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14. ABSTRACT 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.						
15. SUBJECT TERMS mesothelioma, BAP1, asbestos, mechanisms						
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Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Overall Project Summary.....	4
4. Key Research Accomplishments.....	6
5. Conclusion.....	7
6. Publications, Abstracts, and Presentations.....	7
7. Inventions, Patents and Licenses.....	8
8. Reportable Outcomes.....	8
9. Other Achievements.....	8
10. References.....	9
11. Appendices.....	9

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- κ B activity at basal level as well as upon TNF- α treatment (Fig. 1).

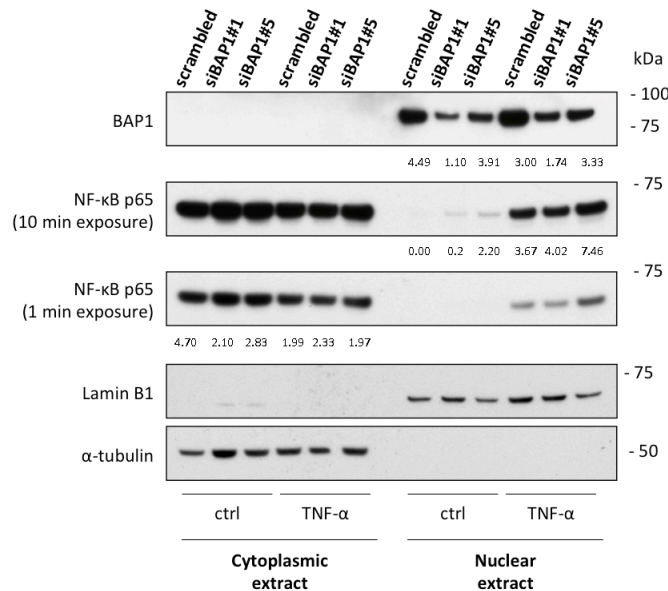


Figure 1. BAP1 silencing in HM increases NF- κ B p65 nuclear levels and its translocation after TNF- α treatment. Primary human mesothelial cells (HM) were seeded in 20% FBS media and then mock- (scrambled) or siRNAs-BAP1 (siBAP1#1 and siBAP1#5) transfected for 24 hours in 1% FBS media. Subsequently, 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).

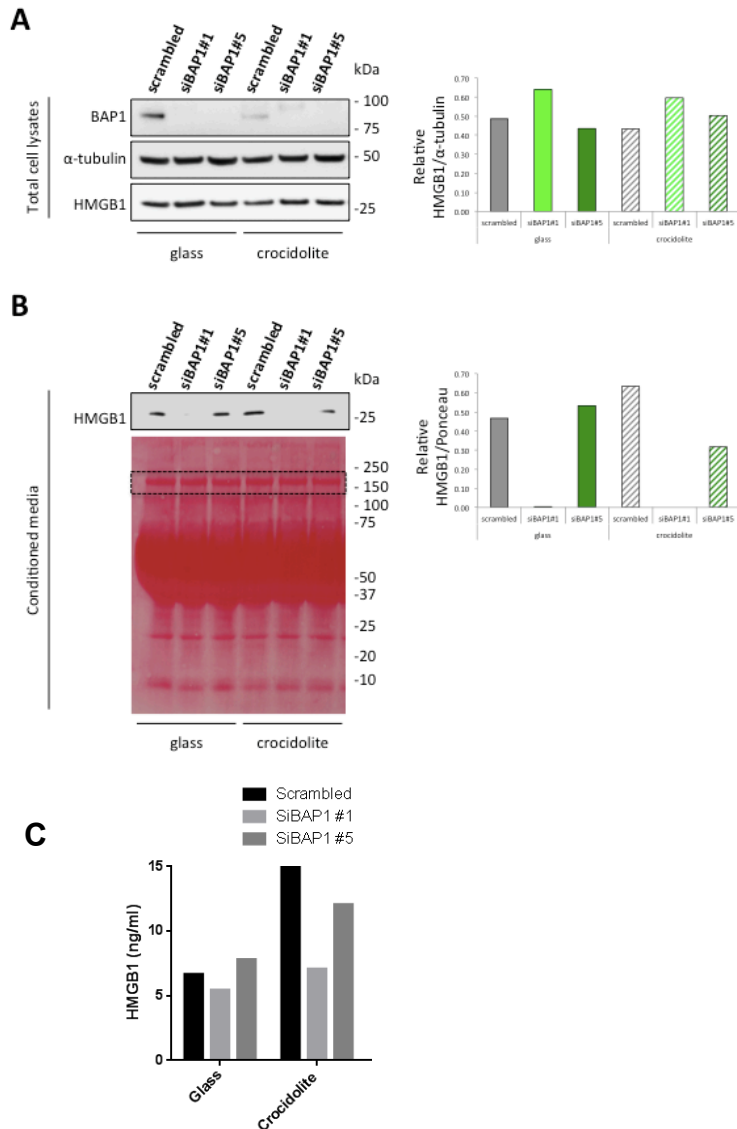


Figure 2. BAP1 silencing prevents HMGB1 release in HM cells upon crocidolite asbestos exposure. HM were seeded in 20% FBS media and then transfected with mock- (scrambled) or siRNAs-BAP1 (siBAP1#1 and siBAP1#5) for 24 hours in 1% FBS media. Subsequently, HM cells were treated with 5 $\mu\text{g}/\text{cm}^2$ 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 H_2O_2 , 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.

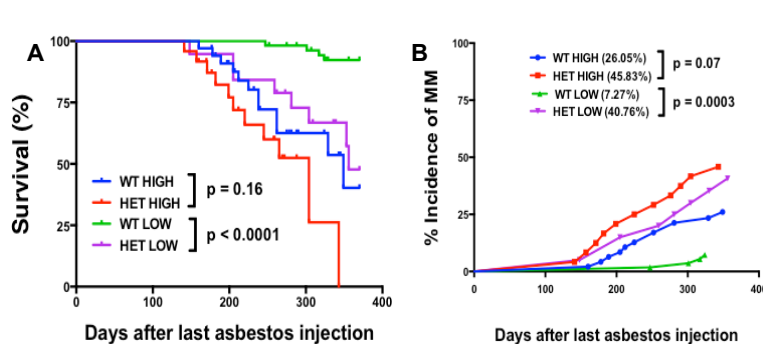


Figure 3. *BAP1*^{+/-} increases susceptibility to asbestos in mice. (A) 40 *BAP1*^{+/+} (Wild Type, WT) and 20 *BAP1*^{+/-} (Heterozygous, Het) mice were injected with a total of 5 mg (high) or 0.5 mg (low) of crocidolite asbestos or glass and followed for MM development. One year from the final asbestos 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.

- (III) 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- κ B 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:

1. Comertpay S, Pastorino S, Tanji M, Mezzapelle R, Strianese O, Napolitano A, Baumann F, Weigel T, Friedberg J, Sugarbaker P, Krausz T, Wang E, Powers A, Gaudino G, Kanodia S, Pass HI, Parsons BL, Yang H, Carbone M. Evaluation of clonal origin of malignant mesothelioma. *J Transl Med.* 2014. Dec 4;12:301. PMID: 25471750
2. Baumann F, Flores E, Napolitano A, Kanodia S, Taioli E, Pass H, Yang H, Carbone M. Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival. *Carcinogenesis.* 2015 Jan;36(1):76-81. PMID: 25380601
3. Nasu M, Emi M, Pastorino S, Tanji M, Powers A, Baumann F, Zhang Y, Gazdar A, Kanodia S, Tiirikainen M, Flores E, Gaudino G, Becich MJ, Pass HI, Yang H and Carbone M. High incidence of somatic BAP1 alterations in sporadic malignant mesothelioma. *J Thorac Oncol.* 2015 Apr;10(4):565-76. PMID: 25658628.
4. Carbone M, Gaudino G, Yang H. Recent insights emerging from malignant mesothelioma genome sequencing. *J Thorac Oncol.* 2015 Mar;10(3):409-11. PMID: 25695218
5. Yang H, Pelegri L, Napolitano A, Giorgi C, Jube S, Preti A, Jennings CJ, De Marchis F, Flores EG, Larson D, Pagano I, Tanji M, Powers A, Kanodia S, Gaudino G, Pastorino S, Pass HI, Pinton P, Bianchi ME and Carbone M. *Cell Death & Disease.* 2015 Jun 11;6:e1786. PMID: 26068794.
6. Napolitano A, Pelegri L, Anwesha D, Larson D, Tanji M, Flores EG, Kendrick B, Lapid D, Powers A, Kanodia S, Pastorino S, Pass HI, Dixit V, Yang H and Carbone M. Minimal asbestos exposure in germline BAP1 heterozygous mice is associated with deregulated inflammatory response and increased risk of mesothelioma. *Oncogene.* 2015 Jun 29. (Epub ahead of print) PMID: 26119930.

(II) Invited Articles:

1. Napolitano A, Jube S, Gaudino G, Pass HI, Carbone M and **Yang H.** Asbestos-induced chronic inflammation and cancer. In: *Cancer and Inflammation Mechanisms: Chemical,*

Biological, and Clinical Aspects. Shousuke Kawanishi, Hiroshi Ohshima and Yusuke Hiraku (Ed.) 2014. ISBN: 978-1-118-16030-5

(III) Meeting Presentations:

1. Nasu M, Napolitano A, Pastorino S, Tanji M, Flores E, Baumann F, Powers A, Gaudino G, Pass HI, **Yang H**, Carbone M. BAP1 mutation in mesothelioma and "BAP1 Cancer Syndrome". 2014 AACR Annual Meeting. April 5-9, 2014. San Diego, CA, USA.
2. Nasu M, Napolitano A, Pastorino S, Tanji M, Flores E, Baumann F, Powers A, Gaudino G, Pass HI, **Yang H**, Carbone M. BAP1 mutation in mesothelioma and "BAP1 Cancer Syndrome". Weinman Symposium, UH Cancer Center, May 2nd, 2014. Honolulu, HI, USA.

7. Inventions, patents and licenses:

1. Carbone M, **Yang H**, Pass, H. I. 2011. Biomarker of Asbestos Exposure and Mesothelioma. 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
2. Carbone M, **Yang H**, Bianchi ME. 2011. Treatment and Prevention of Cancer with HMGB1 Antagonists. 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- κ B 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:

1. Henley SJ, Larson TC, Wu M, Antao VC, Lewis M, Pinheiro GA, and Ehemann C. Mesothelioma incidence in 50 states and the District of Columbia, United States, 2003-2008. *International journal of occupational and environmental health*. 2013;19(1):1-10.
2. Carbone M, Korb Ferris L, Baumann F, Napolitano A, Lum CA, Flores EG, Gaudino G, Powers A, Bryant-Greenwood P, Krausz T, et al. BAP1 cancer syndrome: malignant mesothelioma, uveal and cutaneous melanoma, and MIB1s. *J Transl Med*. 2012;10(1):179.
3. Carbone M, Yang H, Pass HI, Krausz T, Testa JR, and Gaudino G. BAP1 and cancer. *Nature Reviews Cancer*. 2013;13(3):153-9.
4. Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, Cox NJ, Dogan AU, Pass HI, Trusa S, et al. Germline BAP1 mutations predispose to malignant mesothelioma. *Nat Genet*. 2011;43(10):1022-5.
5. Umberto S. *Mesothelioma Carcinogenesis: In Vivo Models*. Springer, New York; 2005.
6. Dey A, Seshasayee D, Noubade R, French DM, Liu J, Chaurushiya MS, Kirkpatrick DS, Pham VC, Lill JR, Bakalarski CE, et al. Loss of the tumor suppressor BAP1 causes myeloid transformation. *Science*. 2012;337(6101):1541-6.

11. Appendices