

BAP1 mutations in high-grade meningioma: implications for patient care

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Abstract

We have recently shown that the breast cancer (BRCA)1-associated protein-1 tumor suppressor gene (*BAP1*) is inactivated in a subset of clinically aggressive meningiomas that display rhabdoid histomorphology. Immunohistochemistry for BAP1 protein provides a rapid and inexpensive method for screening suspected cases. Notably, some patients with BAP1-mutant meningiomas have germline *BAP1* mutations and BAP1 tumor predisposition syndrome (TPDS). It appears that nearly all patients with germline *BAP1* mutations develop malignancies by age 55, most frequently uveal melanoma, cutaneous melanoma, pleural or peritoneal malignant mesothelioma, or renal cell carcinoma, although other cancers have also been associated with BAP1 TPDS. Therefore, when confronted with a patient with a potentially high-grade rhabdoid meningioma, it is important that neuropathologists assess the BAP1 status of the tumor and that the patient's family history of cancer is carefully ascertained. In the appropriate clinical setting, genetic counseling and germline *BAP1* DNA sequencing should be performed. A cancer surveillance program for individuals who carry germline *BAP1* mutations may help identify tumors such as uveal melanoma, cutaneous melanoma, and renal cell carcinoma at early and treatable stages. Because BAP1-mutant meningiomas are rare tumors, multi-institutional efforts will be needed to evaluate therapeutic strategies and to further define the clinicopathologic features of these tumors.

Key words

BAP1 | immunohistochemistry | meningioma | mesothelioma | review

The identification of germline and somatic *BAP1* mutations in meningiomas has defined a new genetic/molecular subtype of meningioma.¹ While this finding allows for the recognition of these tumors and for the implementation of genetic testing and counseling for patients and their families, much still remains to be learned about the clinical and pathologic features of these tumors and their therapeutic vulnerabilities. This review will summarize our current understanding of breast cancer (BRCA)1-associated protein-1 (BAP1) mutant meningiomas and will highlight insights gleaned from other tumors that arise as part of the

BAP1 tumor predisposition syndrome (BAP1-TPDS) (OMIM #6142327).

Meningioma Genetics

Recent genomic studies of meningiomas have elucidated a rich array of subtypes with distinct genetic, clinical, and pathologic features. Anterior skull base meningiomas often harbor mutations in Smoothened or in the ubiquitin ligase tumor necrosis factor receptor-associated factor

7 (TRAF7).²⁻⁵ Mutations in *AKT1*, *PIK3CA*, or *KLF4* often co-occur with *TRAF7* mutations.⁴⁻⁶ Such skull base meningiomas are often of the meningotheelial subtype, and *TRAF7/KLF4*-mutant cases display secretory features.^{4,7} Posterior skull base meningiomas and meningioma of the cerebral convexities often harbor mutations in *NF2*, and such meningiomas tend to display fibroblastic histology.⁴⁻⁶ Angiomatous meningiomas generally lack *NF2* mutations but have multiple chromosomal polysomies.⁸ Inactivation of *NF2* is enriched in higher-grade meningiomas.⁹ Some very aggressive meningiomas have *CDKN2A* inactivation¹⁰ or *TERT* promoter mutations.¹¹⁻¹³

These efforts have defined a molecular framework for improving the management of patients with meningiomas (Fig. 1). These advances should improve diagnostic concordance¹⁴ and help identify patients susceptible to recurrence.^{15,16} A similar framework was recently developed for gliomas, incorporating molecular information into classification and clinical management paradigms, and is strongly supported by the neuro-oncology community.¹⁷ Despite advances in the molecular subtyping of meningiomas and the identification of targetable mutations, pharmacotherapy for meningiomas has remained largely experimental and patients continue to suffer extensive morbidity.¹⁸⁻²¹

Germline and Sporadic BAP1 Mutations Define a Distinct Meningioma Subclass

Germline mutations in the *NF2* tumor suppressor gene are well established in the pathogenesis of meningiomas in individuals with the neurofibromatosis type 2 TPDS.²² More recently, germline mutations in *SMARCE1* have been described in families with spinal clear-cell meningiomas^{23,24}

and in *SMARCB1* in families with schwannomas and multiple meningiomas preferentially arising in the falx cerebri.^{25,26}

The association between meningioma and *BAP1* germline mutations was first reported in case reports of 3 affected families. In one study, a family with p.Q267X truncating mutation had 3 affected individuals, one with uveal melanoma, one with lung adenocarcinoma, and one with meningioma.²⁷ In another study, a family with an extensive history of malignant mesothelioma and a p.Y646X truncating mutation had 2 members who also developed meningioma.²⁸ In a third study, a family with the diagnosis of multiple carcinomas of unknown primary and a p.D404X truncating mutation developed a metastatic neoplasm first diagnosed as a papillary carcinoma but reclassified as a primary papillary meningioma at autopsy.²⁹

In our recent work, in an effort to identify potential genomic aberrations that underlie the rhabdoid meningioma histologic subtype, we collected a multi-institutional set of meningiomas that were clinically annotated as having rhabdoid histologic features.¹ Characterization of such alterations has been challenging because anaplastic meningiomas, especially rhabdoid meningiomas, are uncommon,^{30,31} and there is a significant interobserver variability in the diagnosis of this entity and in the recording and recognition of rhabdoid features. Moreover, while the rhabdoid subtype was originally defined as exclusively high grade and aggressive,³² recent work has shown that in the absence of overt high-grade histologic features, some meningiomas with rhabdoid cytomorphology have indolent behavior akin to World Health Organization (WHO) grade I tumors.³³ This suggested that meningiomas with rhabdoid histologic features may be genetically diverse.

Our collection of tumors was amassed from 60 patients, including those with minimal rhabdoid morphology and

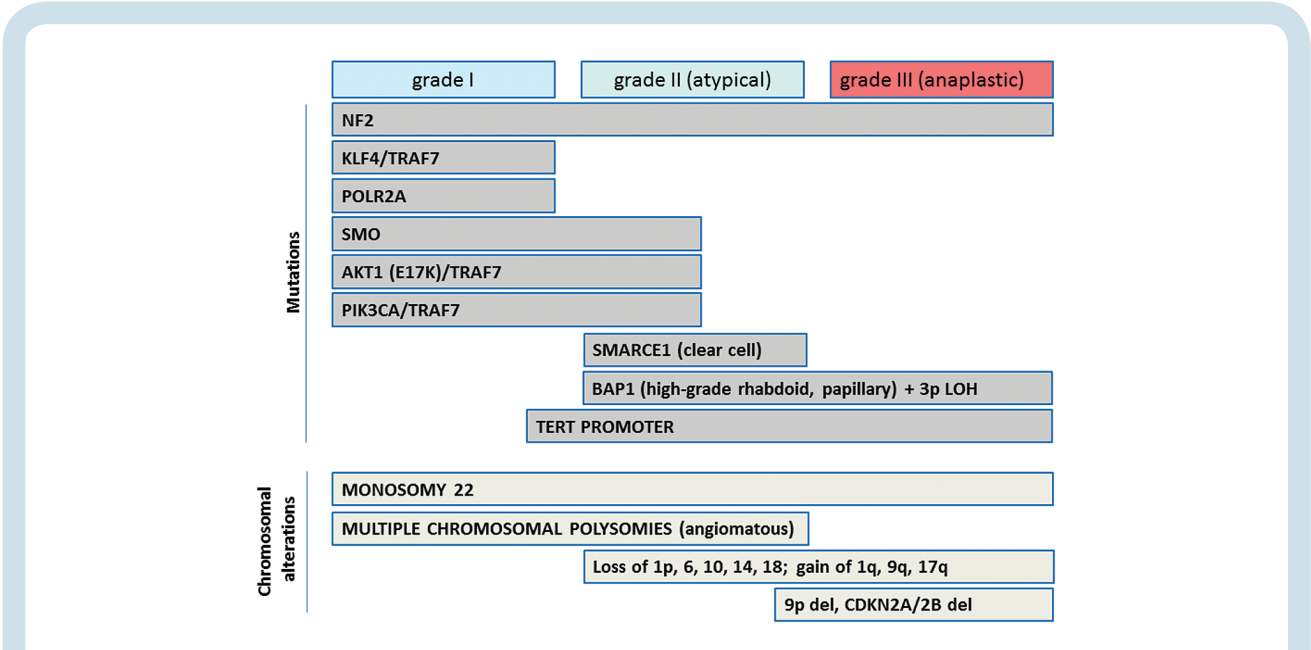


Fig. 1 Summary of major genomic events in meningioma.

low-grade features and others with classic rhabdoid morphology and high-grade features. Among these, we found 6 patients who had tumors with loss of BAP1 protein noted by immunohistochemistry (IHC). Each of these BAP1-negative tumors had inactivating *BAP1* genetic events. These aberrations included focal deletions of individual exons, as well as splice site, nonsense, and frameshift mutations. One case had an intra-chromosomal fusion of genes flanking *BAP1* that led to loss of the *BAP1* allele. All of these aberrations were coupled with single copy loss of chromosomal arm 3p, where the *BAP1* gene resides. Five of 6 cases had *BAP1* inactivated in the earliest samples available for testing. One case, however, only had a single copy 3p loss at first diagnosis and intact BAP1 protein expression. Inactivation of the second allele and loss of BAP1 protein occurred in the first recurrence, demonstrating that meningiomas with single copy 3p loss can develop complete BAP1 inactivation.

Germline *BAP1* mutations may be common events in patients with BAP1-mutant meningiomas. In our samples, germline analysis was only possible on 4 of 6 patients, 2 of whom had germline mutations—one with a c.660 splice site mutation and one with a p.Y173X nonsense mutation. No germline *BAP1* mutation was found in the 2 other BAP1-mutant patients, supporting both germline and somatic inactivation of *BAP1* in rhabdoid meningiomas. In our subsequent clinical work, we identified 2 additional patients with BAP1 IHC negative meningiomas, and both of these patients had germline mutations. Thus, it appears that unlike other cancers with frequent *BAP1* mutations, BAP1-mutant meningiomas may arise more commonly in the setting of germline mutations. While *BAP1* is the most frequently mutated gene in sporadic malignant mesothelioma³⁴ and in metastatic uveal melanoma³⁵ (Fig. 2), only <1%–4% of such BAP1-mutant tumors arise in patients with germline *BAP1* mutation.^{27,34,36–41}

It is unclear whether syndromic and sporadic BAP1-mutant meningiomas are clinically distinct. In malignant mesothelioma, for example, there are significant differences between the clinicopathologic features of sporadic BAP1-mutant tumors and those that arise in BAP1-TPDS. For instance, germline *BAP1* mutations alter the anatomic location of mesothelioma development, with one-half arising in the peritoneum and the other half in the pleura, whereas sporadic mesotheliomas occur much more frequently in the pleura (9:1).^{42,43} Moreover, germline-related BAP1-mutant mesotheliomas arise 20 years earlier than sporadic mesotheliomas, which generally arise in patients in their early seventies. Germline-related BAP1 mesotheliomas occur slightly more frequently in females, whereas sporadic mesotheliomas occur with a male-to-female ratio of 4:1. Also, while *BAP1* mutations are associated with a poor prognosis in patients with clear cell renal cell carcinoma, cutaneous melanoma, and uveal melanoma, somatic *BAP1* mutations in malignant mesothelioma do not appear to be more aggressive than tumors with intact BAP1,^{44,45} with some studies showing less aggressive behavior.³⁸

Characterizing the impact of additional genetic mutations in rhabdoid meningiomas will also be highly important. It will be interesting to identify mutations in genes such as *NF2* and others that can co-occur along with *BAP1* mutations, as well as the mutated genes that drive the development of BAP1 wild-type rhabdoid meningiomas. The genomics of uveal melanoma may provide an example. Early in the development of both indolent and aggressive uveal melanomas, mutations arise in either *GNAQ* or *GNA11* at hotspot residues.^{46,47} Indolent tumors tend to have additional mutations in *SF3B1* or *EIF1AX*,⁴⁸ whereas metastatic tumors have BAP1 inactivation. Thus, there are distinct genetic differences between low and high malignant potential tumors. The presence of *BAP1* mutations in uveal melanoma is strongly associated with metastasis

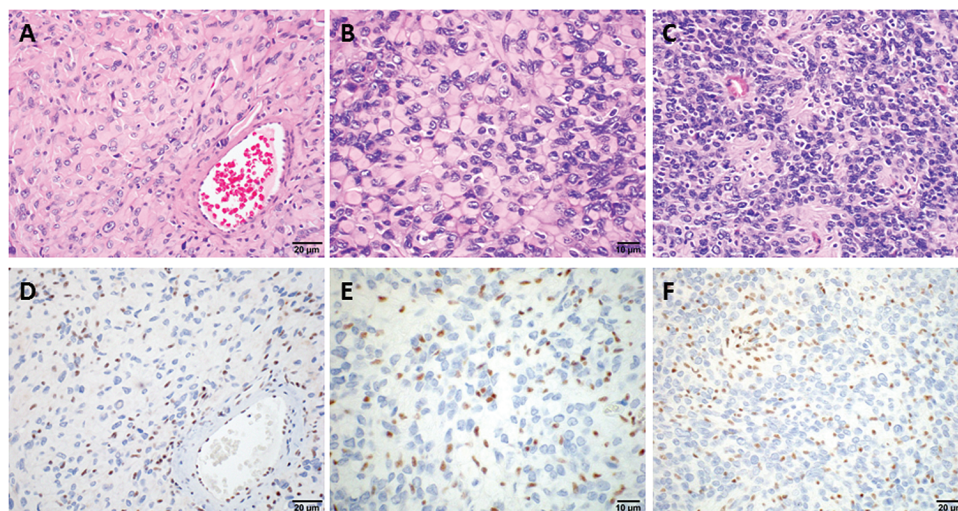


Fig. 2 Histology of BAP1-mutant tumors and BAP1 immunohistochemistry showing diverse histologic features.

and mortality, with relative risks of 10.6 and 9.0, respectively, in one study.⁴⁸

Unlike in uveal melanoma, mutations in *GNAQ*, *GNA11*, *SF3B1*, and *EIF1AX* do not appear to be common in the high-grade rhabdoid meningiomas that have been studied to date by targeted and whole exome sequencing.¹ However, the example of mutually exclusive mutations in good prognosis (*SF3B1/EIF1AX*) and poor prognosis (*BAP1*) uveal melanomas suggests that a similar pattern of good prognosis and bad prognosis driver events may underlie the phenotypes of low-grade and high-grade rhabdoid meningiomas.

Clinical Outcome

Data from our cohort suggest that *BAP1*-mutant meningiomas follow an aggressive clinical course. The mitotic index of these tumors varied between 5 and 28 mitoses per 10 high-powered fields. Thus, all of the cases met the threshold for the designation of at least a WHO grade II atypical meningioma. In 2 cases, mitoses surpassed the 20 per 10 high-powered fields criterion needed for designation as WHO grade III anaplastic meningioma, although that elevated level of mitotic activity was only found in samples that had already recurred more than once. Outcome analysis was limited by sample size but supported that patients with *BAP1*-mutant meningiomas have very poor clinical outcomes, with multiple recurrences and significantly shortened overall survival. Thus, the current data support that such tumors are high grade and clinically aggressive. Several additional *BAP1*-mutant cases that we have identified since the publication have behaved aggressively. Loss of *BAP1* function has been linked to alterations in methylation status.⁴⁹ Such epigenetic alterations have recently been demonstrated to resolve meningioma subtypes into groups with shared natural history.^{50,51} Multi-institutional studies will be needed to further assess prognostic factors in these meningiomas.

Histologic Features

Our understanding of the histologic features of *BAP1*-mutant meningiomas remains at an early stage. Our work suggests that the histologic spectrum of *BAP1*-mutant meningiomas is diverse (Fig. 3). While all 6 of the *BAP1*-mutant meningiomas in our cohort had overt rhabdoid morphology, additional features were also noted. These included large sheets of de-differentiated cells as well as epithelioid-type cells with sharply demarcated cytoplasmic membranes that line fibrovascular papillary cores consistent at least focally with a papillary growth pattern. This pattern of growth seems reminiscent of the case reported by another group in a *BAP1* germline mutation carrier, in which the tumor was reclassified after autopsy as a papillary meningioma rather than a papillary carcinoma.²⁹ Because of these varied histologic features, a high index of suspicion is required to identify some cases that have *BAP1* mutations. While we show that 265 meningiomas without rhabdoid features maintain *BAP1* expression by IHC, some *BAP1*-mutant meningiomas may not have well-developed

and easily recognizable rhabdoid features, and such features may only become overt following tumor recurrence or may be obfuscated by co-occurring histologic patterns. It is also too early to determine whether *BAP1*-mutant tumors that arise in *BAP1*-TPDS will have histologic features that are distinct from sporadic *BAP1*-mutant meningiomas that only have somatic *BAP1* mutations. These are all areas that require further investigation.

Detecting BAP1 Inactivation

BAP1 IHC readily captures the broad range of alterations that can lead to *BAP1* protein deficiency. Because IHC provides a fast, reliable, accessible, and inexpensive method for identifying patients with *BAP1*-deficient tumors, it appears that IHC is the most robust methodology for screening samples. *BAP1* inactivation occurs through a broad range of genomic aberrations, including deletions of entire exons or genomic regions that are even larger. Thus, some nucleotide sequencing methods are unable to identify *BAP1* mutations despite loss of *BAP1* protein by IHC.^{52,53} This discordance may be the result of limitations of the sequencing methods to detect complex genomic aberrations that inactivate *BAP1* but could also be explained by mechanisms such as mutations in promoters, enhancers, or introns that are not covered by commonly used sequencing approaches.⁵⁴ Altered methylation of the *BAP1* promoter has not been detected and likely does not play a role in suppressing *BAP1* expression.^{52,55}

Analysis strategies that simultaneously assess single nucleotide substitutions, indels, gene fusions, and copy number changes are best suited for characterizing *BAP1* in clinical practice and in research studies.^{8,56,57} However, it appears that neither IHC nor sequencing offers 100% sensitivity⁵⁸ and that heterogeneous IHC staining may be seen in cases that harbor *BAP1* mutations.⁵⁸ Thus, in situations where high sensitivity is needed, both IHC and sequencing methodologies can be used to assure optimal sample characterization.

In sequencing data, it is generally straightforward to assess the pathogenicity of indels and frameshift, nonsense, and splice site mutations; however, missense mutations leading to single amino acid substitutions are more problematic to categorize confidently. Single nucleotide variants can indeed lead to pathogenic single amino acid substitutions but can also simultaneously affect mRNA splicing. In lieu of experimental analysis, the effects of such nucleotide alterations can be evaluated using predictive algorithms that calculate the impact of mutations on mRNA splicing, helping explain protein loss and corroborating the importance of a particular mutation on *BAP1* function.

Genetic Counseling

Assessing family cancer history is now a critical part of the evaluation of patients who have meningiomas with rhabdoid morphologies. To date, 4 major malignancies have been associated with *BAP1*-TPDS, including malignant mesothelioma, uveal melanoma, cutaneous melanoma, and

renal cell carcinoma (Table 1). Malignant mesothelioma is the most common malignancy to arise in families with germline *BAP1* mutations, with some families having multiple members with mesothelioma,^{43,59} suggesting a very high susceptibility for mesothelioma in this population. Both patients in our rhabdoid meningioma cohort with germline *BAP1* mutations had family members with mesothelioma.

It is important to note that at least one malignancy has developed in nearly all people over 55 years of age who carry germline *BAP1* mutations, and many have developed multiple tumors.^{59,60} An important hallmark of *BAP1*-TPDS

is the presence of atypical melanocytic tumors,⁶¹ which have been referred to as both “atypical Spitz tumors”⁶² and melanocytic *BAP1*-mutated atypical intradermal tumors, or MBAITs.⁶³ These cutaneous lesions are tan to reddish-brown dome-shaped papules and present in one-half to two-thirds of germline carriers, often appearing well before associated malignancies arise.

Ideally all patients with *BAP1* IHC-negative rhabdoid meningiomas should be screened for germline *BAP1* mutations if resources are available. Annual dermatological and ophthalmological screening may help identify early-stage

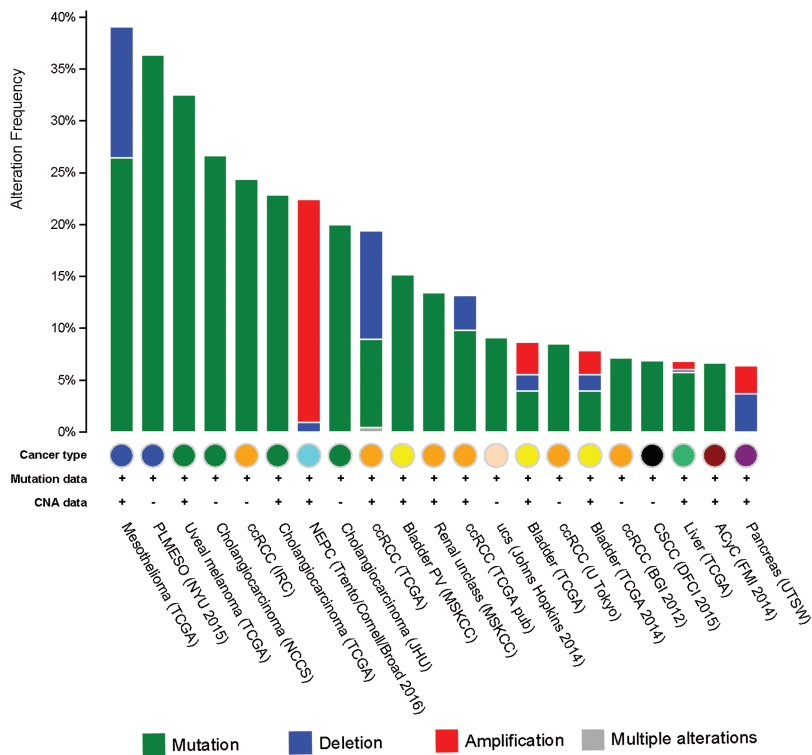


Fig. 3 Cross-cancer alteration summary of *BAP1* alterations from cBioPortal of Cancer Genomics, indicating cancers most commonly harboring *BAP1* aberrations.

Table 1 Frequency of germline and sporadic mutations in tumors with *BAP1* inactivation and frequency of tumors arising in individuals harboring germline *BAP1* mutations

Tumor Type	Prevalence of Germline <i>BAP1</i> Mutations	Prevalence of Somatic <i>BAP1</i> Mutations	Frequency of Tumors Arising in <i>BAP1</i> -TPDS Individuals
Uveal melanoma	1%–2% ⁸⁵	40%–45% ^{48,54}	31%
Mesothelioma	1%–2% ³⁷	23%–64% ^{34,86}	22%
Cutaneous melanoma	0.2%–1% ⁸⁷	2.7% ⁸⁸	13%
Renal cell carcinoma	<1% ⁸⁹	10%–15% ^{83,89}	10%
Basal cell carcinoma	n/a	n/a	6.3%
Cholangiocarcinoma	n/a	25% ⁹⁰	2.3%
Meningioma (all histologies)	<1% ¹	<1% ¹	1.7%

uveal and cutaneous melanomas. Urinalysis and ultrasound may help detect renal cell carcinomas. Other tumors such as rhabdoid meningiomas and mesotheliomas could be more amenable to treatment if identified earlier in the disease course, but appropriate screening approaches for these tumors are unclear.

The age at which to begin dermatological and ophthalmological screening is not clear. Because uveal melanomas can arise before 20 years of age,^{41,59,64,65} screening in the teenage years may be warranted. As the full extent of tumorigenesis in BAP1-TPDS remains incompletely understood, appropriate screening surveillance plans will evolve over time. The importance of screening seems certain due to the high tumor burden of individuals with BAP1-TPDS.⁴⁰

BAP1 Is a Multifaceted Tumor Suppressor

BAP1 has multifaceted roles in tumor biology. *BAP1* is located on chromosome 3p21.1. The gene comprises 17 exons and encodes a 729 amino acid, 90 kDa protein with an N-terminal ubiquitin carboxyl hydrolase (UCH) domain (aa 1–250), a central host cell factor 1 (HCF1) binding domain (aa 365–385), a C-terminal domain with a coiled-coil motif for interacting with addition of sex combs-like 1 and 2 (ASXL1/2) (aa 635–693), and 2 C-terminal nuclear localization sequences (aa 656–661 and aa 717–722). BAP1 functions as a tumor suppressor that requires nuclear localization and its deubiquitinase function. This function is mediated by the multifaceted roles that BAP1 plays as part of the Polycomb deubiquitinase, as a transcriptional coregulator, and as a component of the DNA repair response.

The nuclear localization of BAP1 is influenced by the ubiquitin-conjugating enzyme UBE2O, which multiply mono-ubiquitinates the nuclear localization signal of BAP1 and thereby mediates BAP1 cytoplasmic sequestration.⁶⁶ The inhibitory effect of UBE2O is counteracted by the deubiquitinase function of BAP1 itself. Cancer-associated mutations in BAP1 can increase the interaction with UBE2O and support cytoplasmic retention and inactivation of BAP1.⁶⁶

BAP1 modulates the activity of the transcriptional regulator HCF1, which modulates chromatin architecture by recruiting histone modifying complexes and activating transcription factors, including the E2F family, which controls G1/S phase cell-cycle progression. Loss of BAP1 results in a modest accumulation of HCF1, which promotes transition from G1 to S phase. BAP1 also modulates the function of O-linked N-acetylglucosamine transferase, the transcription factor Ying Yang 1, the mitochondrial respiratory chain component cytochrome *c* oxidase 7C, and the forkhead transcription factors FoxK1/K2.⁶⁷

BAP1 also interacts with ASXL1/2 to form the Polycomb group repressive deubiquitinase complex (PR-DUB). The Polycomb group proteins are critical transcriptional regulators of physiological processes such as stem cell pluripotency, embryonic development, self-renewal, and differentiation. This group of proteins contains Polycomb-repressive complexes (PRCs) that ubiquitinate histones, leading to gene silencing.⁴⁹ Transcriptional balance and control are achieved by ubiquitination by PRCs

and deubiquitination by PR-DUB. Loss of BAP1 has been shown to significantly alter expression of several known Polycomb target genes. Cancer-derived mutations that target the interaction of BAP1 and ASXL1/2 reduce PR-DUB activity, increase ubiquitination of histone 2A, deregulate the cell cycle progression, and hinder cellular senescence.⁴⁹

BAP1 is also important in DNA damage signaling and repair. It interacts with several homologous recombination proteins, including the BRCA1/BARD1 heterodimer and RAD51. The BRCA1/BARD1 complex has E3 ubiquitin ligase activity that regulates the DNA damage response. BAP1 binds and deubiquitinates BARD1, thus modulating the E3 ligase activity of this tumor suppressor complex.⁶⁸

Knockdown of BAP1 in a uveal melanoma line with wild-type BAP1 caused cells to adopt a rounded, epithelioid morphology. These cells grew as multicellular non-adherent spheroids and morphologically resembled the features of metastatic uveal melanoma cells.³⁵

Insights from Animal Models

Studies of genetically engineered mice have revealed important aspects of BAP1 in tumorigenesis.⁶⁷ BAP1 is essential for embryonic development. Conditional biallelic (homozygous) somatic knockout of *BAP1* in adult mice resulted in a myelodysplasia that resembled chronic myelomonocytic leukemia. These mice had monocytosis and neutrophilia along with thrombocytopenia and severe progressive anemia due to ineffective erythropoiesis. The mice had splenomegaly secondary to extramedullary hematopoiesis and expansion of the myeloid lineage.⁶⁷ Interestingly, one patient with myelodysplastic syndrome was identified who carried a somatic heterozygous frameshift mutation in *BAP1*; haplo-insufficiency in mice was shown to cause progressive hematological defects.⁶⁷ However, *BAP1* mutations are rare in myelodysplastic syndrome, unlike *ASXL1* mutations, which are found in 10%–20% of patients with myelodysplastic syndrome.⁶⁹

Mouse models of BAP1-TPDS are highly susceptible to tumor formation. Compared with wild-type littermates, mice with inactivation of one *BAP1* allele (*BAP1* +/-) had a higher incidence of peritoneal mesothelioma upon chronic exposure to asbestos fibers.^{70–72} This predisposition to mesothelioma development was observed in 4 different murine models of BAP1-TPDS, in which one allele of *BAP1* was inactivated in the mouse germline or conditionally in adult mice. In all models, the resulting mesotheliomas showed accelerated onset and were more proliferative and invasive, leading to an earlier death. The tumors which developed had biallelic *BAP1* inactivation.

Notably, mesotheliomas did not arise spontaneously in *BAP1* +/- mice that were monitored between 14 and 20 months,⁷⁰ but they did arise spontaneously in 2 cases upon longer follow-up.⁷¹ In one set of experiments, mice with inactivation of one allele of *BAP1* that were inclined to develop mesothelioma upon exposure to “standard” doses of asbestos were also highly susceptible to mesothelioma upon chronic exposure to very low doses of asbestos (one-tenth of “standard” exposure). These findings in multiple models of BAP1-TPDS support the idea that human

germline carriers of one inactivated *BAP1* allele may have a higher susceptibility to asbestos-induced mesothelioma formation and perhaps to other tumors due to other still undefined environmental exposures. In such people, the levels of asbestos needed to trigger mesothelioma formation may be much lower than those that are carcinogenic in the general population. Consistent with this, individuals with germline *BAP1* mutations who develop mesotheliomas often lack a history of exposure to asbestos.

In addition to asbestos-induced tumor formation, spontaneous tumor formation was also dramatically increased in murine models of *BAP1*-TPDS.⁷¹ The 2 spontaneous mesotheliomas developed at 19 and 29 months, one in the pleura and the other in the peritoneum; neither had high-grade histology or aggressive behavior. Long-term observation (up to 31 mo) of these mouse models demonstrated a marked susceptibility for the spontaneous development of a broad spectrum of tumors. This work further confirmed that *BAP1* is a tumor suppressor gene, yet the tumor types that typically develop in humans with *BAP1*-TPDS were not commonly observed in these mice. Nearly two-thirds of the female mice developed ovarian sex cord-stromal tumors, most commonly granulosa cell tumors. Nearly a third of these sex cord tumors developed distant metastases. Other common tumors in these mice were carcinomas of the lung and mammary glands and cutaneous spindle cell neoplasms.⁷¹ Most tumors developed late in life and a subset of mice had synchronous primaries. Meningiomas, uveal or cutaneous melanomas, and renal cell carcinomas did not develop. Even though these mice do not appear to spontaneously develop meningiomas, they could be useful models for identifying environmental and genetic factors that modulate meningioma development.

Modeling rhabdoid meningiomas in cell culture and in mice will be essential to gain a better understanding of the pathobiology of these tumors and to develop rational approaches for therapy. In uveal melanoma, *BAP1*-deficient lines appear difficult to isolate and are slow growing; however, mesothelioma cell lines frequently harbor *BAP1* mutations.^{73–75} Insights from work in these cell lines can help guide future studies in rhabdoid meningioma. As of yet, there is no direct experience in establishing patient-derived cell lines or xenografts from patients with rhabdoid meningiomas. Developing such resources is a high priority.

Potential Therapeutic Possibilities for *BAP1*-mutant Tumors

Most efforts in therapeutic development have focused on identifying small molecule drugs that can exploit vulnerabilities resulting from *BAP1* loss. Proposals have also been made to develop tumor-specific neo-epitope vaccines, particularly for patients with tumors with *BAP1* frameshift mutations that produce neo-peptides in truncated proteins.⁷⁶

Histone Deacetylase Inhibitors

Preclinical studies have implicated histone deacetylase inhibitors such as valproic acid as agents that can

influence the differentiation state of uveal melanoma cells that have intact or mutated *BAP1*.⁷⁷ Treatment with valproic acid induced changes that are consistent with morphological differentiation, reversed the ubiquitination of histone H2 that results from *BAP1* loss, induced cell cycle exit, and triggered a differentiated melanocytic gene expression profile in cell culture. Moreover, valproic acid demonstrated a modest effect on tumor growth in xenografts, suggesting that histone deacetylase inhibitors may reprogram highly aggressive uveal melanoma cells toward a less aggressive, more differentiated state. Such treatment may slow the progression of micrometastatic disease.⁷⁷ Clinical trials with the histone deacetylase inhibitors vorinostat (NCT01587352) and entinostat (NCT02697630) have been initiated in patients with metastatic uveal melanoma. However, enthusiasm for this approach is tempered by a phase III, randomized, double-blinded, placebo-controlled clinical trial of vorinostat in patients with advanced mesothelioma which revealed very few significant clinical responses,⁷⁸ a rate that is far below what might be expected if *BAP1*-mutated mesotheliomas were preferentially sensitive to vorinostat.

Based on the high frequency of *GNAQ* and *GNA11* mutations in uveal melanoma, 120 patients with metastatic uveal melanoma were treated with selumetinib, a non-ATP competitive inhibitor of mitogen-activated protein kinase (MEK)1 and MEK2. Genomic studies indicate that the majority of these metastatic uveal melanomas will have been *BAP1*-mutant. Despite increased progression-free survival and objective radiographic responses, overall survival was not increased by selumetinib.⁷⁹

Enhancer of Zeste Homolog 2 Inhibitors

One set of studies showed that *BAP1* loss led to profound alterations in chromatin proteins, including increased levels of trimethylated histone H3 lysine 27 (H3K27me3) and decreased histone H4 lysine 20 monomethylation as well as repression of *HoxA* genes.⁴⁹ Moreover, *BAP1* loss led to increased expression of the histone-lysine methyltransferase enhancer of zeste homolog 2 (EZH2) and a dependence on EZH2 for survival. *BAP1*-null cells were dependent on EZH2 for transformation. Notably, mesothelioma cell lines were highly sensitive to EZH2 inhibition either by small interfering (si)RNA or by EPZ011989, a potent and highly selective small molecule inhibitor of EZH2. A structurally related molecule, EPZ-6438 (tazemetostat, 800 mg p.o. b.i.d.) is being tested in a phase II, multicenter, open-label trial in patients with *BAP1*-deficient relapsed or refractory malignant mesothelioma (NCT02860286).

The findings in *BAP1*-deficient mesothelioma cells, however, may be context dependent. A study showed that *BAP1*-deficient uveal melanoma samples⁸⁰ did not have increased EZH2 mRNA levels, that *HoxA* genes were not repressed, and that trimethylated histone H3 lysine 27 was not increased. The authors did not observe differential growth inhibition between *BAP1* wild-type and mutant uveal melanoma cells treated with EZH2 inhibitor EPZ-6438. The differences between

mesothelioma and uveal melanoma cells may be due to critical differences in the effects of BAP1 loss in specific cell lineages and tissues, but could also result from technical differences and challenges in assessing the effects of epigenetic therapeutic approaches in in vitro and pre-clinical studies.⁸¹

Poly(ADP-Ribose)-Polymerase Inhibitors

Recent work has implicated a number of different deubiquitinases as important components of the DNA damage response. BAP1, also referred to as ubiquitin carboxyl-terminal hydrolase 2, belongs to the category of deubiquitinases that contain a UCH domain and has been shown to participate in a number of aspects of the DNA damage response. Studies have shown that BAP1 rapidly localized to sites of DNA damage induced by laser micro-irradiation, in a manner dependent on its UCH domain and its ubiquitin hydrolase catalytic activity.⁸² In doing so, BAP1 regulated accumulation of BRCA1, RAD51, and replication protein A at sites of DNA damage and influenced the efficiency of homologous recombination. BAP1 promoted the repair of double-strand DNA breaks and thereby enhanced survival following DNA damage.

ASXL1, which forms the PR-DUB along with BAP1, was also rapidly recruited to sites of DNA damage in a BAP1-dependent manner. Recruitment of BAP1 to sites of DNA damage appeared to be dependent on Ring finger 8 (RNF8)/RNF168 E3 ubiquitin ligases. Recruitment of BAP1 did not depend on ASXL1 or on ataxia telangiectasia mutated kinase (ATM), even though ATM phosphorylates BAP1 following irradiation. However, recruitment to DNA damage sites is highly dependent upon the activity of poly(ADP-ribose)-polymerase (PARP) 1 and 2. PARP1 or PARP2 siRNA knockdown or inhibition by the small molecule PARP inhibitor AG14361 abrogated BAP1 recruitment to sites of DNA damage.⁸²

Consistent with a role for BAP1 in the DNA damage response, BAP1 knockdown and BAP1-mutant mesothelioma cells were hypersensitive to irradiation.⁸² Notably, knockdown of BAP1 led to increased sensitivity to veliparib (ABT-888), a PARP inhibitor. Similarly, a BAP1-deficient renal cell carcinoma cell line was also sensitive to PARP inhibition.⁸³

Summary

The identification of *BAP1* germline and somatic mutation in meningiomas with rhabdoid histology defines a distinct subset of clinically aggressive meningiomas. The clinical management of such patients requires genetic counseling. Multi-institutional efforts are needed to better define the clinical, pathologic, and genetic features of *BAP1*-mutant meningiomas and to establish cell lines and mouse models needed to advance our understanding of the pathobiology of these tumors and to devise effective treatments.

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