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Biological Mechanisms and Clinical Significance of *BAP1* Mutations in Human Cancer

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Abstract

Among more than 200 *BAP1*-mutant families affected by the "BAP1 cancer syndrome," nearly all individuals inheriting a *BAP1* mutant allele developed one or more malignancies during their lifetime, mostly uveal and cutaneous melanoma, mesothelioma, and clear-cell renal cell carcinoma. These cancer types are also those that, when they occur sporadically, are more likely to carry somatic biallelic *BAP1* mutations. Mechanistic studies revealed that the tumor suppressor function of BAP1 is linked to its dual activity in the nucleus, where it is implicated in a variety of processes including DNA repair and transcription, and in the cytoplasm, where it regulates cell death and mitochondrial metabolism. BAP1 activity in tumor suppression is cell type– and context-dependent. BAP1 has emerged as a critical tumor suppressor across multiple cancer types, predisposing to tumor development when mutated in the germline as well as somatically. Moreover, *BAP1* has emerged as a key regulator of gene–environment interaction.

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Disclosure of Potential Conflicts of Interest

M. Carbone has a patent issued for "Methods for Diagnosing a Predisposition to Develop Cancer." M. Carbone and H. Yang have a patent issued for "Using Anti-HMGB1 Monoclonal Antibody or Other HMGB1 Antibodies as a Novel Mesothelioma Therapeutic Strategy," and a patent issued for "HMGB1 as a Biomarker for Asbestos Exposure and Mesothelioma Early Detection." J.W. Harbour has a patent issued for "Method for predicting risk of metastasis" and for "Compositions and methods for detecting cancer metastasis"; has been a paid consultant for Castle Biosciences, licensee of this intellectual property; and is a consultant/advisory board member for Aura Biosciences, TD2, Castle Biosciences, and Immunocore. No potential conflicts of interest were disclosed by the other authors.

INTRODUCTION

BAP1 is a ubiquitin carboxy-terminal hydrolase (UCH), a member of the deubiquitylase (DUB) family of proteins. BAP1 was identified and named in 1998 as a nuclear protein that supposedly bound to the RING finger domain of the BRCA1 protein (1). However, the extent and significance of this interaction has been called into question. Subsequent studies suggested that BAP1 may not bind directly to BRCA1 but to BARD1 instead, thereby indirectly influencing BRCA1 activity through disruption of the BRCA1–BARD1 complex, thus downmodulating the E3 ligase function of BRCA1 (2). However, a recent inter-actome study based on an affinity purification mass spectrometry approach identified neither BRCA1 nor BARD1 in association with BAP1 and the human polycomb complex (3). Therefore, the association of BAP1 with BRCA1 and BARD1 is currently unclear and needs to be studied in more detail.

BAP1 maps to human chromosome 3p21.3, and the encoded BAP1 protein is found both in the nucleus and in the cytoplasm (4). BAP1 has a nuclear localization signal at its carboxy-terminus; thus, truncating mutations impair BAP1 nuclear translocation. To translocate from the cytoplasm into the nucleus, BAP1 undergoes self-deubiquitylation, such that mutations in the BAP1 catalytic domain result in BAP1 being sequestered in the cytoplasm (5).

Because of the powerful tumor suppressor activity of BAP1 and of its role in modulating "gene–environment" (GxE) interactions in cancer (6), an increasing number of researchers are investigating the biological mechanisms and medical implications of inherited and acquired *BAP1* mutations. This is documented by the mounting number of articles reporting novel BAP1 activities, and clinical studies related to BAP1. As a result, significant progress has been made in recent years. Key mechanisms responsible for BAP1 tumor suppressor activity and its ability to modulate GxE interactions in cancer have been elucidated (Fig. 1). In the clinic, *BAP1* testing has become routine. It is an important component of the pathologic diagnosis of mesothelioma (7, 8) and early-detection clinical trials have been established at the NCI and elsewhere for carriers of germline *BAP1* mutations (NCT03830229), including trials targeting BAP1 (NCT03207347, NCT03531840). Here we review current understanding of how BAP1 suppresses tumorigenesis and how *BAP1* status can inform diagnosis, prognosis, targeted therapy, and cancer prevention in patients with cancer with hereditary and acquired *BAP1* mutations. Moreover, we discuss some puzzling questions, such as why germline *BAP1* mutations are associated with mesothelioma of low aggressiveness, but very aggressive uveal melanoma.

THE DISCOVERY OF THE FAMILIAL BAP1 CANCER SYNDROME

Studying a devastating epidemic in three remote villages in Cappadocia, Turkey, where 50% of villagers died of mesothelioma (9), Carbone and colleagues found that susceptibility to mesothelioma was transmitted in a Mendelian fashion across multiple generations (9, 10). In Cappadocia, the population was exposed to erionite (11), a carcinogenic mineral fiber similar to asbestos (12). However, mesothelioma was detected in some families, but not in others similarly exposed to erionite fibers (9, 13). The investigators proposed that GxE interactions caused the mesothelioma epidemic among genetically predisposed families,

challenging the dogma that mesothelioma was an example of a malignancy caused exclusively by exposure to carcinogenic fibers (9). In addition to families in Cappadocia, Carbone and colleagues investigated several U.S. families with multiple cases of mesothelioma, and proposed the existence of a mesothelioma-predisposing gene (9). Those were pre–next-generation sequencing (NGS) years, and the researchers used array-comparative genomic hybridization (aCGH), linkage analyses, and manual Sanger sequencing to screen germline DNA in two unrelated U.S. families (L from Louisiana and W from Wisconsin) with multiple cases of mesothelioma in which family members neither were exposed to erionite nor had occupational exposure to asbestos. However, environmental exposure to asbestos was very likely among L family members (14). The co-occurrence of uveal melanoma and mesothelioma in an "L" family member pointed the investigators in the right direction. Uveal melanoma and mesothelioma are rare cancers with an estimated probability of co-occurrence in the same individual of approximately $1/10^8$. Both tumors have a high frequency of 3p deletions, and, by sequencing 3p, Carbone and colleagues discovered that the individuals affected by mesothelioma, uveal melanoma, or breast cancer in both families carried truncating *BAP1* mutations, a condition they named the "BAP1 cancer syndrome" (6, 8, 14, 15). A parallel study by Weisner and colleagues reported that *BAP1* germline mutations were causally linked to benign melanocytic tumors developing at a young age, which were initially identified as atypical Spitz tumors (16). Subsequent studies revealed that these benign melanocytic intradermal tumor nodules had unique histologic and molecular characteristics that set them apart from Spitz and other melanocytic lesions, and were thus named "MBAITs" (melanocytic BAP1-associated intradermal tumors; ref. 15). Currently, the detection of MBAITs allows dermatologists to identify potential carriers of germline *BAP1* mutations, a diagnosis that is confirmed by germline DNA sequencing (8, 17, 18). Once these independent studies were published (14, 16), the authors of these articles realized that multiple individuals in mesothelioma families L and W carried MBAITs and vice versa that multiple cases of mesothelioma had occurred in the families in which MBAITs had been discovered by dermatologists, thus confirming the importance of BAP1 in these disease contexts. The obvious question was why BAP1 loss was so powerful in causing cancer, including multiple cancers in affected individuals, and why there was a clear prevalence of certain cancer types.

BAP1 BIOLOGICAL ACTIVITIES

BAP1 Nuclear Activities

In the nucleus, BAP1 binds to several proteins (Fig. 1), including HCF1, YY1, OGT, KDM1B, and FOXK1 and 2 (6). These proteins are assembled in multiprotein complexes that in turn may associate with other tissue-specific transcriptional regulators. Different nonoverlapping complexes may form according to tissue specificity, as observed in clear-cell renal cell carcinoma (ccRCC) and elsewhere (19, 20). As a result of these multiple interactions, BAP1 modulates several cellular activities.

BAP1 activity is critical for normal DNA synthesis as well as DNA replication under stress conditions. BAP1 interacts with and deubiquitylates INO80, playing a critical role in stabilizing and targeting the INO80 chromatin-remodeling complex to the replication forks

(21). By recruiting INO80 to the stalled forks, BAP1 allows stress-induced stalled replication forks to restart, a mechanism that suppresses genome instability and thus cancer development (22).

BAP1 is associated with transcriptionally active chromatin and interacts with several transcription factors and cofactors, thus acting as a chromatin scaffold for chromatin-remodeling complexes (23, 24). It has been proposed that BAP1 may modulate cell proliferation by deubiquitylating the transcriptional regulator HCF1 (23, 25). BAP1 is found in a ternary complex with HCF1 and the transcription factor YY1 and activates YY1-regulated genes in a DUB-dependent manner (24). BAP1 can also be recruited to the DNA via interaction with FOXK2, which promotes local histone deubiquitylation inducing changes in target gene activity (26). Moreover, BAP1 is part of a ternary complex bridging FOXK2 and HCF1 and represses FOXK2 target genes, an effect requiring BAP1 DUB activity but not the interaction with HCF1 binding (27).

BAP1 binds ASXL1, 2, and 3, human homologs of *Drosophila* Polycomb group protein ASX, which is an obligate binding partner for the Drosophila BAP1 ortholog Calypso, which catalyzes the monodeubiquitylation of histone H2A at residue K119 (28). BAP1 forms two mutually exclusive complexes with the product of ASXL1, which is frequently mutated in myeloid leukemia (29), and of ASXL2. These interactions help to maintain physiologic protein levels of BAP1. Furthermore, BAP1 deubiquitylates the deubiquitylase adaptor module DEUBAD of ASXL2, leading to its stabilization (30). Therefore, cancer-associated loss of BAP1 expression results in ASXL2 destabilization, and therefore the BAP1–ASXL2 interaction may play a role that remains to be defined in more detail in tumor suppression (28).

BAP1 promotes double-strand DNA repair by homologous recombination (HR), a key process to reduce genetic damage and prevent cancer (31, 32). The involvement of BAP1 in HR may be regulated by the BAP1–BARD1 binding (2).

BAP1 Regulates Cell Death

Recent evidence revealed that in the cytoplasm BAP1 regulates cell death and mitochondrial metabolism (Fig. 1). Studying primary fibroblast cell cultures derived from skin-punch biopsies of individuals from two separate families carrying heterozygous *BAP1* mutations (*BAP1*$^{+/-}$), and wild-type *BAP1* (*BAP1*$^{+/+}$) control fibroblasts (matched for sex and age from individuals from the same families), it was found that BAP1 localizes to the endoplasmic reticulum (ER), where it deubiquitylates and thus stabilizes the type 3 inositol-1,4,5-trisphosphate receptor (IP3R3; ref. 4). IP3R3 mediates the release of Ca^{2+} from the ER into the cytoplasm, in proximity to the mitochondria-associated membranes. Ca^{2+} is driven into the mitochondrial matrix by the voltage-dependent anion channels (VDAC) and the mitochondrial uniporter channel (MUC), which are located at the outer and inner mitochondrial membranes, respectively (33). The increase in mitochondrial Ca^{2+} concentration triggers the release of cytochrome *c* that in turn activates apoptosis (33). In heterozygous *BAP1*$^{+/-}$ conditions, as in family members affected by the BAP1 cancer syndrome, the reduced BAP1 levels impair both DNA repair, making their cells accumulate more DNA damage, and the apoptotic response. This dual effect favors the accumulation of

cells with higher levels of DNA damage so that over time some may become transformed and eventually give rise to cancer (4). These mechanisms were elucidated in human fibroblasts and in mesothelial cells, and it remains to be determined whether they apply to other cell types.

Very recently, studying a human cancer cell line, Zhang and colleagues discovered that BAP1 promotes ferroptosis, a nonapoptotic form of cell death that contributes to the tumor suppression function of BAP1 (34). Using chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq), Zhang and colleagues identified an array of BAP1-targeted genes, many of them associated with metabolism. Among these genes, *SLC7A11* (encoding the Solute Carrier Family 7 Member 11), an antiporter that imports cystine and exports glutamate (35), was repressed by BAP1. SCL7A11 downregulation caused a reduction of the uptake of cystine, a key metabolite for the synthesis of reduced glutathione, which in turn reduced antioxidant activity and lipid peroxidation, inducing ferroptosis. It remains to be studied whether the two distinct types of programmed cell death linked to BAP1, apoptosis and ferroptosis, are controlled in a synchronized or an independent manner (36) and whether BAP1 regulates additional mechanisms of cell death.

Although physiologic BAP1 levels are required for cells to execute apoptosis and ferroptosis, BAP1 may also exert a prosurvival role in response to metabolic stress via repression of the unfolded protein response (UPR). UPR can be induced by glucose starvation, leading to metabolic stress at the ER that, if not resolved, leads to programmed cell death. BAP1 exerts a prosurvival role by suppressing the UPR gene-regulatory network, repressing ATF3 and CHOP. The transcriptional repression of ATF3 and CHOP is dependent upon the deubiquitinylation of H2A (at K119) by BAP1 (37).

By engineering a knock-in mouse model expressing the catalytically inactive C91A *Bap1* mutant, He and colleagues showed that the loss of function of BAP1 has a proapoptotic effect in mouse embryonic stem cells, fibroblasts, liver, and pancreas, but not in melanocytes and mesothelial cells, the cells that give rise to the cancer types most commonly associated with *BAP1* mutations in humans (38). Studies in *Xenopus* revealed that BAP1 is required for the epigenetic switch from pluripotency to differentiation in the ectoderm, mesoderm, and neural crest through its regulation of epigenetic marks, such as histone 3 lysine 27 acetylation (H3K27ac). BAP1 loss causes transcriptional silencing and failure of H3K27ac to accumulate at promoters of genes regulating pluripotency-to-commitment transition (39).

These findings suggest a complex role for BAP1 in cancer that is context- and lineage-dependent and may differ among cancer types and species.

BAP1 Modulates Cellular Metabolism

Metabolomics analysis in the serum from $BAP1^{+/-}$ family members and *in vitro* validation in primary fibroblast cultures from these individuals revealed that a reduction of BAP1 protein levels shifted cell metabolism from oxidative phosphorylation (e.g., Krebs cycle) to aerobic glycolysis (e.g., Warburg effect; ref. 40). Moreover, in primary fibroblasts from individuals with heterozygous $BAP1^{+/-}$ mutations, aerobic glycolysis/lactate secretion were increased and mitochondrial respiration/ATP synthesis were decreased compared with

fibroblasts obtained from *BAP1*$^{+/+}$ volunteers from the same families that were age- and gender-matched (40). This effect may be linked to the reduced intramitochondrial Ca^{2+} required by several enzymes that regulate oxidative phosphorylation (ref. 40; Fig. 1). This evidence suggests a potential new tumor-promoting role for the Warburg effect that predates malignancy. In this scenario, *BAP1*-mutant cells operating in aerobic glycolysis are favored in their invasive growth into the nearby tissues even if they are in a hypoxic environment (40). Moreover, using a genetically engineered inducible *Bap1* knockout murine model, it has been demonstrated that the deletion of *Bap1* altered several metabolic pathways. Cholesterol biosynthesis was increased, whereas gluconeogenesis and lipid homeostasis proteins were decreased in the liver. The downregulation of mitochondrial proteins in the pancreas was accompanied by pancreatitis (41). Furthermore, through the O-GlcNAc transferase/HCF1 complex, BAP1 regulates gluconeogenesis by modulating the stability of the transcriptional coactivator PGC1α (42). Therefore, BAP1 contributes in maintaining metabolic homeostasis.

BAP1 MUTATIONS AND HUMAN CANCER

In 2010, Harbour and colleagues reported that 26 of 31 metastasizing uveal melanomas carried inactivating somatic *BAP1* mutations, and one of the patients also carried a germline *BAP1* mutation (43). In 2011, Carbone's team reported that germline *BAP1* mutations predisposed to mesothelioma and uveal melanoma (14), the BAP1 cancer syndrome (6). In 2012, Brugarolas and colleagues reported that 15% of ccRCCs carried somatic *BAP1* mutations, and subsequently found that some patients also had inactivating germline mutations (20, 44). Since then, there has been an exponential increase in studies linking BAP1 to human cancer.

BAP1 Germline Mutations

Numerous studies have now confirmed and expanded on the direct link of *BAP1* germline mutations to a cancer syndrome characterized by a predisposition to mesothelioma (45–49), uveal melanoma (43), and less frequently cutaneous melanoma (50), as well as ccRCC (20, 44, 51, 52), which are the core cancer types in the BAP1 cancer syndrome (6, 15). Although the term "mutations" has been widely used to encompass different types of genetic damage, most *BAP1*-mutant families carry truncating *BAP1* mutations (8, 53–55). Moreover, breast cancers and basal cell carcinomas are also quite frequent and will likely be included among the "core cancers" as more data accumulate (8, 55–57). Less frequently associated cancers include cholangiocarcinoma (58), meningioma (59), and others (ref. 55; Fig. 2A). Some of the BAP1-associated cancers, such as mesotheliomas and skin melanomas, are strongly linked to environmental carcinogens (60), suggesting GxE interaction in some instances (8, 18, 55, 61).

The hypothesis that GxE interaction increased the incidence of mesothelioma (61) was tested by exposing *Bap1*$^{+/-}$ mice to very low doses of asbestos fibers (total of 0.5 mg, compared with 3–5 mg usually used in such studies). Following this low exposure, *Bap1*$^{+/-}$ mice developed mesothelioma at a comparable rate to wild-type mice (*Bap1*$^{+/+}$ mice) exposed to ten times higher doses of asbestos (62). Parallel studies reported evidence of GxE

interaction in mice (63) and also in one *BAP1*-mutant family (64). Recently, Badhai and colleagues (65) reported that the combined deletion in the mesothelial cell lineage of *Bap1, Nf2*, and *Cdkn2ab* caused mesothelioma in 100% of mice. *Bap1* deletion alone caused mesothelioma in 5% of unexposed mice, and combined *Nf2* and *Cdkn2ab* deletion alone did not. In summary, inherited *BAP1* mutations cause cancer in mice and in humans, and cancer incidence increases upon exposure to asbestos or other carcinogenic fibers and when other mutations are present. However, the spontaneous development of mesotheliomas in $Bap1^{+/-}$ mice not exposed to asbestos (65, 66), and the development of multiple cancer types in carriers of *BAP1* mutations (Fig. 2B), including tumor types that have not been associated with known carcinogens (53, 67), suggests that *BAP1* mutations also drive tumor growth independently of genotoxic stress, perhaps by favoring the accumulation of age-related DNA damage.

Environmental carcinogens clearly associated with uveal melanoma have not been definitively identified. However, patients with uveal melanoma with germline *BAP1* mutations always have an initiating mutation in the G-alpha-q (Gq) pathway, suggesting that additional genetic variants play a critical role in the development of uveal melanoma (68).

Germline mutations are either inherited or *de novo* mutations. For example, among individuals affected by the Li-Fraumeni syndrome, approximately two thirds were inherited and one third were *de novo* mutations (69–73). Instead, among more than 200 families with multiple members carrying germline *BAP1* heterozygous mutations, all mutations for which family information was available demonstrated heritability, some tracing back more than 600 years (53), suggesting a low rate of *de novo* germline mutations (8, 67).

In summary, *BAP1* is a powerful tumor suppressor gene. Carriers of germline *BAP1* mutations often develop multiple cancers during their lifetime. The overall penetrance for cancer is at least 85% and approaches 100% with increasing age (8, 54, 55). The fact that most BAP1-associated cancers arise in middle-age and older individuals, and that the penetrance for any particular cancer type is less than 100%, suggests that genomic aberrations in addition to BAP1 loss are required for cancer formation, for example, mutations in the Gq signaling pathway (68).

Somatic *BAP1* Mutations

The first cancer in which somatic (i.e., acquired) *BAP1* mutations were found to be common was uveal melanoma, where these mutations are present in approximately 45% of primary tumors and are highly correlated with the poor prognosis class 2 transcriptional signature and metastatic phenotype (ref. 43; Fig. 3A). In uveal melanoma, BAP1 loss may drive malignant progression by removing epigenetic restraints imposed on differentiated cells (Fig. 3B; ref. 39). Other cancers in which acquired somatic *BAP1* mutations are common include mesothelioma (60%–70% of them; ref. 8) and ccRCC (15% of them; Fig. 4; ref. 20), the core cancers of the BAP1 cancer syndrome (6, 15). The parallel between the tumor types developing most frequently in carriers of germline *BAP1* mutations and the tumor types that most frequently contain somatic *BAP1* mutations underscores the increased susceptibility of uveal, mesothelial, and kidney cells to BAP1 loss. Somatic *BAP1* mutations are also present in other malignancies (52), although at lower rates: thymic carcinoma (13%),

cholangiocarcinoma (7%), cutaneous melanoma (5%), basal cell carcinoma (4%), and others (ref. 74; COSMIC database, https://cancer.sanger.ac.uk/cosmic). The role of *BAP1* as a two-hit tumor suppressor gene is underscored by the fact that in humans they are accompanied by monoallelic loss of 3p, or by biallelic deletions of the *BAP1* locus (LOH), including broad deletions of 3p21, narrow deletions of several exons, or loss of the entire *BAP1* allele (75, 76).

Initial studies underestimated the frequency of *BAP1* mutations in mesothelioma as 22% to 23% (14, 77). A subsequent study using a comprehensive integrated genomic approach that included Sanger sequencing, multiplex ligation–dependent probe amplification (MLPA), copy-number analysis, and cDNA sequencing, combined with IHC and DNA methylation analyses in mesothelioma biopsies, found that >60% carried biallelic somatic *BAP1* mutations (78). This study revealed that *BAP1* was inactivated both by point mutations (which were detected by Sanger sequencing and not by MLPA) and by larger deletions (which were detected by MLPA but not by Sanger sequencing). This is because of the frequent occurrence of minute *BAP1* deletions in the range of 250 to 3,000 kb, which are not reliably detected by targeted NGS (tNGS), by whole-exome sequencing (WES), or by Sanger sequencing, but are instead detected by MLPA. IHC proved to be the most sensitive and specific test to detect *BAP1* mutations (78). Surprisingly, DNA methylation analyses found no evidence for epigenetic *BAP1* inactivation (78). These findings were supported by a study in which high-density aCGH to detect deletions larger than 250 bp, and tNGS to detect nucleotide level mutations, resulted in a much higher prevalence of *BAP1* mutations in human mesothelioma biopsies than either technique alone (~50%). This was because aCGH missed point mutations and tNGS missed larger deletions (79). Additional studies confirmed that *BAP1* is the most frequently mutated gene in mesothelioma; however, studies that relied only on tNGS or WES invariably underestimated the true incidence of *BAP1* mutations (80–82).

Similarly, Harbour and colleagues used an integrated DNA/RNA-sequencing approach and a customized bioinformatics pipeline to improve the detection of *BAP1* mutations in uveal melanoma, identifying *BAP1* mutations in approximately 45% of uveal melanomas, twice the rate detected by previous NGS approaches (68). Many of the mutations that were previously undetected consisted of large insertions/deletions (indel) that were missed by standard indel realignment tools (68). In addition to *BAP1*-mutated cancers, these studies are relevant to all human malignancies where mutations are assessed by tNGS or WES, as these techniques underestimate the extent of genetic damage.

A detailed analysis of 3p deletions in sporadic mesotheliomas revealed that deletions are not contiguous but rather preferentially occur in *BAP1* and in some nearby genes (*SETD2, PBRM1*, and *SMARCC1*), alternating with segments showing oscillating copy-number changes along the 3p21 chromosome, findings suggestive of chromothripsis (79). The occurrence of chromothripsis in mesothelioma may be favored by BAP1 inactivation (79), and it has been confirmed by mate-pair sequencing (MPseq) analyses (83) and by WES (84). In addition, chromothripsis has been observed in ccRCC where it results in a t(3;5) derivative chromosome, leaving a wild-type chromosome 3 and two copies of wild-type chromosome 5 (85).

A potential clinical interest of *BAP1* NGS analysis is the identification of germline mutations not predicted by the clinical guidelines, as in those identified in mesothelioma by a large universal sequencing study conducted in more than 1,000 cases (86). Of note, mesothelioma and uveal melanoma have among the lowest mutational burdens of all cancers in The Cancer Genome Atlas, a finding that is in part a consequence of the technical approach used (NGS) that underestimates the amount of genetic damage (79).

CLINICAL IMPLICATIONS

Diagnosis

BAP1 IHC is now an integral part of the routine of diagnostic pathology of the pleura and peritoneum and it is also extensively used in research (8). About 60% of mesotheliomas show tumor cells with an epithelioid morphology, 10% show a spindle cell sarcomatoid morphology, and 30% a biphasic morphology, that is, a mixture of epithelioid and spindle cells. When clear evidence of invasion is lacking it is not possible to determine whether the lesions are benign/reactive, such as a chronic pleuritis, or malignant. When invasion is present, it is difficult to decide whether the spindle cells are benign or malignant, and thus whether a lesion represents an epitheliod or a biphasic mesothelioma (7, 8). BAP1 IHC is very helpful in both cases. The correct interpretation of BAP1 IHC is critical in cancer diagnosis and also in research; therefore, we will review this issue, which, in our experience, is confusing to many (Fig. 5). Immunostaining produces a nuclear and cytoplasmic stain in stromal cells and in tumor cells containing wild-type BAP1 (Fig. 5A and B). On the other hand, nearly all biallelic *BAP1* mutations result in the absence of nuclear staining because either there is no BAP1 protein or the mutated BAP1 protein cannot enter the nucleus (Fig. 5C–F). Negative BAP1 nuclear staining by IHC, regardless of cytoplasmic staining, is found in about 60% to 70% of mesotheliomas and is a reliable, rapid, and economical approach to identify biallelic *BAP1* inactivating mutations. Instead, BAP1 nuclear staining is evidence of wild-type BAP1 (8). Note that BAP1 nuclear staining is found in the normal cells of carriers of germline *BAP1* mutations because the remaining wild-type allele produces a normal BAP1 protein. Therefore, IHC, which is not a quantitative assay, cannot help to identify carriers of germline *BAP1* mutations.

As for the cytoplasm, two scenarios are possible. In most tumor cells showing no nuclear staining (e.g., mutated BAP1), there is also no staining in the cytoplasm (Fig. 5C and D). At times, however, a mutated, biologically inactive BAP1 (4) can accumulate in the cytoplasm where it forms amyloid (87), and it may produce cytoplasmic staining without accompanying nuclear staining (Fig. 5E and F; ref. 78).

In summary, the absence of BAP1 nuclear staining is a valuable diagnostic test to distinguish benign (positive nuclear staining) from malignant (negative nuclear staining) mesothelial cells, including distinguishing benign pleural effusions, benign atypical mesothelial hyperplasia, and benign chronic pleuritis from mesothelioma (7, 8, 88–91). Moreover, negative BAP1 nuclear staining in both epithelioid and spindle cells confirms the diagnosis of biphasic mesothelioma (Fig. 5F). Negative BAP1 nuclear staining is also helpful in the often difficult differential diagnosis between primary mesothelioma (most of them BAP1-

negative) and metastatic lung carcinomas to the pleura, which instead are nearly always BAP1-positive (92, 93).

The IHC assay is similarly effective in ccRCC. In a study of 176 ccRCC at The University of Texas Southwestern (UTSW), where both IHC and DNA sequencing were performed, IHC results were interpretable in 175 tumors (20). Nuclear BAP1 protein was detected in 150 tumors and 148 were wild-type for *BAP1*. The two discordant samples had missense mutations in the catalytic domain (p.Gly13Val and p.Phe170Leu). Unlike other contexts, however, the mutant BAP1 protein still localized in the nucleus in these two samples. Twenty-five samples were negative by IHC and 22 of these had *BAP1* mutations. In addition, Western blot analyses of an IHC-negative sample with wild-type *BAP1* failed to reveal detectable BAP1 protein (20), suggesting that *BAP1* may have been inactivated through mutations eluding detection by conventional Sanger sequencing, as described in mesothelioma (78, 79). Overall, the positive and negative predictive values of the IHC test for ccRCC were >95% (20). This IHC test has been utilized extensively (more than 3,000 RCCs) at UTSW (94–98) and there is a Clinical Laboratory Improvement Amendments (CLIA)–certified IHC test being implemented in routine clinical practice (7, 8, 78, 90, 91).

Prognosis: Germline Mutations

Baumann and colleagues reported that the presence of germ line *BAP1* mutations strikingly increased the 5-year survival rate of patients with mesothelioma by 7-fold [47% (95% confidence interval [CI], 24–67) vs. 6.7% (95% CI, 6.2–7.3)], indicating that mesothelioma is less aggressive when it occurs in the context of the BAP1 cancer syndrome (67). In these individuals, normal cells contain 50% of the BAP1 protein, whereas tumor cells show biallelic *BAP1* mutations and thus do not contain a biologically functional BAP1 protein (4, 78). In a follow-up prospective study, this research team tested 79 patients, all tumor stages, diagnosed with mesothelioma at an early age (<50 years of age) and/or with a family history of either mesothelioma or any of the core tumors of the BAP1 cancer syndrome. These characteristics were selected to identify patients who were likely carriers of germline mutations. These 79 patients were tested for germline mutations of *BAP1* and of an additional 55 genes that included tumor suppressor genes, oncogenes, genes involved in DNA repair, and genes commonly found mutated in mesothelioma (54). Most subjects (43/79) carried germline *BAP1* mutations; 12 of 79 carried germline mutations in other tumor suppressors; and 5 of 79 carried mutations in *BAP1* and also in other cancer-related genes. These 79 patients selected for possible cancer heritability had a significantly prolonged survival of 5 to 10+ years ($P < 0.001$) compared with patients with sporadic mesothelioma from the SEER cohort, who had a median survival of 8 months (all stages combined), and 11 months in patients with stage I mesothelioma (54). These results were supported by two independent and parallel studies. An analysis conducted at the University of Chicago on the germline DNA of 198 patients with mesothelioma using targeted capture and NGS of 85 cancer susceptibility genes revealed that 12% of patients within this cohort carried pathogenic germline mutations; *BAP1* was the most commonly mutated gene (47). A similar survey conducted at the NCI on a cohort of 385 patients using a panel of 73 genes involved in DNA repair and tumor suppression demonstrated that 12% of them carried germline mutations (mostly germline *BAP1* mutations). The presence of inherited mutations

significantly increased median overall survival compared with patients without these mutations (7.9 years vs. 2.4 years, $P = 0.001$; ref. 99). Together, these studies validate the prognostic significance of germline mutations that confer a significantly improved survival to patients with mesothelioma (Fig. 6).

The improved survival was also observed among patients who, in addition to mesothelioma, developed other aggressive malignancies, which, rather than an exception, is the norm among those carrying germline *BAP1* mutations. At times, five aggressive cancers were diagnosed in the same patient, and, surprisingly, they survived 5 to 10 or more years (refs. 53, 54, 67; Fig. 2B). Because almost all *BAP1*-mutated cancers contain biallelic *BAP1* mutations, regardless of whether they are sporadic or occur in carriers of germline mutations, the markedly improved prognosis of mesotheliomas occurring in carriers of germline *BAP1* mutations does not seem to be related only to the mutation in the tumor cells. Therefore, the improved prognosis of mesothelioma and other cancer types in carriers of germline *BAP1* mutations may be influenced by the microenvironment and/or the immune system. Whether a microenvironment with reduced BAP1 levels would be more effective in controlling tumor growth is an area of intense investigation across multiple laboratories, as it may help to find novel ways to fight cancer.

In contrast to mesothelioma, a study of 8 patients with uveal melanoma with germline *BAP1* mutations found an increased risk of metastasis (p.003) compared with uveal melanoma patients with wild-type *BAP1* in their germline, confirming that *BAP1* mutations induce a metastatic phenotype (100). This study did not compare survival among metastatic uveal melanomas with germline or with somatic mutations. Somatic *BAP1* mutations in the tumor biopsy are the strongest known risk factor for uveal melanoma metastatic death (43, 101). These phenotypic differences among cancer types associated with *BAP1* mutations suggest that there are cell type- and context-dependent differences in the role of BAP1 in biology and cancer. It is not yet clear whether germline *BAP1* mutations are associated with improved survival in RCC.

Prognosis: Somatic Mutations

Mesotheliomas with acquired *BAP1* mutations are mostly of the epithelial type and may have a slightly improved prognosis of a few months compared with mesotheliomas of similar histologic type with wild-type *BAP1* (102–104); however, some studies did not support this finding (105).

Intriguingly, the opposite correlation with survival was observed in uveal melanoma (43, 100, 101, 106, 107), ccRCC (96, 108, 109), and cholangiocarcinoma (110), where somatic biallelic *BAP1* mutations were associated with a metastatic phenotype and poor prognosis. In uveal melanoma, detection of *BAP1* mutations directly by sequencing, or indirectly via the class 2 transcriptional signature or other methods, is now a routine part of patient care, with high-risk patients stratified for increased surveillance and clinical trial entry (111–113). The recent finding that BAP1 loss leads to defective differentiation and an arrested primitive phenotype in vertebrate development and uveal melanoma (39) suggests that BAP1 loss can promote tumor progression by inducing cell dedifferentiation and stem-like behavior (Fig. 3B; ref. 114).

Brugarolas and colleagues found that ccRCCs with somatic *BAP1* mutations were associated with high-grade tumors. They discovered that mutations in *BAP1* were mutually exclusive with mutations in *PBRM1* (20, 115). *PBRM1*, which encodes a switching defective/sucrose nonfermenting (SWI/SNF) nucleosome remodeling complex protein (BAF180), is inactivated in approximately 50% of ccRCC (20, 116). In contrast to *BAP1*-mutant tumors, *PBRM1*-mutant tumors tend to be of low grade (20, 98). To determine whether BAP1 and PBRM1 directly affect tumor grade, mice were generated with targeted inactivation of *Bap1* or *Pbrm1* in the kidney using the same Cre driver (117). Inactivation of *Bap1* or *Pbrm1*, along with *Vhl*, which is uniformly inactivated in ccRCC, led to the development of ccRCC (117, 118). Similar to humans, BAP1-deficient tumors were of high grade, and PBRM1-deficient tumors were of low grade (Fig. 4). Furthermore, PBRM1-deficient tumors developed after a significantly longer latency period. Overall, these data suggest that the differences in grading observed in ccRCC are directly related to the loss of BAP1 and PBRM1. These differences in grade also translate into differences in survival. Patients with BAP1-deficient tumors have a three-fold higher risk of death than patients with PBRM1-deficient tumors (98, 119). The *PBRM1* gene, like the *BAP1* and *VHL* genes, is located on chromosome 3p (19). Following *VHL* inactivation, which is the signature and initiating event in ccRCC (85, 120, 121), a mutation of the second copy of *BAP1* or *PBRM1* likely leads to tumors of different grade and prognosis (19). A fourth tumor suppressor gene in the same 3p region, *SETD2*, is also mutated in ccRCC and is associated with poor prognosis (94). Whereas mutations in *BAP1* and *PBRM1* tend to be mutually exclusive, mutations in *PBRM1* and *SETD2* appear to cooperate and are found at higher-than-expected frequencies (115).

Although mutation exclusivity in cancer often identifies genes encoding for proteins that act in the same pathway, where mutations at two different levels may offer little added advantage, this is unlikely the case in ccRCC. Indeed, BAP1- and PBRM1-deficient tumors differ not only in grade and prognosis, but also in gene expression (20, 119), and the mouse models show ccRCCs that are histologically quite different.

In summary, somatic mutations have only mild to no beneficial survival effect in mesothelioma, and are instead associated with a much more aggressive tumor phenotype and reduced survival in uveal melanoma and ccRCC (20, 43, 90, 91, 102–104, 108, 121, 122). These findings are puzzling and if addressed may provide critical information to develop novel therapeutic approaches.

Therapy

Mesothelioma, metastatic uveal melanoma, metastatic RCCs, and other BAP1-related malignancies are resistant to current therapies, and thus it is important to develop novel therapeutic approaches (8, 43, 96, 108, 109, 123–126). Numerous ongoing studies are exploring the possibility of targeting *BAP1* mutations or using *BAP1* status as a biomarker for sensitivity to different therapies.

The incorporation of the active metabolite of gemcitabine into DNA causes replication arrest and apoptosis, making this drug one of the most used chemotherapeutic agents against several cancers, including mesothelioma, for which it is approved as second-line treatment

(125). Two recent independent studies proposed that *BAP1* status may be predictive of sensitivity to gemcitabine. Upon treatment with this agent or with hydroxyurea, the viability of mesothelioma spheroids expressing nonfunctional C91A BAP1 was significantly higher compared with wild-type BAP1 counterparts (127). Similarly, mesothelioma cells expressing wild-type BAP1 were more sensitive to gemcitabine-induced apoptosis and cell-cycle derangement, compared with mesothelioma cells expressing nonfunctional BAP1 or silenced for BAP1 (128). These findings underscore the biological relevance in relation to cancer of BAP1 regulation of cell death (Fig. 1). The evidence generated by these studies (127, 128) suggests that *BAP1* status is a promising candidate predictor for sensitivity to gemcitabine therapy in patients with mesothelioma and possibly other *BAP1*-mutated cancers. *BAP1* status may equally predict sensitivity to other chemotherapeutic agents that cause DNA damage and cell death.

Recently, Webster and colleagues demonstrated that *Bap1* loss cooperated with active oncogenic $BRAF^{V600E}$ in supporting melanoma growth in a mouse model. The mice bearing *Bap1*-deficient tumors had a complete response to the combination treatment with vemurafenib (BRAF inhibitor) and cobimetinib (MEK inhibitor). This combined therapy is the standard of care for $BRAF^{V600E}$-mutant human melanoma (129). If the results are confirmed in humans, *BAP1* status may help to identify those patients more likely to respond to this therapy. In addition, a large study in more than 100 cases of metastatic RCC demonstrated that *BAP1* mutational status did not correlate with clinical benefit upon rapalog therapy (130), despite the significantly higher aggressiveness of RCC in carriers of *BAP1* mutations (131).

Histone deacetylases (HDAC), including class I HDAC1 and HDAC2, are epigenetic regulators of gene expression, which can be dysregulated in cancer, and have been proposed as targets by using specific inhibitors such as suberoylanilide hydroxamic acid (SAHA) or vorinostat for mesothelioma (132) and uveal melanoma (133) therapy. Upon BAP1 loss, HDAC1 is increased and HDAC2 is reduced, an effect that was observed across several lung cancer and mesothelioma cell lines (134). This suggested that *BAP1* status may help to identify patients who may be responsive to HDAC inhibitors. However, patients with mesothelioma—not selected for *BAP1* status—treated in the second or third line in the randomized phase III VANTAGE-014 trial with vorinostat did not show improved survival (135).

HDAC inhibitors can reverse dedifferentiation associated with BAP1 loss and induce cell-cycle exit in uveal melanoma cells (133). BAP1 modulates the development of the neural crest, from which melanocytes arise, and this role is dependent on an indirect effect of BAP1 on acetylation of H3K27 (Fig. 3B), which is associated with increased transcription (39). The BAP1-deficient developmental phenotype could be rescued using SAHA or specific depletion of HDAC4. Although results of HDAC inhibitors in metastatic uveal melanoma have been disappointing, there may be a role for such compounds in uveal melanoma in the adjuvant setting, with the goal of delaying or preventing the outgrowth of micrometastasis in high-risk patients (133).

The chromatin-associated PARP enzyme is involved in the recovery of cells from DNA damage, and PARP inhibitors selectively target cancer cells with defective DNA repair. In the context of mutations in *BRCA1* or *BRCA2* genes in patients with breast, ovary, prostate, or pancreatic cancers, PARP inhibitors have shown antitumor activity, and three agents are currently in the clinic. BAP1 loss impairs HR and double-strand break repair, promoting error-prone nonhomologous end-joining, with consequent genomic instability (Fig. 1). Therefore, BAP1-deficient cells may be more sensitive to PARP inhibitors (32). BAP1 loss may sensitize ccRCC cells to PARP inhibitors (20). Several studies proposed to test the efficacy of PARP inhibitors in mesothelioma, although patients were not selected for *BAP1* status (136, 137). A *BAP1* mutant by alternative splicing resulting in a 54-bp deletion increased sensitivity to the PARP inhibitor olaparib (138). A recent study on HR defects in a cohort of patients with mesothelioma showed both *in vitro* and by digital gene-expression analysis that loss of BAP1 increased sensitivity to PARP inhibitors (139). Presently, two ongoing clinical trials are testing the hypothesis that *BAP1* mutations increase sensitivity to PARP inhibitors in mesothelioma (NCT03207347, NCT03531840). However, very recently Hassan and colleagues reported that *BAP1* status does not determine sensitivity to PARP inhibitors in patient-derived mesothelioma cell lines (140). We propose that these results may indicate that the increased resistance to cell death caused by *BAP1* mutations in tumor cells over-come whatever increased DNA damage may be induced by PARP inhibitors in these same cells, making them resistant to this therapy.

Studies in RCC have led to the identification of a link between BAP1 loss in tumors and an inflammatory microenvironment. To characterize the tumor microenvironment (TME) in RCC, Wang and colleagues (141) undertook an innovative approach involving RNA-seq. RNA-seq datasets were generated from patients' RCC as well as from the corresponding tumor implanted orthotopically in mice. Subsequently, RNA-seq reads from the tumorgraft corresponding to the murine genome were subtracted. These reads correspond to the tumor stroma, which is replaced by the mouse, as only human tumor cells propagate in the mouse. Comparative analyses were then performed between the patients' transcriptome, corresponding to tumor and stroma, and the tumorgraft transcriptome, corresponding to the tumor only. By subtracting the tumorgraft transcriptome from the patient tumor transcriptome, Wang and colleagues were able to empirically define the TME in RCC (141). According to this signature, RCCs could be divided into an inflamed subtype and a noninflamed subtype. Interestingly, the inflamed subtype was enriched for *BAP1* mutations ($P < 0.0001$). What drives the inflammation in BAP1-deficient ccRCCs is unclear, but a recent study found a link between BAP1 loss and the expression of endogenous retroviruses (142). In contrast, the noninflamed subtype was enriched for angiogenesis-related genes. These findings may provide cues to help identify the patients with ccRCC most likely to respond to checkpoint versus angiogenesis inhibitors.

Similarly, in peritoneal mesothelioma, two subsets of tumors can be distinguished based on the presence of an inflammatory TME, and this difference correlated with *BAP1* haploinsufficiency. *BAP1* haploinsufficiency was characterized by a distinct expression profile including genes related to chromatin remodeling, DNA repair, and activation of immune checkpoint receptors, a pattern associated with the inflammatory TME. This evidence makes *BAP1* a candidate predictive biomarker for immunotherapy in peritoneal

mesothelioma (143) and possibly also in pleural mesothelioma (144). Moreover, based on the structural rearrangements induced by chromothripsis, which may be favored by *BAP1* mutations (79), Mansfield and colleagues predicted and validated *in vitro* the expression of altered peptides that may act as neoantigens and thus potentially increase mesothelioma immunogenicity and responsiveness to immunotherapy (145). BAP1 loss results in increased global trimethylation of histone H3 lysine 27 (H3K27me3), which is catalyzed by the polycomb repressive complex 2 enzyme EZH2 (39, 146). Mesothelioma cells deficient for BAP1 were found to be sensitive to EZH2 inhibitors (146). However, this effect was not seen in neural crest–derived uveal melanoma cells (147). In addition, depletion or pharmacologic inhibition of EZH2 in BAP1-deficient *Xenopus* embryos did not rescue a neural crest developmental phenotype (39). Thus, the role of EZH2 inhibitors in BAP1-deficient cancers remains unclear, and the results of a clinical trial on tazemetostat in mesothelioma that has been completed are being evaluated (ref. 8; NCT02860286).

CONCLUDING REMARKS

Although the BAP1 cancer syndrome and the driving role of acquired *BAP1* mutations in human cancer were discovered less than a decade ago, much progress has been made to elucidate critical mechanisms of BAP1 activities (Fig. 1), and consequently why reduced or absent BAP1 protein levels cause and favor cancer progression. BAP1 plays an important role in regulating both DNA repair by HR and cell death in some cell types. Therefore, reduced levels of BAP1, as observed in carriers of heterozygous *BAP1* mutations, increase the amount of genetic damage that occurs spontaneously as cells divide, or that occurs in response to exposure to environmental carcinogens (4). In parallel, the reduced levels of BAP1 impair apoptosis (4), ferroptosis (34), and possibly other mechanisms of cell death, resulting in an accumulation of cells with DNA damage, which normally would be eliminated by these mechanisms. These DNA-damaged cells can eventually become malignant. Moreover, BAP1 loss favors tumor growth by inducing a Warburg effect (i.e., aerobic glycolysis) that provides the metabolic building blocks to support cell division and at the same time helps cancer cells to grow in a hypoxic environment. A 50% reduction of BAP1 protein levels is sufficient to induce a Warburg effect in “normal” cells; thus, cells from those carrying germline mutations are primed to malignant growth once genetic damage causes malignant transformation (40). Therefore, the combined nuclear and cytoplasmic BAP1 activities account for the very high incidence of cancer in carriers of germline *BAP1* mutations (Fig. 1).

BAP1 has emerged as a critical regulator of GxE interaction (61). BAP1’s role in increasing susceptibility to asbestos, UV light, and ionizing radiation has been established in primary human fibroblasts and mesothelial cells in culture (4), and it has been demonstrated in mice exposed to asbestos (62, 63). Because *BAP1*-mutant uveal melanoma does not show strong evidence of DNA damage repair defects or increased mutation burden (148), further work is needed to determine which of the possible functions of BAP1 are relevant to each cancer type in which it is mutated.

All published data support the notion that BAP1 is a potent tumor suppressor, as almost all carriers of pathogenic germline *BAP1* mutations developed one or more cancers during their

lifetime. LOH for *BAP1* is observed in 100% of human tumors developing in carriers of germline *BAP1* mutations, as well as in sporadic mesotheliomas with somatic *BAP1* mutations, underscoring the potent tumor suppressor activity of BAP1. Intriguingly, LOH for *BAP1* is not always observed in tumors developing in mice carrying germline *Bap1* mutations (62, 65).

Some of the BAP1 activities may be more or less impactful depending on the cell type and species. This critical question shall be addressed in the coming years to understand why germline *BAP1* mutations cause or are present as somatic mutations more frequently in mesothelioma, uveal melanoma, and ccRCC, rather than in other cancer types. This information will help to design more effective preventive and therapeutic strategies for patients carrying germline *BAP1* mutations or cancers with somatic *BAP1* mutations.

An additional question that will be addressed in coming years is why *BAP1* mutations have phenotypic and prognostic implications that are cell type– and context-dependent: Germline mutations confer a better prognosis in mesothelioma (54, 67, 99), whereas somatic mutations induce a worse prognosis in uveal melanoma (42, 100, 101, 106, 107) and ccRCC (96, 108, 109). The difference in survival is quite significant, with median survival of 5 to 7 years for mesothelioma developing in carriers of heterozygous *BAP1* mutations compared with 1-year median survival in sporadic mesotheliomas, cancers characteristically resistant to therapy (54, 67, 99). Some of these patients with mesothelioma have a normal life 20 years after diagnosis, their cancers still detectable. They appear to respond to any therapy, which raises the question of whether they respond to therapy or whether these tumors would have remained indolent regardless of therapy.

Adding to this puzzle, in sporadic mesotheliomas acquired biallelic *BAP1* mutations are frequent, yet these mesotheliomas are not associated with significantly improved survival (8). These findings in the same tumor type suggest that the improved survival in carriers of germline *BAP1* mutations may be linked to the microenvironment, the immune system, and maybe in part to early diagnosis, as family members are being enrolled in early-detection screening programs. However, the improved survival predates screening, as it was also observed in family members who were diagnosed before the discovery of the BAP1 cancer syndrome (67).

What if germline *BAP1* mutations render the host capable to fight mesothelioma growth by affecting the tumor microenvironment? There is some preliminary experimental evidence that supports this hypothesis. *BAP1* mutations may influence the response to immunotherapy by increasing the propensity to chromothripsis, by deregulating the expression of genes that modulate immune checkpoints, and by promoting a proinflammatory tumor microenvironment (62, 141, 145). By studying patients and mice with germline *BAP1* mutations, we may learn how to treat mesotheliomas and maybe other cancers more effectively.

The information about the effects of BAP1 on mitochondrial respiration and cell metabolism has provided researchers with a range of potential targets and tools for prevention and therapy that should be investigated in the coming years. For example, the role of metformin,

a drug that reprograms cell metabolism by restraining aerobic glycolysis and promoting mitochondrial respiration (149), could be explored in the existing mouse models carrying heterozygous *Bap1* mutations and in xenografts of *BAP1*-mutated tumors.

Currently, we recommend germline and tumor cell *BAP1* testing for all patients with mesothelioma, uveal melanoma, and RCC, as it helps in diagnosis, has prognostic relevance, and may also guide clinicians to target therapies, as several clinical trials are becoming available for patients with *BAP1*-mutant tumors (see the previous section). Family members of carriers of germline *BAP1* mutations should be tested for *BAP1* mutations, and those found to carry mutations should be enrolled in early-detection clinical trials (NCT03830229) that can help identify malignancies at an early stage, when they can be cured by surgery (uveal melanoma, RCC, cutaneous melanoma, etc.), or when they may be more susceptible to therapy (mesothelioma). Moreover, *BAP1* mutant carriers should limit exposure to diagnostic and therapeutic ionizing radiation that in these individuals may carry a higher cancer risk than in the population at large. Instead, they should be screened preferentially using sonography and MRI (8) according to the same guidelines used for patients affected by the Li-Fraumeni cancer syndrome (73).

In summary, the discoveries made during the past decade allow us to implement preventive and early-detection programs that improve the survival of carriers of *BAP1* germline mutations. Furthermore, with future advancements in understanding the fundamental biology of BAP1 and the development of novel technologies, targeted therapies for BAP1-deficient cancers should lead to substantial clinical improvements.

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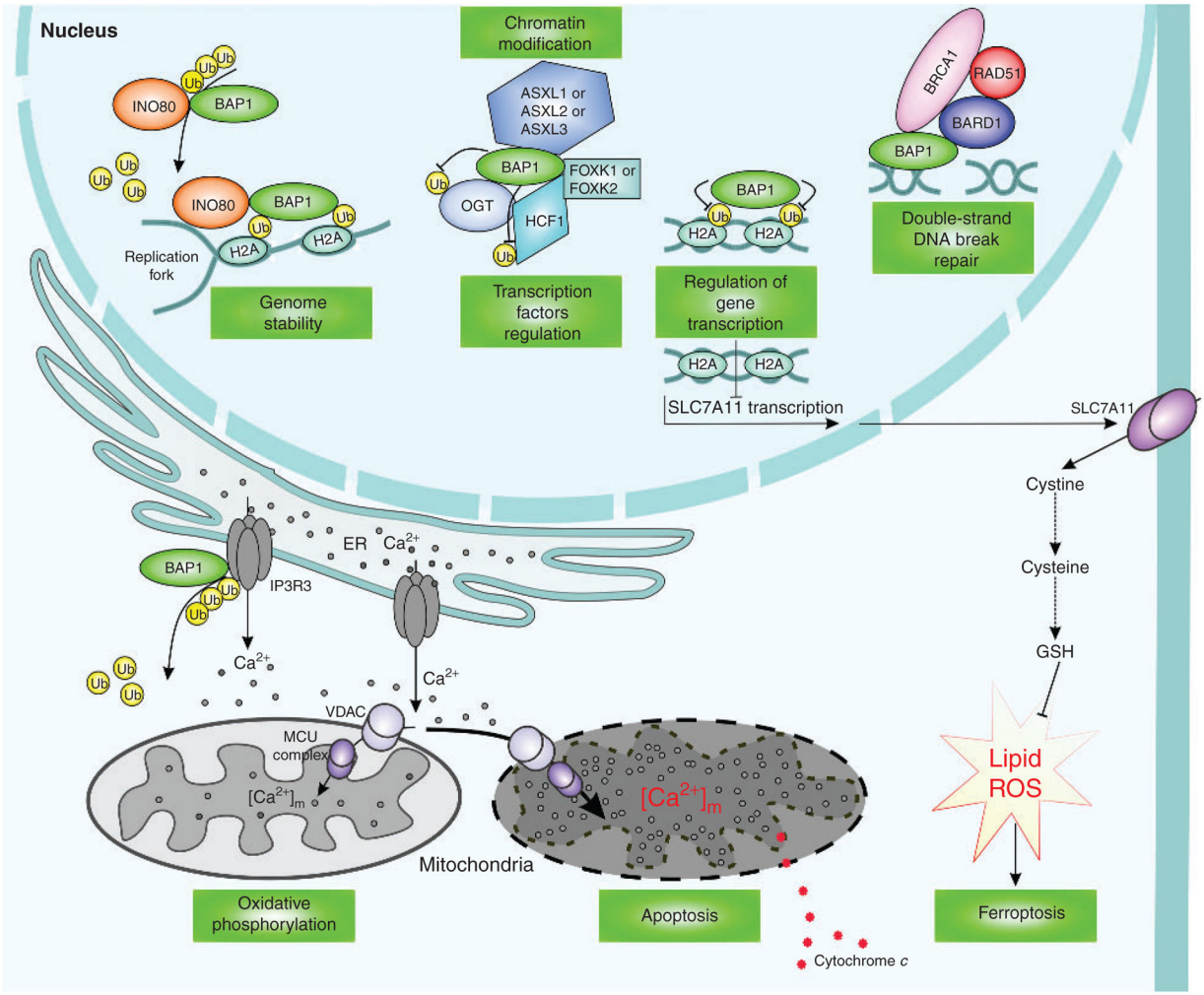

Figure 1.

BAP1 nuclear and cytoplasmic physiologic activities. BAP1 nuclear activities (top). BAP1 stabilizes and recruits INO80 to replication forks, via the interaction with H2A-Ub, for efficient replication fork progression and DNA replication, thereby ensuring genome stability (21, 22). Nuclear BAP1 regulates gene expression through effects on a number of epigenetic modifications and interaction with transcription factors. The interaction of BAP1–ASXL1 with HCF1–FOXK1 allows deubiquitylation and thereby stabilization of OGT, HCF1, and potentially other unknown targets to regulate transcription. The BAP1–ASXL1–HCF1–OGT complex localizes on numerous gene-regulatory elements, possibly through factors such as FOXK1, and functions as either a gene-specific activator or repressor complex on distinct genes (23–30). BAP1 suppresses tumor development by repressing SLC7A11 expression through regulation of H2A-Ub levels on the SLC7A11 promoter and inducing ferroptosis. SLC7A11 imports extracellular cystine, which is subsequently converted to cysteine in cells; cysteine is a rate-limiting precursor for glutathione (GSH) biosynthesis; GSH is used as a cofactor by glutathione peroxidase 4 to reduce lipid reactive oxygen species (ROS) to lipid alcohols; overproduction of lipid ROS in cells results in ferroptosis (34). By binding BARD1 (2), BAP1 participates in the double-strand DNA break

repair process (31, 32). This RAD51-dependent DNA repair pathway is highly regulated and includes many proteins that, in addition to BARD1, may also be substrates for BAP1-mediated ubiquitin hydrolysis. Exposure to DNA-damaging agents, such as asbestos, UV light, and ionizing radiation, induces DNA damage that is rapidly repaired with the help of nuclear BAP1. BAP1 cytoplasmic activities (bottom). The integrity of the IP3R3 ER channels requires the presence of normal amounts of BAP1 that remove ubiquitin from IP3R3. The balance between ubiquitylation mediated by FBXL2 (150) and deubiquitylation mediated by BAP1 (4) maintains a proper amount of IP3R3 required for Ca^{2+} transfer from the ER to the mitochondria. Mitochondria need Ca^{2+} for oxidative phosphorylation; however, higher than physiologic Ca^{2+} concentrations in the mitochondria cause apoptosis, a mechanism used to eliminate cells that accumulate extensive DNA damage that cannot be repaired. This mechanism prevents cells with DNA damage from propagating, thus preventing cancer development (4). MCU, mitochondrial calcium uniporter.

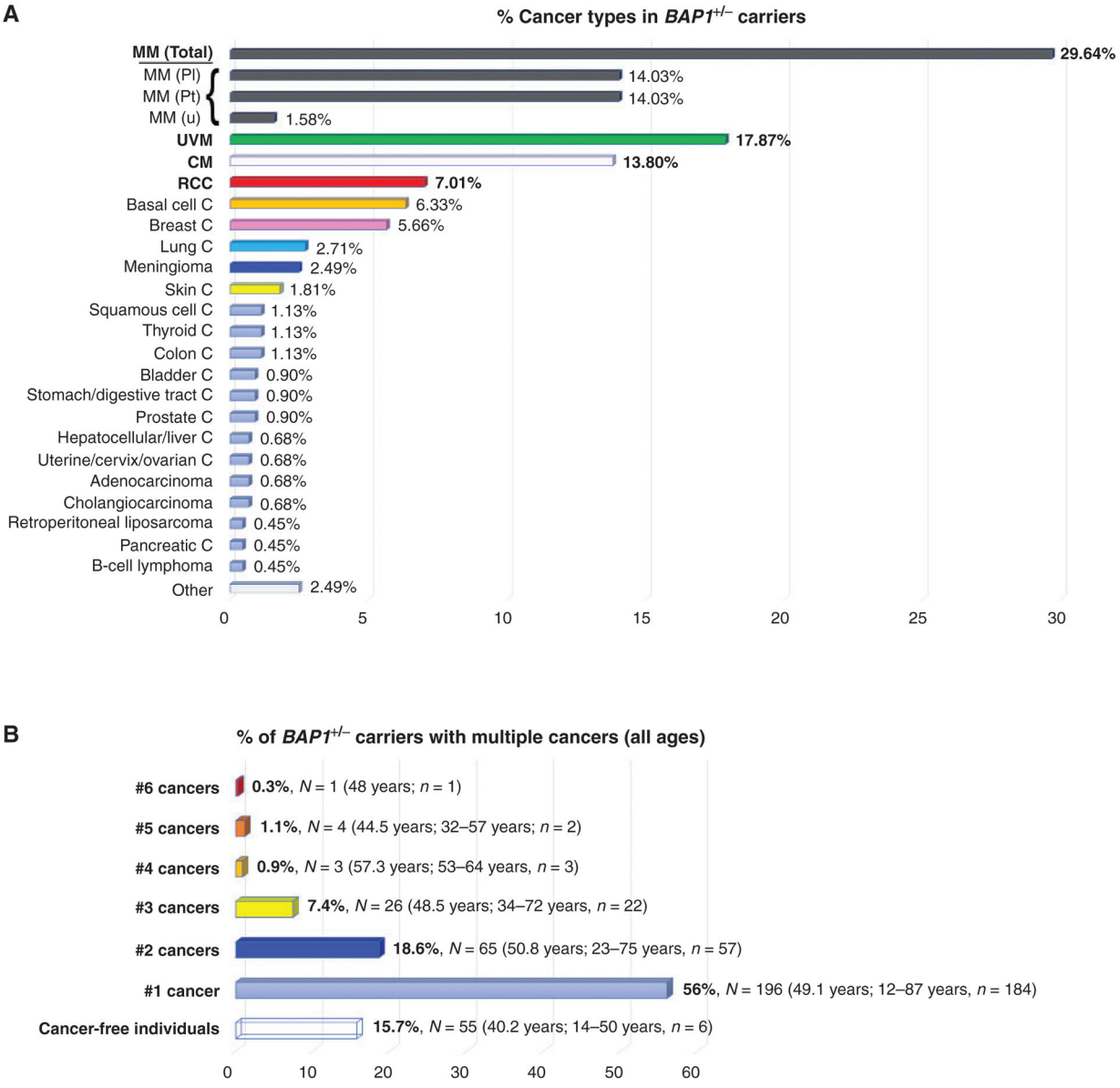

Figure 2.

Cancer types and age of onset of tumors presenting in *BAP1*$^{+/-}$ carriers. **A,** Occurrence of cancer types expressed as percentage. Data were collected from 45 articles published up to September 30, 2019 (4, 14, 16, 18, 44–47, 50, 51, 53, 54, 56–58, 64, 99, 151–177), for a total of 350 *BAP1*$^{+/-}$ carriers (all ages); of them, 295 (84.3%) developed cancer, for a total of 442 different cancers (as several of them developed more than one cancer). Note that to avoid the risk of including nonpathogenic *BAP1* variants, we used very stringent criteria to select these patients: Only germline *BAP1* mutation carriers with a family history of BAP1-core cancers were included. Specifically, the cohort shown in this figure includes cases from 140 published families. Core cancers of the BAP1 cancer syndrome are indicated in bold. CM, cutaneous melanoma; MM, malignant mesothelioma all sites; Pl, pleural malignant mesothelioma; Pt, peritoneal malignant mesothelioma; u, malignant mesothelioma, site not

specified; UVM, uveal melanoma; C, carcinoma. **B,** The percentage of *BAP1*$^{+/-}$ carriers of all ages with one or multiple cancers. The average age of diagnosis of the first cancer, and relative range in years, are shown in parentheses. Individuals in the group "Cancer-free individuals" are 50 years of age or younger, and thus have not reached the age when cancer has occurred in most *BAP1*$^{+/-}$-mutant carriers. *N*, number of individuals; *n*, number of individuals whose age at diagnosis was known. Example: 7.4% of patients developed 3 different cancers (total of 26 patients). The age of onset of tumors was known for only 22 of 26 of them; the median age of first tumor onset was 48.5 years; the range of first tumor development was between 34 and 72 years old.

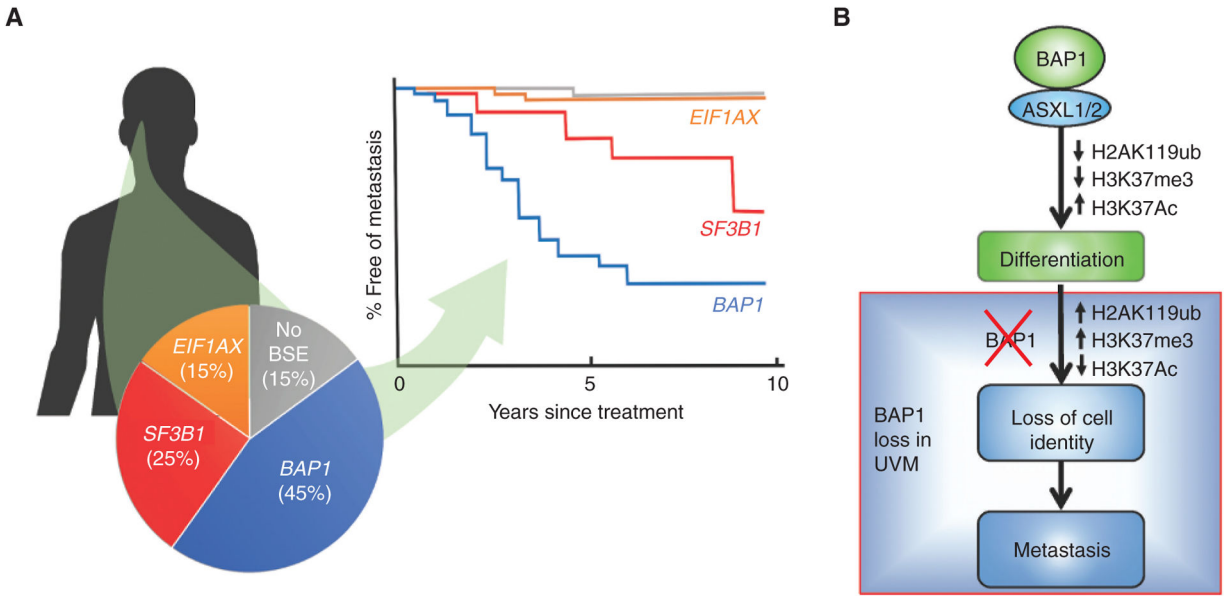

Figure 3.

BAP1 mutations in uveal melanoma (UVM). **A,** Uveal melanomas arise in the iris, ciliary body, and choroid of the uveal tract of the eye. Their metastatic potential is determined by mutually exclusive "BSE" progression mutations in *BAP1, SF3B1* (and rarely in other splicing factors), and EIF1AX. Inactivating mutations in *BAP1*, when coupled with the loss of the other copy of chromosome 3, result in high metastatic risk associated with the class 2 gene-expression profile. Hemizygous mutations in *SF3B1* (or rarely in other splicing factors) retain the class 1 gene expression profile and are associated with intermediate metastatic risk. Hemizygous mutations in the translation initiation factor *EIF1AX* also retain the class 1 gene expression profile and are associated with low metastatic risk. Uveal melanomas without BSE mutations have a prognosis similar to those with *EIF1AX* mutations. This figure represents a synopsis of published data (68, 101, 178). **B,** Recent work indicates that BAP1 regulates the switch from progenitor to differentiated cell types in vertebrate development, not only through effects on H2A ubiquitination but perhaps more importantly by repressing HDACs and allowing acetylation of H3K27 to activate genes involved in differentiation in neural crest and other lineages. Loss of BAP1 abrogates this differentiation switch in development that parallels phenotypic and transcriptomic alterations observed in association with *BAP1* mutation in uveal melanoma (114).

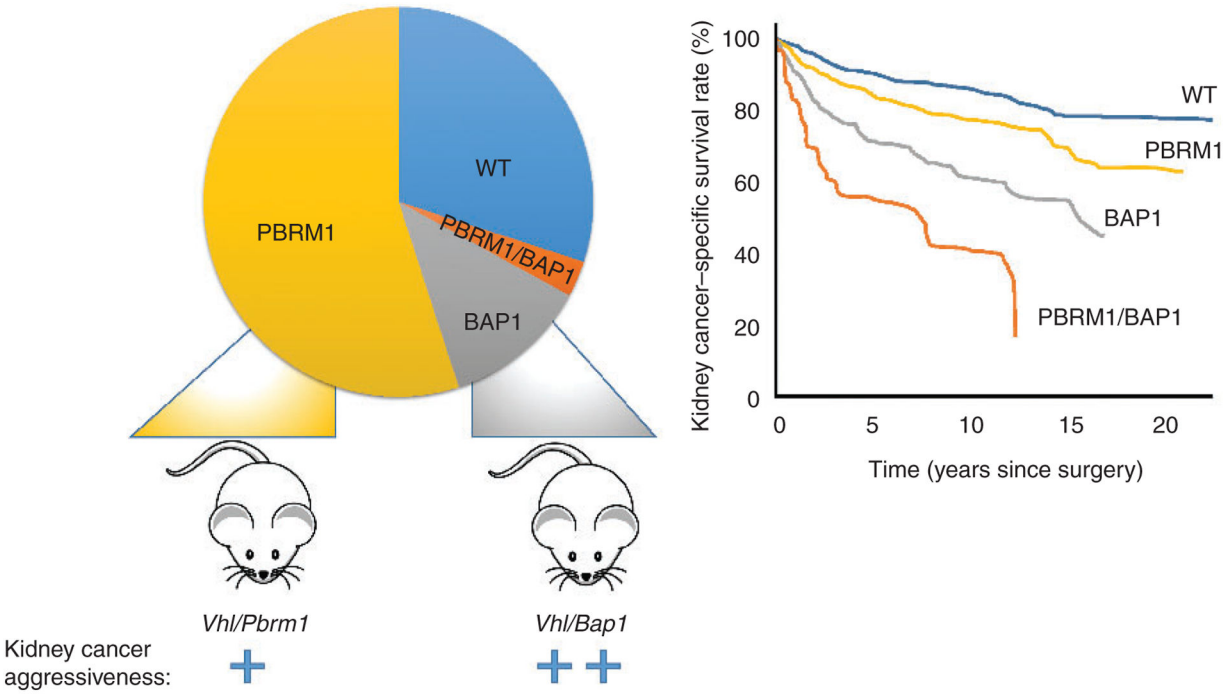

Figure 4.

BAP1 and PBRM1 establish the foundation for a molecular genetic classification of renal cancer with prognostic implications. ccRCC can be classified into 4 subtypes according to *BAP1* and *PBRM1* status, and these subtypes are associated with differential kidney cancer–specific survival in patients (20, 98). Targeted disruption of *Vhl* and either *Pbrm1* or *Bap1* genes in the mouse kidney induces ccRCC of low and high grade, respectively, similar to human tumors (117). The pie chart shows the inactivation of PBRM1, as shown by loss of protein expression, in 55% of cases. By measuring protein expression, we are able to integrate both mutational and epigenetic mechanisms of gene inactivation.

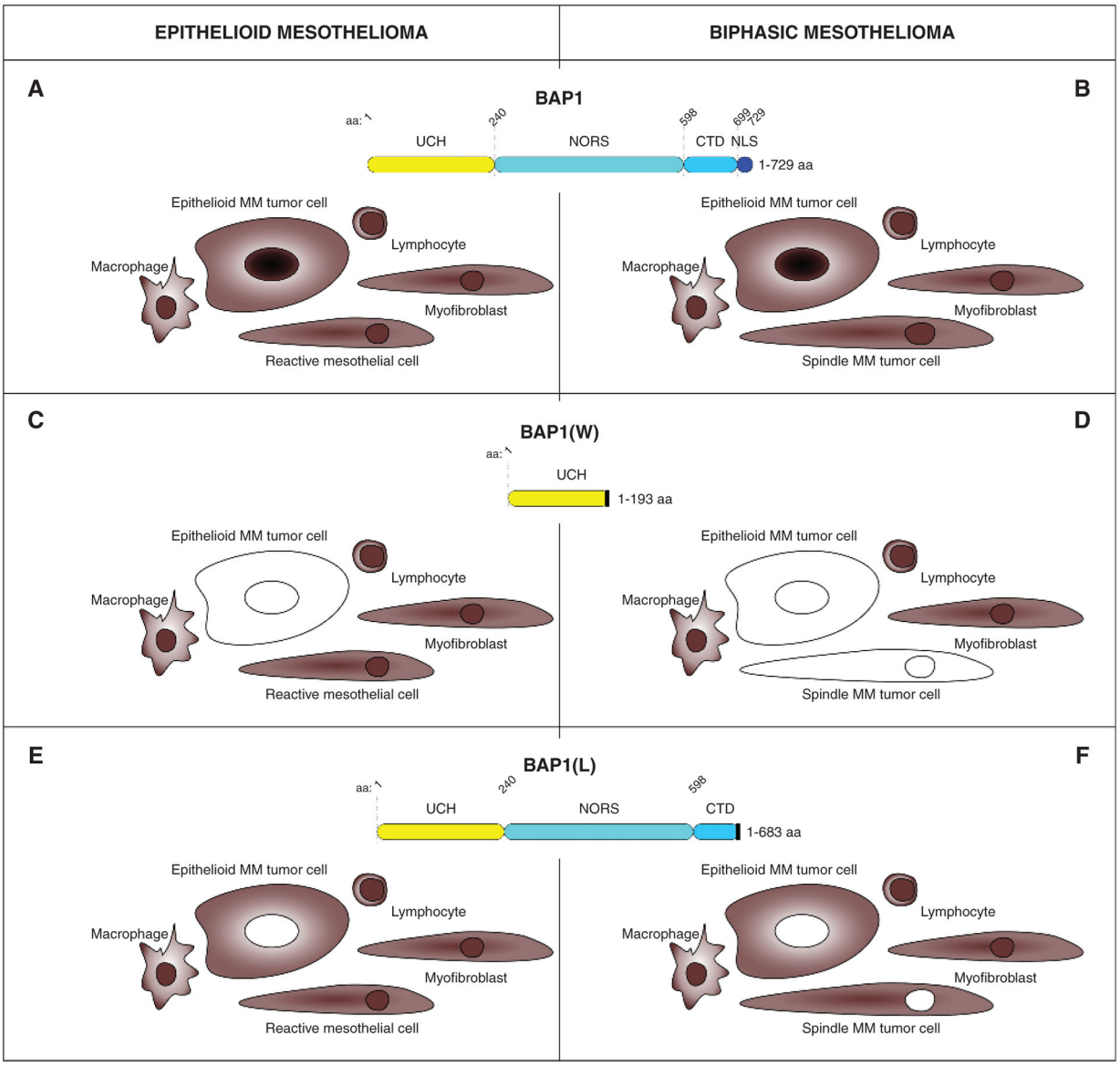

Figure 5.

BAP1 immunostaining in mesothelioma. Wild-type *BAP1.* Epithelioid (**A**) and biphasic (**B**) mesotheliomas with wild-type *BAP1* found in about 30% of cases show both nuclear and cytoplasmic BAP1 staining, as observed in nearby benign reactive cells. Nuclear and cytoplasmic staining in these cases is strong evidence of wild-type *BAP1* but is not helpful in the differential diagnosis. **C** and **D,** Mutated *BAP1.* Negative nuclear and cytoplasmic BAP1 staining is found in about two thirds of the mutated cases, and it is associated with positive staining in nearby stromal and inflammatory cells. This is strong evidence of malignancy and supports the diagnosis of mesothelioma over other cancer types that can metastasize to the pleura/peritoneum. This IHC pattern is seen mostly in tumors carrying truncating mutations resulting in large *BAP1* deletions. **C,** Epithelioid mesothelioma; only the epithelioid mesothelioma cells lost BAP1 staining. **D,** The presence of spindle tumor cells (BAP1-negative) supports the diagnosis of biphasic mesothelioma. W, representative

BAP1 truncating mutation found in the W family. **E** and **F,** Mutated *BAP1.* Negative nuclear staining but positive cytoplasmic BAP1 staining is found in about one third of the mutated cases together with positive nuclear and cytoplasmic staining in nearby stromal and inflammatory cells. As for **C** and **D**, this is also strong evidence of malignancy and supports the diagnosis of mesothelioma over other cancer types that can metastasize to the pleura/peritoneum. This IHC pattern is seen mostly in tumors carrying truncating mutations resulting in small *BAP1* deletions. **E,** Epithelioid mesothelioma; only the epithelioid mesothelioma cells lost BAP1 nuclear staining. **F,** The presence of spindle tumor cells with negative nuclear BAP1 staining supports the diagnosis of biphasic mesothelioma. Note that BAP1 is retained in the nuclei of background reactive benign mesothelial spindle cells; the latter have a slightly smaller size and bland nuclear features. L, representative *BAP1* truncating mutation found in the L family.

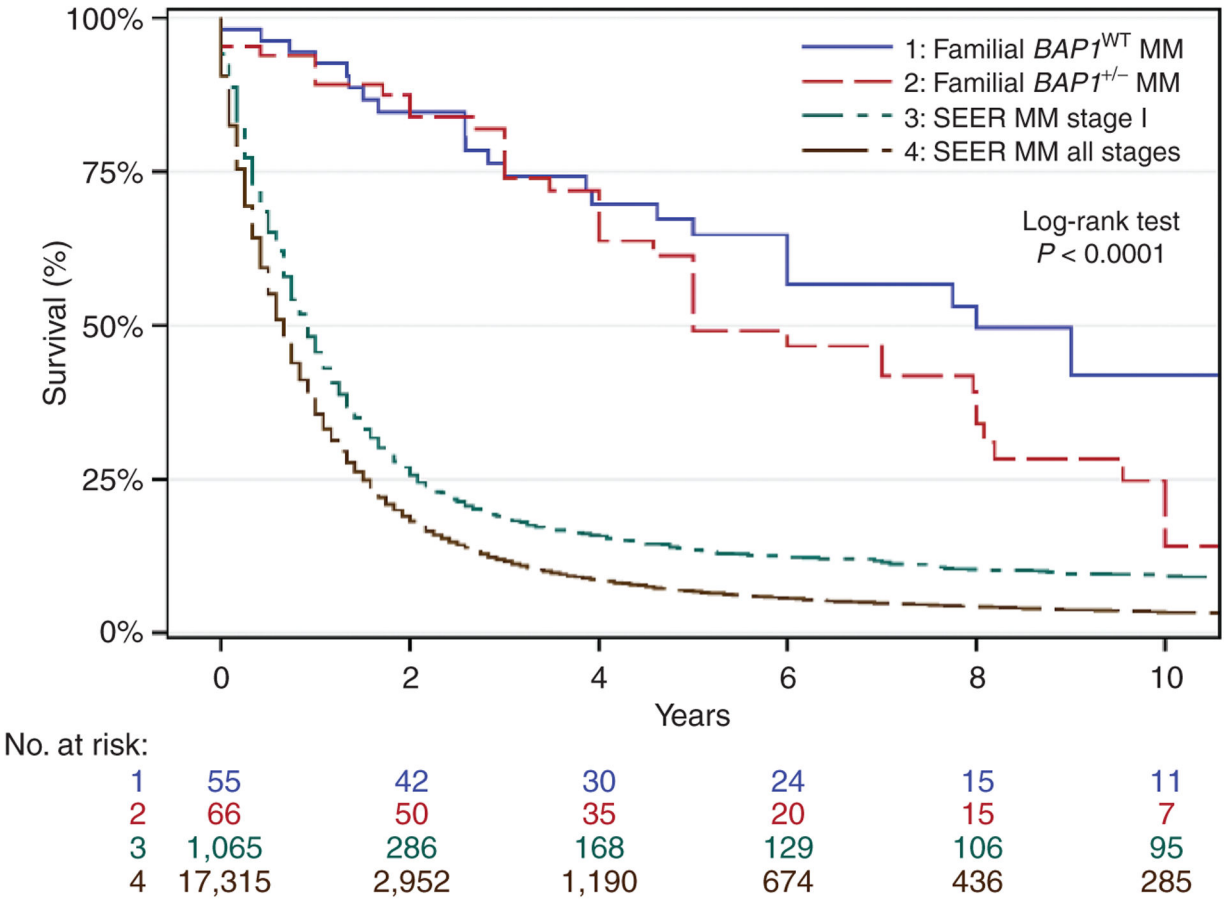

Figure 6.

Survival analysis of individuals with sporadic mesothelioma or familial mesothelioma by *BAP1* mutation status. Kaplan–Meier survival probability versus years with number at risk. Survival data combine results from references: (54, 67, 99). Blue, familial $BAP1^{WT}$ mesothelioma (median survival, 8 years; 10-year survival, 42.0%); red: familial $BAP1^{+/-}$ mesothelioma (median survival, 5 years; 10-year survival, 14.1%); green: SEER, stage I (median survival, 11 months; 10-year survival, 9.2%); brown: SEER, all stages (median survival, 8 months; 10-year survival, 3.3%). Rows below the graph indicate the number of patients at risk in each cohort per year. $BAP1^{+/-}$, heterozygous BAP1-inactivating mutations; $BAP1^{WT}$, wild-type BAP1; MM, malignant mesothelioma.