the release of HMGB1 in HM cells upon asbestos exposure (Fig. 2).

Figure 2. BAP1 silencing prevents HMGB1 release in HM cells upon crocidolite asbestos exposure. HM were seeded in 20% FBS media and then transfected with mock- (scrambled) or siRNAs-BAP1 (siBAP1#1 and siBAP1#5) for 24 hours in 1% FBS media. Subsequently, HM cells were treated with 5 µg/cm² crocidolite, or equal amount of glass as control, in 1% FBS media for 24 hours. Total cell lysates (A) were prepared, and the conditioned cell cultures media were concentrated with an Amicon centrifugal filter (Millipore) (B). Cell lysates and conditioned media were analyzed by Western Blot. Bar plots on the right show HMGB1 relative densitometry units (conditioned media HMGB1 levels are expressed relative to the Ponceau staining bands enclosed in the dotted rectangle). (C) HMGB1 concentrations in cell conditional media measured by ELISA assay.

In the meantime, we were setting the experiments to assess the impact of BAP1 in the process of asbestos-induced HM transformation in vitro, as proposed in Aim 2. Very interesting, we observed that after silencing BAP1 in HM, the cells seem to be more resistant to asbestos induced cytotoxicity, which made us wonder whether BAP1 could regulate certain cell signaling pathways that are related to cell death. In order to understand the mechanisms, we further performed various experiments using HM and human fibroblasts that are derived from BAP1 wild type and mutant carriers, and we induced the cell death using H₂O₂, which is much easier to control the dose compared to asbestos. We obtained very novel and exciting results that we would like to report in detail. The data are listed in the third section.
(2) To assess the impact of BAP1 in the process of MM development and progression in vivo, as proposed in Aim 3, we performed animal experiment using transgenic mice model. As we know, in some of the families with hereditary BAP1 mutations, there is a high prevalence of MM compared to other cancers and MM is the cause of death in ~50% of carriers (4). The etiology of the most common malignancies found in BAP1+/− carriers, MM and melanoma, has been closely linked to environmental carcinogens. We hypothesize that BAP1 mutations increase susceptibility to modest amounts of mineral fiber exposure that are usually not associated with an overall increase of MM in the general population. Accordingly, in families that were exposed to even modest amounts of mineral fibers, MM may be the predominant malignancy, whereas in families with negligible mineral fiber exposure, other malignancies may prevail. Our hypothesis is supported by the fact that MM induction by asbestos in mice (all strains) is dose dependent and amounts of 1 mg or less do not induce MM, or do so only rarely and after a prolonged latency (5), a finding reproduced by us (Carbone et al. unpublished results). The in vivo data we obtained recently revealed that BAP1+/− mice, kindly provided by V. Dixit (6), develop MM following low dose asbestos exposure that very rarely, causes MM in BAP1+/+ mice (Fig. 3), which is exactly as we expected.

Figure 3. BAP1+/− increases susceptibility to asbestos in mice. (A) 40 BAP1+/+ (Wild Type, WT) and 20 BAP1+/− (Heterozygous, Het) mice were injected with a total of 5 mg (high) or 0.5 mg (low) of crocidolite asbestos or glass and followed for MM development. One year from the final asbestos injection, the survival of BAP1+/− mice is lower compared to wt littermates (left). This difference is highly significant for the low asbestos exposed groups (p<0.0001). (B) The incidence of MM also differed. In groups injected with the high dose of asbestos, 26.05% of BAP1+/+ (blue line) and 45.83% of BAP1+/− mice (red line) mice developed MM (p = 0.07). In the low asbestos exposed groups, 40.76% of BAP1+/− mice developed MM (magenta line), compared to 7.27% of BAP1+/+ group (green line). This difference is highly significant (p=0.0003). No MM was observed in the glass-exposed group (data not shown), indicating increased susceptibility of BAP1 mutation carriers to asbestos.

We are very excited with the results. We will further investigate the sequence of genetic alterations that lead to MM in BAP1+/− mice and study whether similar genetic alterations are found in human BAP1+/− MM, which will help us address whether and how BAP1 mutations influence susceptibility to asbestos-induced MM.

4. Key Research Accomplishments:

(I) BAP1 status influences NF-κB activity at basal level as well as upon TNF-α treatment.

(II) We found that BAP1 status influences the release of HMGB1 in HM cells upon asbestos exposure.
We found that monoallelic BAP1 loss increases susceptibility to low doses of asbestos by using a mouse model.

5. Conclusion:

We have reported that germline BAP1 mutations cause a novel cancer syndrome characterized by very high incidence of MM, uveal melanoma, and other cancers in carrier families. During this year, we investigated the BAP1 cancer syndrome with a focus on MM to elucidate the mechanism of MM pathogenesis. We found that BAP1 can regulate NF-κB activity as well as HMGB1 release. We also found that monoallelic BAP1 loss increases susceptibility to low doses of asbestos. We will further study the mechanisms responsible for the increased susceptibility of BAP1+/− mutants to low doses of asbestos, the set of genetic alterations that occur in the early stages of MM development in BAP1 mutation carriers exposed to low doses of asbestos.

6. Publications, Abstracts, and Presentations:

(I) Peer-Reviewed Scientific Journals:


(II) Invited Articles:

(III) Meeting Presentations:


7. Inventions, patents and licenses:

   PCT patent application filed 6/7/12
   US National phase patent application filed 12/3/13

   US Provisional patent applications filed 6/7/11 and 6/28/11
   PCT patent application filed 6/7/12
   US National phase patent application filed 12/3/13

8. Reportable Outcomes:

Our results support our hypothesis that BAP1 status influence HMGB1 release and NF-κB activity, and that germline monoallelic BAP1 loss increases susceptibility to low doses of asbestos. This finding represents a scientific advance in the field of MM carcinogenesis, providing a possible explanation for the increased susceptibility of BAP1 mutant individual to MM, and potentially opening new avenues for discovery of new MM treatments.

9. Other Achievements:

(I) Promotion to Associate Professor (Tenure Award in 07/01/2014). Dept. of Cancer Biology, University of Hawaii Cancer Center, University of Hawaii at Manoa, Honolulu, HI.
10. References:


11. Appendices