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BAP1 and Cancer

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Preface

BAP1 is a deubiquitylase that is found associated with multi-protein complexes that regulate key cellular pathways, including the cell cycle, cellular differentiation, cell death, gluconeogenesis and the DNA damage response (DDR). Recent findings indicate that germline *BAP1* mutations cause a novel cancer syndrome, characterized, at least in the affected families studied so far, by the onset at an early age of benign melanocytic skin tumours with mutated *BAP1*, and later in life by a high incidence of mesothelioma, uveal melanoma, cutaneous melanoma and possibly additional cancers.

For the past 15 years we have investigated a mesothelioma epidemic in Cappadocia, Turkey, where we discovered that, in some families, susceptibility to mesothelioma, and possibly to mineral fibre carcinogenesis, was transmitted genetically in an autosomal dominant manner[1-5]. These studies led us to search for a putative mesothelioma susceptibility gene or genes. To isolate these putative genes, we worked with two unrelated US families, referred as “L” and “W”, for their Louisiana and Wisconsin residency, that experienced a high incidence of mesothelioma and had only minimal potential exposure to asbestos[6]. Our first important clinical observation was that two members in one of these families developed uveal melanoma (UVM): one of them died of the disease and the other was treated at an early stage and cured, but subsequently developed mesothelioma[4, 6]. There are about 3000 mesotheliomas and a similar or smaller number of UVMs diagnosed annually in the US. The likelihood of both malignancies occurring in more than one individual in the same family was estimated at 36 per trillion[6]; this linkage suggested a common genetic denominator. Chromosome microarray and genetic linkage analyses on the L and W families implicated

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Competing Interests Statement. MC and JRT have a pending patent application on BAP1.

chromosome region 3p21[6], a locus frequently altered in both UVM[7] and mesotheliomas[8, 9]. Sequencing this region of chromosome 3 led to the identification of *BAP1* as the gene mutated and associated with high rates of mesothelioma in the L and W families (experiments are in progress to address whether BAP1 also causes the high incidence of mesothelioma in Cappadocia)[6].

BAP1 is a member of the ubiquitin C-terminal hydrolases (UCH) subfamily of deubiquitylating enzymes (DUBs) and was mutated in each family member who had developed mesothelioma, UVM and other cancers. None of these tumour types was detected in family members who did not carry BAP1 mutations[6]. Moreover, among several cases of sporadic mesotheliomas, we found that 2/26 patients had germline *BAP1* mutations[6] and importantly we found that both of these patients had been previously diagnosed with UVM. None of the 24 patients with sporadic mesothelioma who had wild-type germline *BAP1* had been diagnosed with UVM. On the basis of these findings, we have proposed that germline *BAP1* mutations cause a new cancer syndrome characterized by mesothelioma and UVM[6].

In this Progress article, we discuss this potential inherited cancer syndrome, the possible mechanisms through which mutations in *BAP1* might lead to tumour development and the clinical implications raised by detection of germline *BAP1* mutations.

Benign melanocytic tumours in BAP1 families

In parallel with our paper[6], Wiesner *et al.*[10] reported that germline *BAP1* mutations caused benign atypical melanocytic tumours. In a subsequent paper[11], they noted that these melanocytic lesions resembled histologically “atypical Spitz tumours (ASTs)”. ASTs are a heterogeneous group of melanocytic tumours with overlapping histological characteristics between benign Spitz nevi and malignant melanoma and are formed by large melanocytes with mitotic activity not only in the superficial but also in the deep part of the lesion. They studied 32 sporadic ASTs and found that 9 had loss of BAP1 expression and 8 of these had concomitant BRAF mutations. Only 1 of 23 ASTs that expressed BAP1 had a BRAF mutation ($P < 0.0001$) [11]. *BRAF* mutations are common in melanocytic nevi (80%) and in cutaneous melanoma (65%) but are rare in ASTs[12]. Njauw *et al.*[13] noted the same type of skin tumours arising in families with germline *BAP1* mutations and referred to them as nevoid melanoma-like melanocytic proliferations (NEMMPs). Thus, pathologists have used different names to identify these pink to tan skin tumors of about 0.2-1.0 cm in diameter, which macroscopically resemble dermal nevi. Together with a team of dermatologists, pathologists and dermatopathologists at 3 different US National Cancer Institute (NCI)-designated Cancer Centers we reviewed the published morphology and histology of all of these melanocytic tumours, including some of the original tissue sections kindly provided by Weisner and colleagues[10]. In addition, we examined the skin tumours we detected in the members of the L and W families. We found that all the melanocytic tumors in these *BAP1* mutant patients had the same histological and molecular characteristics. In our review of the skin tumours in patients with mutant *BAP1* we found that these are nevus-like lesions usually formed by a conventional junctional, compound or dermal nevus composed of small melanocytes expressing BAP1. Next to these conventional nevus cells there is a dermal lesion formed by large epithelioid BAP1 negative and most frequently BRAF mutated melanocytes with virtually no mitotic activity. This intradermal component with these unique features and the nearby conventional nevus-like lesion, to our minds, clearly sets these tumours apart from ASTs and other melanocytic lesions. Acknowledging that these lesions are characteristic of, although possibly not unique to, *BAP1* mutation carriers, we proposed naming them melanocytic *BAP1*-mutated atypical intradermal tumours (MBAITs)[12].

Malignant tumours in BAP1 families

Wiesner and colleagues noted that in the families that they studied there was a possible increased risk of cutaneous melanoma and UVM[10] and subsequently they confirmed the association with mesothelioma in 2 out of the 3 European families they studied[14]. Such cancer clustering has been noted in other studies. A 1972 case report described an individual who developed UVM, mesothelioma and meningioma[15]. Harbour *et al.*[7] reported inactivating mutations of *BAP1* in 26 of 31 aggressive (class 2) UVMs, among which a germline mutation was found in 1 of 26 patients.

Abdel-Rahman *et al.*[16] investigated *BAP1* status in 53 unrelated patients with UVM who had a high risk of hereditary cancer. One of the 53 patients had a germline truncating *BAP1* mutation that segregated in other family members. In this family, there were 7 *BAP1* mutation carriers (5 tested and two inferred) and only one was cancer free at age 55; the other six had developed UVM (2 cases), cutaneous melanoma, mesothelioma, meningioma, lung and other types of carcinoma. The two patients treated for UVM subsequently developed a second malignancy[16]. Njauw *et al.*[13] sequenced *BAP1* from 50 patients with metastatic UVM and 50 patients with non-metastatic UVM. *BAP1* was mutated in the germline of 8% of patients with metastatic UVM: none of the patients with non-metastatic UVM had *BAP1* germline mutations. Also, they found that 2 of 7 probands from families with UVM-cutaneous melanoma carried germline *BAP1* mutations, compared to 1 of 193 probands from families with a history of cutaneous melanoma and no UVMs (p=0.003). Aoude *et al.*[17] reported that 2 of 66 (3%) patients with UVM unselected for family history carried germline *BAP1* mutations. Additional tumour types, including renal, breast and lung carcinomas were also found among *BAP1* mutation carriers[6, 13, 16]. Very recently, *BAP1* mutations have been reported in sporadic renal carcinoma[18]. Moreover, a novel inactivating germline splice mutation was reported in a family with UVM and cutaneous melanoma. Members of this family were also affected by multiple myeloma, paraganglioma and breast cancer[19].

Table 1 summarizes the reports on families with *BAP1* mutations as of January 17, 2013. To date, 53 of 76 *BAP1* germline mutation carriers have developed malignancies; 13 of these 53 developed two or more cancers. Table 1 and our meta-analysis[12], indicate that mesothelioma, UVMs and cutaneous melanomas are clearly associated with the putative BAP1 cancer syndrome (Box 1). In addition, 35 *BAP1* mutation carriers were investigated for the lesions that we call MBAITs, and 19 of them were found to have one or more (Table 1). These benign skin lesions may occasionally progress to cutaneous melanoma[14].

In the *BAP1* mutation families, there is an exceptionally high incidence of malignancy overall (69.74% cancer incidence among 76 mutation carriers). Some of these malignancies developed at an earlier age than observed in the general population (Table 1), as expected in familial cancer syndromes. All of the mutation carriers unaffected by cancer are younger than 55 years old and may develop malignancies later in life. However, the relatively high frequency in the general population of the carcinomas that develop in *BAP1* mutation carriers does not allow us yet to distinguish with certainty if the association with carcinomas is causal. It should also be noted that population-based studies have shown that mutation carriers in the general population may have a lower risk than suggested by estimates obtained from families with multiple tumours, as for example observed for *CDKN2A* mutations[20]. Therefore, large population based studies will be required to validate the association of germline *BAP1* mutations with the exceptionally high incidence of malignancies detected in the *BAP1* mutant families.

Most autosomal dominant cancer syndromes cause tumours in specific sites and tissues, and do not result in an overall increase in cancer risk. However, the Li-Fraumeni syndrome, caused by inherited mutations of the *TP53* tumour suppressor gene, is an autosomal dominant cancer syndrome characteristically associated with the development of multiple tumour types [21]. Is the BAP1 cancer syndrome, similarly to the Li-Fraumeni syndrome, associated with an overall general increase of cancer risk? If so, why do mesotheliomas, UVMs, cutaneous melanomas predominate? As we expand our studies to include additional families that have mutant *BAP1* genes we will address these questions. In the section below we discuss the putative functions of BAP1 and how they might contribute to cancer. Most data about BAP1 function were published in the past 2 years, following a renewed interest in this gene caused by the discovery of its association with human mesothelioma and melanomas, and possibly with other cancer types. Therefore, some of the most recent studies have still to be independently verified. Overall the data indicate that BAP1 can influence multiple cellular pathways, at times suppressing and at times promoting cell growth, possibly in a cell type and species specific fashion.

BAP1 has multifaceted functions

Protein ubiquitylation was initially seen as a mechanism to label proteins for degradation; however, this notion has evolved as we have come to understand that ubiquitylation and DUBs regulate various cellular processes, including DNA repair, gene transcription, cell membrane trafficking, cell cycle progression, stress response, cell communication, differentiation and apoptosis, and that they have a role in cancer development[22, 23].

The *BAP1* gene is located at chromosome region 3p21.1[24], a genomic region that is deleted in several human malignancies, including approximately 30-60% of mesotheliomas [6, 8, 9, 25] and 85% of metastasizing UVMs[10]. BAP1 was discovered in a yeast two-hybrid screen owing to its interaction with the RING finger domain of the tumour suppressor BRCA1. Initial data suggested that BAP1 was a tumour suppressor gene because BAP1 suppressed the growth of MCF7 human breast cancer cells in soft agar[24]. However, despite the fact that auto-ubiquitylated BRCA1 is a DUB substrate, subsequent experiments have shown that BAP1 does not influence the deubiquitylation of BRCA1[26]. BRCA1 forms a heterodimer through its RING domain with BRCA1 associated RING domain 1 (BARD1), and this BRCA1–BARD1 tumour-suppressor complex has E3 ubiquitin ligase activity that regulates the DNA damage response (DDR)[27]. BAP1 binds and deubiquitylates BARD1, thus modulating the E3 ligase activity of BRCA1–BARD1[28]. Indeed, inhibition of BAP1 by short hairpin RNAs (shRNAs) impaired the DDR and caused HeLa cells to become hypersensitive to ionizing radiation resulting in S-phase retardation, a phenotype similar to BRCA1 deficiency. Therefore, BRCA1-mediated ubiquitylation and BAP1-mediated deubiquitylation may coordinately regulate these cellular processes[28].

Ventii *et al.* reported that in human NCI-H226 non-small cell lung cancer cells that do not express BAP1, its exogenous expression through lentivirus infection accelerated passage through the G1/S checkpoint. This effect might have impaired DNA repair leading to DNA damage and in the induction of cell death both by apoptosis and necrosis[29]. Accordingly, NCI-H226 cells expressing exogenous wild-type BAP1 grew poorly in cell culture and when injected into athymic nude mice, they formed tumours that were about 10-15-fold smaller than those obtained with cells infected with vector alone or expressing BAP1 mutants lacking deubiquitylating activity (C91A substitution) or the second nuclear localization signal, NLS2, that is required for the nuclear compartmentalization of BAP1[29]. BAP1-mediated growth suppression in this *in vivo* model was BRCA1-independent. These data were interpreted as further evidence that *BAP1* is a tumour suppressor gene, and that both

nuclear localization and deubiquitylating activity are required for BAP1-mediated tumour suppressor activity[29].

When in the nucleus, BAP1 interacts with several proteins including host cell factor 1 (HCF1; also known as HCFC1). HCF1 is a cell cycle regulator that contains a Kelch domain that binds to a conserved peptide sequence known as the HCF1 binding motif (HBM) common to several transcription factors. Although HCF1 does not bind DNA directly, transcription factors that contain a HBM domain recruit HCF1 to specific promoters, where it regulates transcription. HCF1 binds BAP1 through a HBM sequence present in the middle portion of BAP1[30, 31]. HCF1 is ubiquitylated on lysine residues, preferentially through K48-linked and K63-linked chains. BAP1 hydrolyzes K48-linked chains[30, 31]. Overexpression of mutated, catalytically inactive BAP1[30] and BAP1 silencing[31] resulted in a modest accumulation of HCF1, indicating that HCF1 K48 ubiquitylation does not necessarily label HCF1 for degradation.

A critical role of HCF1 is to sustain the formation of complexes between chromatin modifying enzymes and transcription factors. For example, by recruiting histone methyltransferases to the E2F1 transcription factor, HCF1 allows the transcription of E2F1 target genes, including genes that control the cell cycle and cellular proliferation[32]. It has been proposed that ubiquitylation of HCF1 blocks E2F-responsive promoter activity, and that HCF1 deubiquitylation by BAP1 would remove this inhibition and promote cell proliferation[33]. In addition, co-immunoprecipitation experiments have shown that BAP1 directly associates with E2F family members, suggesting a possible additional direct control of these transcriptional factors by BAP1[33]. Therefore, the ability of BAP1 to influence progression through the cell cycle may be mediated, at least in part, by E2F family members[33].

The *Drosophila* Polycomb group (PcG) gene *calypso* is homologous to the human *BAP1* gene. PcG proteins are transcriptional repressors that control cellular differentiation and development[34]. These proteins form multiprotein complexes that bind to chromatin and together repress gene expression. The two main families of PcG protein complexes are Polycomb repressive complex 1 (PRC1) and PRC2. Compaction of nucleosomes and ubiquitylation of histone 2A (H2A) are two of the mechanisms through which PRC1 silences gene expression[35]. Mono-ubiquitylation of H2A is a critical mechanism in the control of transcription initiation and elongation, transcription silencing and DNA repair[36]. In *Drosophila*, Calypso binds additional sex combs (ASX) to form the Polycomb repressive deubiquitinase (PR-DUB) complex that specifically removes mono-ubiquitin from H2A. Similarly, in humans BAP1 binds ASXL1 through its carboxy-terminus and deubiquitylates H2A[34]. It appears possible that PR-DUB may help fine-tune gene expression levels by preventing the hyperubiquitylation of H2A by PRC1 and BRCA1. BRCA1 maintains the ubiquitylated status of H2A, a mechanism that is critical for BRCA1 tumour suppressor activity because it antagonizes the transcriptional de-repression of satellite DNA repeats that contribute to breast cancer growth[37].

Thus, PcG activity may not simply be ascribed to its gene suppressive functions, but may rather result from the delicate balance between H2A ubiquitylation by PRC1 and BRCA1 (which suppress transcription) and H2A deubiquitylation by PR-DUB (which should re-activate transcription). PRC2 might also be important in tumours that have mutant *BAP1*. The PRC2 proteins EZH2 and EED are frequently overexpressed in mesothelioma and Kemp *et al.* proposed inhibiting PRC2 as a possible therapeutic strategy for treating mesothelioma[38].

Yu *et al.*[39] reported that almost all cellular BAP1 is found in high-molecular weight multi-protein complexes, which include HCF1, ASXL1 and ASXL2, O-linked N-acetylglucosamine transferase (OGT) and the forkhead transcription factors FOXK1 and FOXK2, in what they called “the BAP1 core complex” (Figure 1a). This core complex, in turn, associates with additional regulators and transcription factors to form specific functional complexes that may be cell type specific[39]. In addition, recent studies have shown that BAP1, HCF-1 and OGT (which is essential for Polycomb mediate gene repression in Drosophila[40]) exist as a multiprotein complex that regulates itself and also regulates several targets (Figure 1b). For example, BAP1 deubiquitylates and thus stabilizes OGT, which O-GlcNacylates HCF1 and activates it[41, 42]. Activated HCF1 recruits OGT to O-GlcNacylate peroxisome proliferator activator receptor γ coactivator 1α (PGC-1α. This modification enables BAP1 to bind and deubiquitylate PGC-1α which is stabilized and can promote gluconeogenesis[42] (Figure 1b). Moreover, chromatin immunoprecipitation (ChIP) assays identified a total of 9128 BAP1 peaks (5926 located near the transcription start sites of 5731 genes); 85% of promoters occupied by BAP1 also contained HCF1, and BAP1, HCF-1 and OGT were recruited as a complex on 1827 different promoters[41].

BAP1 has also been shown to form a ternary complex with HCF1 and the transcription factor Yin Yang 1 (YY1) that controls the transcription of genes involved in cell proliferation. Specifically, BAP1 interacts with the zinc fingers of YY1 through its coiled-coil motif and it is recruited together with HCF1 to the promoter of the *COX7C* gene that encodes a component of the mitochondrial respiratory chain[39].

Thus, it can be expected that BAP1 influences a wide array of cellular functions, and consistent with this hypothesis, BAP1 depletion by RNA inhibition induced significant changes in the expression of many genes that control various cellular pathways[39].

In summary, despite the findings implicating BAP1 in suppression of proliferation[24, 29], some groups have reported that BAP1 promotes cell proliferation[25, 30, 33]. These conflicting results suggest that BAP1 effects may be cell-type specific and/or that they could be influenced by the experimental approach used. BAP1 activity could vary in different cells because the BAP1 core complex[39] is likely to associate with different transcription factors and thus influence different pathways in a cell-type specific manner. Also, some of the proteins that normally bind BAP1 may have different activities in its absence. For example, YY1 acts as a repressor or an activator of *COX7C* depending on whether it is bound to the BAP1–HCF1 complex[39].

All the germline mutations found in the BAP1 cancer syndrome encode BAP1 proteins lacking the nuclear localization sequence and most of them have an intact UCH domain (Figure 2). In the tumours that develop in *BAP1* mutant carriers, BAP1 expression is either absent because of loss of heterozygosity (LOH), resulting in biallelic inactivation, or the BAP1 protein is localized in the cytoplasm[6, 16] where it may retain DUB activity, with as yet unknown possible effects on the cellular ‘ubiquitinome’.

What do BAP1 associated cancers have in common?

Because germline *BAP1* mutations are associated with mesotheliomas, UVMs and cutaneous melanomas, there should be some common pathways controlled by BAP1 that are of particular importance to the development of these malignancies. The finding that somatic *BAP1* mutations are common in these cancers supports this interpretation.

Somatic *BAP1* mutations were found in 84% of metastasizing UVM biopsies[7] and in 22%[6] and 23%[25] of sporadic US mesothelioma biopsies. Instead, Yoshikawa *et al.* found somatic inactivating *BAP1* mutations in 61% of Japanese mesothelioma biopsies[43]. To address this

discrepancy, in collaboration with our Japanese colleagues we are currently investigating whether the higher incidence of *BAP1* mutations that they detected was caused by ethnic differences or by the different technical approach used, that is multiplex ligation-dependent probe amplification (MLPA)[43] compared with PCR[6].

Studies in mice[41] revealed that *Bap1* deletion is lethal during embryogenesis. When *Bap1* was deleted in adult mice that expressed the tamoxifen-inducible recombinase cre–ERT2 ubiquitously and had *Bap1* exons 4 and 5 flanked by lox sites, these mice developed splenomegaly caused by extramedullary hematopoiesis, monocytosis, neutrophilia, and progressive anemia[41], findings comparable to the human myelodysplastic syndrome (MDS). The absence of mesothelioma, uveal and cutaneous melanoma in these mice is somewhat puzzling, and may be related to species differences. In addition, at the level of disease causation, the common denominator among mesotheliomas and melanomas is a well-defined role of environmental carcinogens. We are presently testing the hypothesis that germline *Bap1* mutations increase the susceptibility to mineral fibre-induced and UV light-induced carcinogenesis; this may explain the absence of mesothelioma and melanoma in mice that were not exposed to these carcinogens. Mineral fibres are well-documented causes of mesothelioma[1-5]. Deposition of these fibres in tissues induces a chronic inflammatory reaction that is started by the release of high mobility group box 1 (HMGB1) by mesothelial cells undergoing programmed cell necrosis[2, 44]. Extracellular HMGB1 starts the inflammatory process by recruiting macrophages and other immune cells that in turn actively secrete HMGB1 and other cytokines, and release mutagenic oxygen and nitric radicals, promoting carcinogenesis and mesothelioma growth (reviewed in[2]). In such an environment, an impaired DDR caused by *BAP1* deletion, might be expected to favour the accumulation of genetic damage that eventually gives rise to a malignant clone. However, Bott *et al.*[25] found no consistent differences in RAD51 or BRCA1 complex formation between *BAP1* wild-type and *BAP1* deficient cell lines exposed to ionizing radiation, suggesting no critical role for BAP1 in the formation of these DNA repair complexes. Moreover, we[6] found very minimal and Wiesner et al[14], found no evidence of exposure to asbestos or erionite among the patients who developed mesothelioma in the families with BAP1 mutations, findings that do not support gene-environment interaction in causing mesothelioma, at least in these families. A separate mechanism, by which HMGB1 might influence mesothelioma especially in cells lacking *BAP1*, is the capacity of HMGB1 to induce nucleosome assembly[45]. High levels of HMGB1 may increase gene repression by PRC1 in a mutated *BAP1* background.

Ultraviolet (UV) light exposure increases the risk of cutaneous melanoma. The role of UV light exposure in causing UVM is still debated[46] however, it seems possible that *BAP1* mutation carriers are at an increased risk because of impaired DDR. Because *BAP1* mutation carriers represent only a fraction of patients who develop UVM (3-4%)[13, 17], the overall association of UVM with UV light exposure should be re-evaluated in *BAP1* mutations positive versus *BAP1* mutation negative patients.

Moreover, BAP1 is a UV-inducible substrate of the ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) kinases. ATM and ATR regulate the DNA damage checkpoint that inhibits cell cycle progression in response to genomic insults – such as UV – to allow time to damage repair or, if the damage cannot be repaired, to enter apoptosis[47].

We anticipate that as more studies uncover the precise mechanisms by which *BAP1* mutations cause mesothelioma and melanomas, specific preventive and therapeutic approaches for these malignancies will be developed (Box 1). It is worth noting that a recent paper showed that histone deacetylase inhibitors (HDACIs) impair the *in vitro* and *in vivo*

growth of UVM metastatic tumours with *BAP1* inactivating mutations[48]. Specifically, HDAC inhibitors block H2A ubiquitylation through inhibition of PRC1 and so reverse H2A hyperubiquitylation caused by BAP1 loss. HDACIs also induce differentiation of Class II UVM to Class I UVM[48], which is less aggressive.

Conclusions

The apparent ability of *BAP1* mutations to cause multiple tumour types and the high tumour phenotype penetrance (Table 1) suggests that this gene has a major role in influencing cancer cell growth. The pleiotropic effects of BAP1 can account for this finding. Not only is *BAP1* the human homologue of the *Drosophila* PcG gene *calypso*, but it also contains a HBM domain, which is absent in Calypso, that allows BAP1 to bind HCF1, a chromatin associated protein that similar to PcG regulates the expression of a plethora of different genes. Thus, by regulating the activities of PcG and HCF1 target genes, BAP1 has a central role in regulating gene expression in mammalian cells.

The clinical implications of these recent findings have yet to be fully established[12]. The presence of specific skin lesions that we have called MBAITs is an important clinical feature that might help to identify *BAP1* mutation carriers. We think that these patients require close monitoring for early detection and curative resection of uveal and cutaneous melanoma, and of mesothelioma and other malignancies.

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Glossary

Uveal melanoma [UVM]	Uveal melanomas are the most common primary intraocular malignancy and they account for about 13% of melanoma deaths.
Proband	Individual that, by seeking medical/scientific attention, allows the detection of a genetic disorder in a family.
Autosomal dominant	A genetic disease inherited as a result of having a single copy of the mutated gene, located on one of the 22 non-sex chromosomes.
Asbestos	This term identifies 6 different fibrous minerals – among about 400 present in nature – that were used commercially. Exposure to asbestos as well as to other mineral fibres, such as erionite, can cause mesothelioma.

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Box 1 Clinical considerations

Wiesner et al[10] noted that in *BAP1* mutation carriers benign melanocytic tumors develop during the second decade of life and their number increases with age, a finding confirmed in the *BAP1* mutant families we are studying[12]. The occurrence of cutaneous melanoma in some *BAP1* mutation carriers, including one that appeared to originate from these melanoctytic tumors[14], suggests that occasionally these benign tumors may undergo malignant transformation. This finding may account, at least in part, for the overall high risk of cutaneous melanoma detected among BAP1 germline mutation carriers (Table 1). We propose that these melanocytic tumors that for the reasons described in the text and in ref. [12] we term MBAITs, could be viewed as a specific phenotypic marker of *BAP1* mutation carriers. Other autosomal dominant cancer syndromes also have phenotypic markers, such as adenomas in familial adenomatous polyposis families and Lisch nodules and café-au-lait spots in families with neurofibromatosis type 1. Suspected MBAITs could be removed and tested to identify patients with BAP1 mutations. If this approach was found to have clinical relevance it could allow for the close monitoring of these individuals and enable early detection of cutaneous melanoma, uveal melanoma (UVM), mesothelioma and possibly other cancers[12]. Both cutaneous melanoma and UVM can be cured when detected at an early stage and are fatal when they have metastasized. Early diagnosis also benefits mesothelioma patients because when they are diagnosed and treated at a very early stage, survival for 5 or more years is not uncommon[12, 49]. Thus, the detection of families with inherited mutations of *BAP1* offers opportunities for early cancer detection and more effective therapeutic approaches.

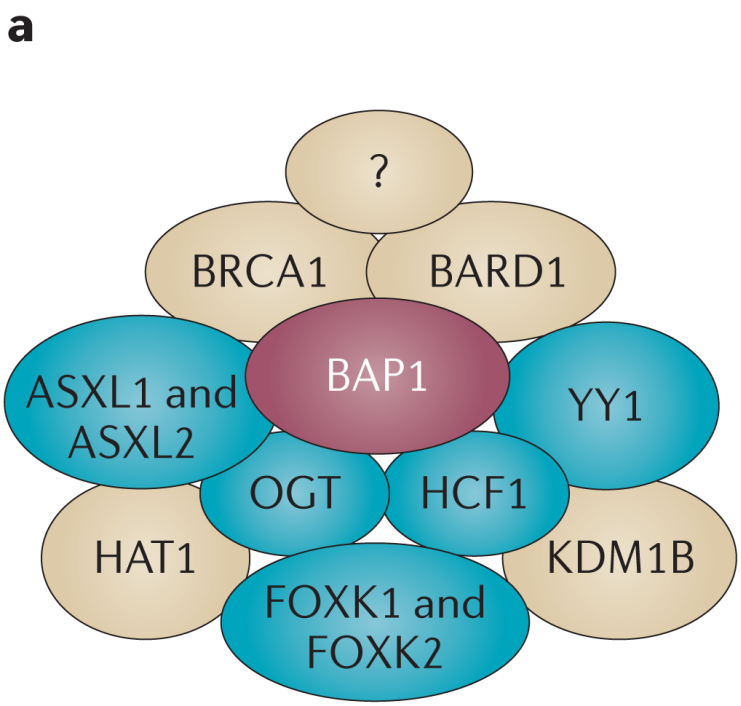

Figure 1a. BAP1 protein partners

Putative BAP1 protein partners, identified by co-immunoprecipitation and/or affinity-capture mass spectrometry. Proteins forming the putative BAP1 core complex are shown in blue[39]. Other putative BAP1 partners of hypothetical new complexes are shown in beige[28, 39, 41]. Protein partners shown may vary in different cell types and under different conditions.

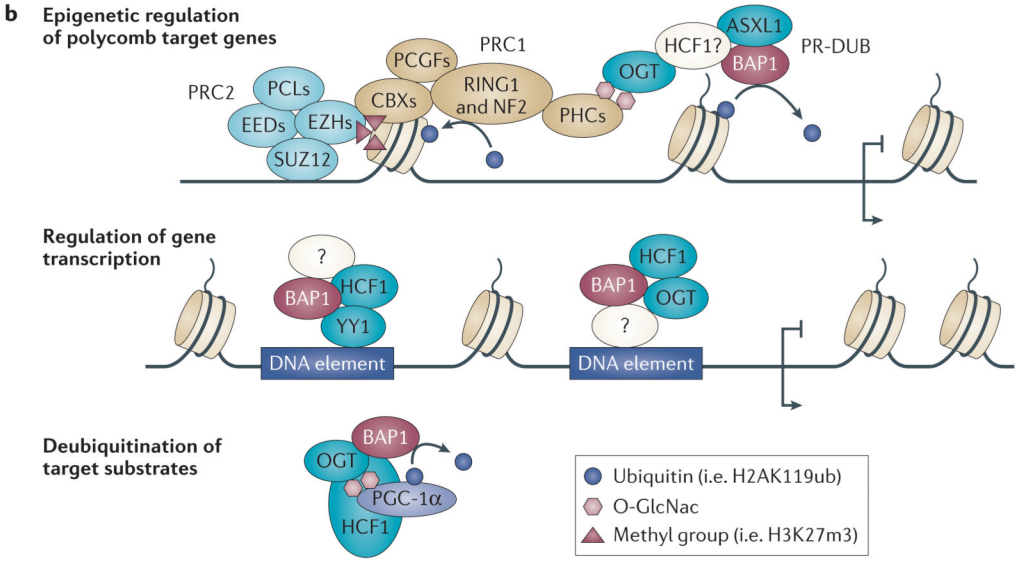

Figure 1b. Possible mechanisms of BAP1 function

I) Epigenetic regulation of Polycomb target genes is achieved via the cooperation of multiple protein complexes. The signature of gene silencing mediated by PRC2 is the trimethylation of H3K27. PRC1 can be recruited to H3K27me3 sites and contributes to gene silencing via monoubiquitylation of H2AK119[35]. The BAP1-ASXL1 complex (PR-DUB) has H2A-K119ub deubiquitylase activity opposite to PRC1[34]. OGT contributes to the fine tuning of these epigenetic marks via O-GlcNacylation of PHCs[40]. Hypothesis: PR-DUB might be recruited to Polycomb target genes because of the interaction between BAP1, HCF1 and OGT.

II) BAP1 regulates gene transcription via association with other protein partners, e.g. HCF1 and YY1[39] or HCF1 and OGT[41]. Other putative BAP1 partners are shown in grey. Hypothesis: BAP1, HCF1, YY1 and OGT might also be part of one large protein complex.

III) BAP1-HCF1-OGT complex increases the stability of PGC-1α, regulating gluconeogenesis and possibly mitochondrial biogenesis[42].

ASXL1/2, Additional sex combs like 1/2; BAP1, BRCA1-assiciated protein 1; BARD1, BRCA1-associated RING domain protein 1; BRCA1, breast cancer type 1 susceptibility protein; CBXs, mammalian chromobox protein homologues; EZHs, enhancer of zeste homologs; FOXK1/2, Forkhead box protein K1/2; HAT1, Histone acetyltransferase 1; HCF1, Host cell factor 1; KDM1B, lysine (K)-specific demethylase 1B; OGT, UDP-glucose-dependent O-glucosyltransferase; PCLs, Polycomb Like proteins; PGC-1α, Peroxisome proliferator-activated receptor gamma coactivator 1α; PHCs, Polyhomeotic-like proteins; PRC1, Polycomb repressive complex 1; PRC2, Polycomb repressive complex 2; PR-DUB, Polycomb repressive deubiquitinase; RING1, Really interesting new gene 1; RNF2, RING finger protein 2; YY1, Ying-Yang 1.

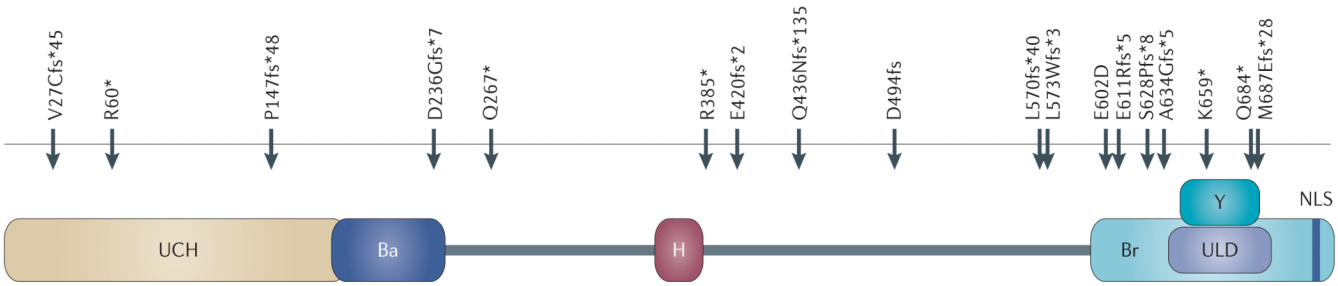

Figure 2. Schematic representation of BAP1 domains and locations of the reported BAP1 germline mutations

All the germline BAP1 mutations found in families with UVM and mesothelioma encode BAP1 proteins lacking the nuclear localization sequence. Most of them have an intact ubiquitin carboxyl-terminal hydrolase (UCH) domain[6, 7, 10, 13, 14, 16, 17, 19].

Ba, BARD1 binding domain; H, HCF-1 binding domain; Br, BRCA1 binding domain; Y, YY1 binding domain; ULD, UCH37-like domain; NLS, nuclear localization signal; Red arrows, germline mutations.

Table 1

Description of tumors rates and age at diagnosis in the *BAP1*-mutated cohort and 2005-2009 US cancer incidence and median age at diagnosis (*source: SEER*)

					Age at diagnosis			US statistics	
Tumor	**N total**	**N cases**	**Ref.**	**% (*95%CI*[*])**	**N age known**	**Range (years)**	**Mean (s.d.) (years)**	**Incidence per 100,000 p.yr**	**Median age (years)**
Malignant mesothelioma	76	22	6,10,14,16,19	28.95 (*19.96-39.96*)	22	37-85	55.2 (15.5)	0.1-1	74
Uveal melanoma	76	21	6,7,10,12,16,19	27.63 (*18.84-38.37*)	20	18-72	52.8 (12.8)	0.6	56
Cutaneous melanoma	76	11	10,12,16,19	14.47 (*7.79-24.84*)	10	21-57	41.7 (10.0)	21.9	61
Lung cancer	76	5	12,16,19	6.58 (*2.45-15.34*)	4	46-57	52.0 (5.35)	64.5	70
Breast cancer	43	4	6,12,19	9.30 (*3.68-21.16*)	3	37-75	51.7 (20.4)	124.3	61
Ovarian cancer	43	2	6,16	4.65 (*1.28-15.45*)	2	59-69	64.0 (7.1)	12.7	63
Renal carcinoma	76	2	6,12	2.63 (*0.72-9.09*)	2	46-57	51.5 (7.8)	15.1	64
Non-melanoma skin c.	76	2	6	2.63 (*0.72-9.09*)	0	-	-	-	-
Meningioma	76	1	16		1		59	3	-
Cholangiocarcinoma	76	1	12		1		47	<1	8th decade
Leiomyosarcoma	76	1	6		1		33	<1	5th decade
Neuroendocrine tumors	76	1	16		1		52	4.4 to 6.5	-
Pancreatic cancer	76	1	6		1		72	12.3	71
Paraganglioma	76	1	19		1		42	0.2 to 0.8	3rd to 5th decade
Malignant Fibrous Histiocytoma	76	1	19		1		45	3	6^{rth} to 7th decade
Cancer non specified	76	1	16						
Patients with at least 1 malignancy	76	53	6,7,10,12,14,16,	69.74 (*57.99-79.48*)	47	18-85	51.7 (14.1)	477	66
Patients with 2 or more malignancies	76	13[**]	19	17.11 (*10.28-27.10*)				8% of all cancer survivors	
MBAITs	35	19	10,12	54.29 (*38.19-70.78*)					

Abbreviations: N: number; 95% CI: 95% confidence interval; s.d.: standard deviation; p.yr: person year; - data not available.

Criteria of inclusion: Published data of individuals tested genetically and found to carry *BAP1* germline mutations.

[*] The confidence intervals were calculated using the Wilson method, without correction for continuity.

[**] 13 persons among the 53 with malignancies had 2 or more malignant tumors; that is 24.53%, 95%CI: (14.94%-37.57%), compared with 8% in US.