Research Highlight

Important recent insights into the genetics and biology of malignant pleural mesothelioma

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Knowledge of the disease-specific genetic mutations in malignant pleural mesothelioma (MPM) has the potential to lead to rational targeted therapies. Recent studies have reported previously unknown recurrent genetic alterations in the *BAP1* and *LATS2* genes. Additional work has increased our understanding of the mechanism by which inactivating mutations in *NF2* cause tumorigenesis. This review will highlight these recent discoveries and their relevance to MPM therapeutics.

BAP1

BRCA-associated protein 1 (BAP1) is a 729 amino acid protein encoded by the *BAP1* gene at chromosome 3p21. A recent integrated genomics analysis identified somatic mutations and genomic losses of *BAP1* in approximately 25% of MPM (1). Subsequent groups have identified germline mutations of *BAP1* in families with a high incidence of MPM, uveal melanoma (UM), melanocytic tumors, cutaneous melanoma (CM), and other cancers (2,3). Somatic mutations of *BAP1* occur in approximately 84% of metastasizing UM, 14% of clear cell renal cell carcinoma (RCC), and a small subset of lung and breast cancer (4-7).

Functional studies of BAP1 have characterized the protein as a nuclear-localized deubiquitinase (DUB) and member of the ubiquitin carboxy-terminal hydrolase (UCH) family of DUBs. Mass spectrometry studies have identified host cell factor 1 (HCF1) and additional sex combs like protein 1 (ASXL1) as the major BAP1 binding partners. Together with HCF1, BAP1 modulates the expression of genes whose promoter regions are bound by the transcription factors E2F and YY1, or other as yet undefined transcription factors. HCF1 recruits histone methyltransferases to confer activating histone marks on the chromatin at promoter regions, thereby increasing gene transcription (8-10). As a binding partner with ASXL1, BAP1 forms the Polycomb repressive deubiquitinase (PR-DUB) complex, which cleaves ubiquitin from histone H2A. Histone H2A monoubiquitinated at lysine 119 is a regulatory mark in the Polycomb protein complex-mediated system of gene regulation (11). Polycomb proteins guide differentiation during embryogenesis, and defects in various subunits of the Polycomb protein complex have been found in a variety of cancers (12). Knockdown of BAP1 using siRNA has been shown to alter the expression of E2F and YY1-regulated genes and Polycomb-associated genes (1,10). Other possible functions of BAP1 include a role in DNA damage repair, but this remains to be better defined (5). Although the BAP1 protein was originally discovered using a yeast two-hybrid screen with the RING finger domain of BRCA1 as bait, the association between BAP1 and BRCA1 remains unclear.

Based on BAP1's apparent role in histone ubiquitination and the known functional inter-relationships between different histone modifications, agents targeting another type of chromatin modification, histone acteylation, have been tested in UM and MPM cell lines. The histone deacetylase inhibitors Vorinostat (a.k.a. SAHA), trichostatin A, and valproic acid (VPA) all caused growth arrest in BAP1 wild type (WT) UM cell lines and reverted the gene expression profile to a well-characterized less aggressive state. BAP1 shRNA knockdown in UM cell lines increased

sensitivity to VPA and reduced cell proliferation, but similar work using SAHA in the MPM cell lines 211H, HMeso, and H2373 [all BAP1 wild-type] as well as H28 (BAP1 deficient) failed to show a simple relationship between BAP1 loss and increased sensitivity to histone deacetylase inhibitors (13). (R. McMillan, M. Ladanyi, unpublished data) Furthermore, the large Phase III VANTAGE trial of SAHA as a second-line chemotherapy failed to show a survival benefit or clinically significant increase in progression free survival (PFS) (14). Interestingly, studies in RCC cell lines have shown increased sensitivity to a PARP inhibitor in cells when BAP1 levels were reduced by treatment with *BAP1* shRNA (5).

The *BAP1* mutation is a sensitive and specific marker for metastatic potential in UM and correlates with higher tumor grade in RCC (4,5). *BAP1* loss has yet to be linked to a more aggressive phenotype in MPM, though it may be associated with higher rates of tobacco use (M. Zauderer, unpublished data). A recent study of patients with *BAP1* germline mutations underscores the importance of dermatologic and ophthalmologic surveillance examinations in these individuals for secondary prevention of CM and UM (15).

NF2

Inactivating mutations in the neurofibromatosis 2 (*NF2*) gene have been reported in 35-40% of MPM. *NF2* encodes an ERM (ezrin, radixin, and moesin) domain protein also known as Merlin, which acts as a tumor suppressor mediating contact inhibition of proliferation (16,17). *NF2* resides on chromosome 22q11, and was originally identified as the causative mutation of familial neurofibromatosis. Additional studies have identified *NF2* mutations in sporadic schwannomas, ependymomas, meningiomas, MPM, and a smaller number of RCC and CM (18).

ERM proteins link membrane proteins to the cortical actin cytoskeleton, and for this reason NF2 had been postulated to function primarily at the cell cortex, the cytoplasmic region on the inner face of the cell membrane (19). Functional studies have established that NF2 regulates Rac-PAK signaling, the EGFR-RAS-ERK pathway, the PI3K-Akt pathway, and FAK-Src signaling (20,21). However, unlike other ERM proteins, NF2 lacks a canonical, carboxy-terminal actin-binding motif and the active form of NF2 localizes to the nucleus (22). NF2 is active in its "closed" conformation, which is formed by intramolecular bonding between its N-terminal FERM domain and its C-terminal tail, rather than in its "open" conformation like other ERM proteins. Phosphorylation of NF2 at S518 disrupts intramolecular binding, resulting in NF2 adopting an "open", inactive conformation (17).

A recent mass spectrometry study identified the E3 ubiquitin ligase CRL4 as a major binding partner of WT NF2 but not mutated forms of NF2 found in cancer. WT NF2 binds to the DCAF1 subunit of CRL4 where it inhibits CRL4-mediated ubiquitination of histones and other target proteins. Without NF2 inhibition, CRL4 activates a broad oncogenic program leading to cell hyperproliferation, though the substrates of CRL4 have yet to be fully identified (22). The common link between NF2, BAP1, and ubiquitination of histones presents an intriguing possibility of interaction between these two major MPM tumor suppressor genes.

The additional discovery that NF2 loss leads to mTORC1 activation independent of the AKT pathway offers an avenue for targeted inhibition of this pathway (23). Preclinical studies using everolimus as well as a combination of kinase inhibitors and rapamycin show increased sensitivity in cell lines with NF2 loss compared to NF2 wild-type (23,24). The Phase II Southwest Oncology Group (SWOG) study of everolimus as a single-agent, second-line chemotherapy failed to meet its primary endpoint of 4 month PFS, but the patients enrolled were unselected for NF2 loss (25).

LATS2

A comparative genome hybridization study of MPM cell lines recently led to the discovery of recurrent mutations in the Large tumor suppressor 2 (*LATS2*) gene at chromosome 13q12. The incidence of *LATS2* mutations in tumor samples was lower than that found in cell lines—7 mutations in 20 cell lines versus 3 mutations in 25 tumor samples—though additional groups have reported *LATS2* mutations in MPM tumors (1,26). The LATS2 protein is a serine threonine kinase that phosphorylates Yes-associated protein (YAP) and is a member of the Hippo signaling pathway. The Hippo pathway controls organ-growth during embryogenesis, and alterations of the pathway have been implicated in tumorigenesis by impairing contact inhibition of cell growth. YAP is the main downstream mediator of the Hippo pathway, functioning as a transcription factor which is active and nuclear-localized in its dephosphorylated state. YAP overexpression in the nucleus has been noted previously in MPM as well as hepatocellular carcinoma, lung cancer, and colon cancer. Phosphorylation of YAP by

LATS2 inactivates the transcription factor and sequesters YAP in the cytoplasm (27,28). Interestingly, NF2 loss has also been associated with increased nuclear expression of YAP. Also, *NF2* cDNA transfection in NF2-deficient MPM cell lines results in increased YAP phosphorylation (29). However the MPM cell line Y-Meso-14 harbors both *NF2* and *LATS2* inactivating mutations, and transfection with plasmid encoding LATS2 but not NF2 is sufficient to restore YAP phosphorylation, suggesting NF2 acts upstream of LATS2 (26).

Conclusions

Disease-specific mutations in cancer offer the potential for rational targeted therapeutics. Previous discoveries in MPM genetics such as the frequent homozygous deletion of P16/*CDKN2A* at 9p21 have been correlated with patient outcome (30). New findings such as *BAP1* germline mutations may identify groups at greater risk for MPM who might benefit from increased surveillance and early intervention. The identification of pathways altered in MPM such as the PI3K-AKT-mTORC1 and the Hippo pathway may lead to targeted therapies that could be more effective than current therapies. A Phase I trial of the PI3K-AKT-mTORC1 inhibitor GDC-0980 has shown activity in patients with MPM and may represent an example of this sort of therapy (31). Further advances in our understanding of the molecular biology of MPM are likely to emerge in the near future as more cases will be subjected to next-generation sequencing of whole exomes, whole transcriptomes, and whole genomes.

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