The Targetable Epigenetic Tumor Protein EZH2 is Enriched in Intraocular Medulloepithelioma

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PURPOSE. Intraocular medulloepithelioma (IM), the second most common primary neuroepithelial tumor of the eye, can lead to blindness in the affected eye and in rare cases, is deadly. Intraocular medulloepithelioma lacks targetable biomarkers for potential pharmacologic therapy. The purpose of this study was to identify actionable, tumor-specific proteins for potential diagnostic or therapeutic strategies. We hypothesize that the tumor-specific epigenetic enzyme EZH2 is selectively expressed in IM.

METHODS. We conducted a retrospective case series study of five IM from five eyes of four children and one adult. Hematoxylin and eosin (H&E) stains of sections from formalin-fixed, paraffin-embedded blocks of IM tumors were used to localize IM tumor cells in each case. Using an EZH2-specific antibody for immunohistochemistry, we semiquantitatively calculated the proportion of IM tumor cells positive for EZH2, and also assayed for EZH2 staining intensity.

RESULTS. We found that EZH2 was expressed in all IM cases but this protein was absent in nontumor ciliary body or retinal tissues. However, not all IM tumor cells expressed EZH2. Similar to retinoblastoma, moderately to poorly differentiated (primitive appearing) IM tumor cells strongly expressed EZH2; expression was weaker or absent in areas of well-formed neuroepithelial units.

CONCLUSIONS. To our knowledge, this is the first study to identify an actionable tumor-specific maker, EZH2, in IM. Our findings point to the possibility of exploring the potential of EZH2 inhibitors, already in clinical trials for other cancers, for IM.

Keywords: medulloepithelioma, epigenetics, EZH2
and ocular adnexal lymphoma, and cutaneous basal cell carcinoma.  

In those reports, we discovered that the epigenetic enzyme EZH2 is a key biomarker for aggressive forms of these cancers, including retinoblastoma, which, like IM, arises from neuroepithelial progenitor cells. EZH2, like KMT2D, is a chromatin modifier that methylates lysine residues on histone H3, which regulates gene expression, including tumor suppressors and oncogenes. Gain of function EZH2 mutations and EZH2 overexpression are frequent among cancers, and several small molecule EZH2 inhibitors have recently entered early phase clinical trials for nonophthalamic cancers such as lymphoma and advanced solid tumors. The goal of the current study is to explore whether the targetable epigenetic protein EZH2 is specifically enriched IM.

### METHODS

This study was approved by the University of Michigan (Ann Arbor, MI, USA) institutional review board. Deidentified slides from IM cases were sent from Wills Eye Hospital (Thomas Jefferson University, Philadelphia, PA, USA) and Washington University in St. Louis (MO, USA). Slides recut from archived, paraffin-embedded, formalin-fixed blocks from one iridocyclectomy were analyzed. Each slide was stained with hematoxylin and eosin (H&E) and EZH2. As previously described, EZH2 staining was qualitatively scored by two pathologists (SEA and AMS) on the basis of proportion of nuclei with positive staining (% of nuclei with EZH2 staining).

### RESULTS

This report focuses on the histopathologic findings relating to EZH2 immunohistochemistry in IM; limited clinical information (Table) was available for each case. All five IM tumors showed classic hallmarks of IM, including multilaminar rosettes or neurotubules, cords or fustoons of cells, and small cellular units that were either solid or displayed different-sized lumens, surrounded by a loose mesenchymal tissue.

In our recent report of EZH2 expression in retinoblastoma (RB), we found that while EZH2 appears to be a specific marker for RB, it did not mark all RB cells. We noted that EZH2 protein is low or absent in focal regions of photoreceptor differentiation (e.g., fleurettes). Similarly, EZH2 does not mark many IM tumor cells. This targetable epigenetic factor, EZH2, is a histone methyltransferase that catalyzes formation of H3K27me3, which is known to silence tumor suppressor gene expression, thereby promoting tumorigenesis.

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implications. One, EZH2 may serve as a diagnostic biomarker to detect invasive IM tumor cells in the ciliary body, retina, iris, and possibly optic nerve or extraocular tissues, similar to retinoblastoma. Second, because EZH2 is specifically expressed in IM tumor cells, but not other nontumor cells, EZH2 represents an attractive avenue for targeted therapy for intraocular cancers. Indeed, we found that small molecule EZH2 inhibitors related to those currently in early phase clinical trials, uniquely target retinoblastoma tumor cells but spare nontumor RPE cells in vitro. EZH2 inhibitors might have a similar effect on IM cells; however, no in vitro or in vivo models for IM exist. Future derivation of IM cell lines from enucleated eyes or establishment of animal models with conditional intraocular deletions of DICER1 or KMT2D could enable a preclinical platform to test the efficacy of EZH2 inhibitors and other targeted strategies.2,6

The study of epigenetics in ocular and orbital diseases is in its infancy. The recent discovery of recurrent mutations in another histone methyltransferase, KMT2D, in IM highlights the importance of epigenetics in the tumorigenesis of this uncommon tumor.2 From studies of cancers in other parts of the body, therapies that target tumor addiction to epigenetic dysregulation, such as recurrent EZH2 overexpression or gain of function mutations, have emerged as one of the most promising strategies against cancer. For instance, in the last 2 years, several clinical studies (in the public domain, https://clinicaltrials.gov/ct2/results?term=ezh2) evaluating the use of EZH2 as a biomarker or therapeutic target in lymphoma and a
variety of solid cancers have emerged. Our seminal studies detailing EZH2 dysregulation in RB, orbital and ocular adnexal lymphoma, and cutaneous basal cell carcinoma have uncovered “the tip of the iceberg” of the potential of using epigenetics to better understand how ocular, ocular adnexal, and orbital tumors form, and to develop novel therapeutics.\textsuperscript{6–8}

Taken together, this study indicates EZH2 is a biomarker for IM, and highlights the possibility that EZH2 could be exploited as a therapeutic target for this pediatric tumor, a cancer that lacks biologically targeted treatments.

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