Smaller protein, larger therapeutic potential: PPM1D as a new therapeutic target in brainstem glioma

“The epidemiology of *PPM1D* alterations in all types of cancer – which is still being uncovered – will illuminate the function of the mutations and guide therapeutic development work.”

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Gain-of-function *PPM1D* mutations in brainstem gliomas

Brainstem gliomas are a heterogeneous group of tumors of the midbrain, pons, medulla and/or cervical spine. One of the most frequent types of brainstem gliomas is the diffuse intrinsic pontine glioma, a devastating childhood tumor [1]. Genome-wide analyses aimed at understanding brainstem glioma biology, stratifying patients for clinical trials and discovering therapeutic targets have recently culminated with five high-profile reports [2–6]. A total of 9–23% of diffuse intrinsic pontine gliomas were found to harbor heterozygous, somatic mutations in *PPM1D* (also called *WIP1*) [2–4]. This discovery provides a potential therapeutic target for a deadly brain tumor, and adds a new dimension to the complex role for the *PPM1D* proto-oncogene in human cancer.

Normal function of *PPM1D*

PPM1D dephosphorylates multiple DNA damage response (DDR) components, including p53, Chk2, ATM and γ -H2Ax. By doing so, it antagonizes these checkpoint proteins to promote re-entry into the cell cycle following activation of the DDR. Inappropriate activation of *PPM1D* *in vitro* has been linked to transformation and impairment of apoptosis by suppressing downstream DDR modulators [7]. The genetic landscape of brainstem gliomas makes it clear that *PPM1D* mutations disrupt the DDR axis:

mutations of *PPM1D* and *TP53*, and rare mutations of *ATM* and *CHEK2*, occur in a nonoverlapping fashion [2,4,5].

Cancer-derived mutations truncate & stabilize *PPM1D*

The *PPM1D* mutations in brainstem gliomas truncate *PPM1D* to express a shorter, more stable *PPM1D* protein. Most mutations are nonsense mutations clustered in exon 6 around codons 460–560 [2]. These mutations leave the phosphatase domain (residues 1–370) intact and functional, but remove a C-terminal tail that probably regulates turnover of the protein [8,9]. The truncated protein has a longer half-life in cells, and the persistent mutant *PPM1D* protein promotes dephosphorylation of p53, Chk2 and γ -H2Ax [2,9–11]. The C-terminal tail of *PPM1D* contains numerous functional motifs [8], raising the possibility that truncating mutations could have additional effects on the function or localization of *PPM1D*.

A constellation of *PPM1D* alterations in cancer

The epidemiology of *PPM1D* alterations in all types of cancer – which is still being uncovered – will illuminate the function of the mutations and guide therapeutic development work.

Mutations in nonbrainstem tumors

Somatic, truncating *PPM1D* mutations have been found at low frequencies in gli-

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mas outside the brainstem [2,3], in uterine endometrioid carcinomas and a handful of other tumors outside the CNS [10], and in a colon adenocarcinoma and an osteosarcoma cell line [9]. These scattered mutations may be the ‘tip of the iceberg’, and additional tumor types with frequent *PPM1D* mutations may be found with focused resequencing efforts. Somatic missense mutations that inactivate *PPM1D* have also been observed in several cancers [10], but it is unclear whether these rare inactivating mutations play a biological role in cancer.

“This puzzling finding of an allele that predisposes to cancer yet disappears in the tumor itself warrants further study.”

Germline mutations

PPM1D mutations have been discovered in germline tissues and in a germline mosaic distribution in cancer patients. The germline *PPM1D* mutations – also truncating, activating mutations – were present in peripheral blood collected from approximately 1% of patients with colorectal and breast cancers, implicating *PPM1D* as a cancer predisposition gene [9,11]. Surprisingly, the *PPM1D* mutations found in those patients’ germlines were not always present in their tumors. This puzzling finding of an allele that predisposes to cancer yet disappears in the tumor itself warrants further study.

Amplification

PPM1D was first established as a proto-oncogene over a decade ago after it was found to be a target of copy number amplification and overexpression in breast and other tumor types [7]. One brainstem glioma harbored a *PPM1D* amplification in our study [2], indicating that amplification and truncating mutations have overlapping oncogenic functions.

Splicing variant

A natural alternative splicing variant of *PPM1D*, termed *PPM1D430*, is only expressed in testes and leukocytes [12]. *PPM1D430* comprises the first 420 residues common to full-length *PPM1D*, including the entire phosphatase domain and a unique ten C-terminal residues – a very similar configuration to cancer-derived truncated *PPM1D*. Understanding the physiological role of *PPM1D430* in these proliferative tissues may provide clues about the role of truncated *PPM1D* mutants in tumor cells.

Mutant *PPM1D* as a therapeutic target

Early studies implicate mutant *PPM1D* as a therapeutic target, and experimental small molecules targeting it could already be available.

Mutant *PPM1D*: an oncogenic driver

Truncated *PPM1D* mutants oppose γ -H2Ax, p53 and Chk2 activation in response to ionizing radiation [2,9–11]. This finding indicates that the mutations drive tumor growth by opposing the DDR and allowing tumor cells to progress through the cell cycle and avoid apoptosis. We found that removing the *PPM1D* mutation found in a colon adenocarcinoma cell line by genetic recombination led to slowed cell line growth – indicating that *PPM1D*-mutated cancer cells depend on mutant *PPM1D* function for continued proliferation [2]. Together, these findings point to inhibition of mutant *PPM1D* as a therapeutic strategy. Genetically engineered mice completely lacking *PPM1D* are viable into adulthood (although with reproductive and immune system defects), which bodes well for the side-effect profile of any therapy targeting *PPM1D* [13,14].

Existing *PPM1D* inhibitors

The discovery of oncogenic *PPM1D* amplifications in breast cancer spurred the development of small molecule *PPM1D* inhibitors, which predate the discovery of the *PPM1D* mutations in brainstem gliomas. Molecules reported to date include a cyclic phosphopeptide inhibitor [15], a compound identified in a screen that specifically kills *PPM1D*-overexpressing cell lines [16,17], a unique uncompetitive inhibitor [18], and an orally active small molecule which allosterically inhibits *PPM1D* and which showed inhibition of a mouse lymphoma tumor graft model with diploid *PPM1D* [19]. Since they target the phosphatase domain of *PPM1D*, these small molecules can probably inhibit truncated mutants of *PPM1D* found in brainstem gliomas and could act as prototypes for new therapies.

Future directions

The discovery of *PPM1D* mutations in brainstem gliomas opens the door to promising clinical and therapeutic studies.

Clinical correlates & tumor heterogeneity

Follow-up of a larger cohort of brainstem glioma patients will be needed to determine whether *PPM1D* mutations associate with different baseline clinical characteristics or different median survival. Also, an open question remains as to whether *PPM1D* mutation is present throughout brainstem glioma tissues or only in subclonal populations, and whether the mutation is stable after tumor progression. These questions must be resolved by analysis of microdissected and recurrent tumors to help prioritize *PPM1D* as a therapeutic target.

Preclinical studies

It remains to be formally confirmed that compounds designed to inhibit full-length PPM1D can inhibit truncated PPM1D mutants. Also, it is critical to develop *PPM1D*-mutated brainstem glioma models, including genetically engineered animals that express mutated PPM1D in tumors, and animals with tumor grafts derived from *PPM1D*-mutated human gliomas. These models will enable testing whether drugs targeting PPM1D can cure *PPM1D*-mutated brainstem gliomas in combination with radiotherapy or other drugs. Nonglioma cell lines that harbor *PPM1D* mutations represent important tools that are already available to interrogate the function of mutant *PPM1D* [9].

Future clinical trials?

Brainstem gliomas present several challenges for testing investigational new drugs. The tumors are rare,

and CNS drug availability must be considered. If an investigational new drug targeting PPM1D is developed, other cancer types may provide a more ideal setting for early clinical trials. Thus, focused resequencing to determine whether other tumor types harbor frequent *PPM1D* mutations is the first step to prioritize (and encourage) preclinical work.

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