Experimental and Molecular Pathology 100 (2016) 476–481

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Mutational status of IDH1 in uveal melanoma☆

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ARTICLE INFO

Article history:
Received 17 February 2016
and in revised form 26 April 2016
Accepted 2 May 2016
Available online 4 May 2016

Keywords:
Melanoma
IDH1
Isocitrate dehydrogenase
Uveal melanoma
Ophthalmic pathology

ABSTRACT

Uveal (intraocular) melanoma is an uncommon malignancy that comprises a small percentage of all melanoma cases. While many uveal melanomas harbor mutations in the *BRCA-Associated Protein 1* (*BAP1*) gene, the genetics of non-*BAP1* associated tumors are not completely understood. Recent studies have shown that a small subset of non-uveal melanomas hold mutations in *isocitrate dehydrogenase* (*IDH*), but the mutational status of *IDH* in uveal melanoma is unclear. Mutations in *IDH* are strongly prognostic and predictive of tumor behavior in other cancers, mainly diffuse gliomas, which commonly contain the IDH1-R132H mutation. For this study, we hypothesized that uveal melanoma may contain the IDH1-R132H mutation, similar to non-uveal melanoma and other cancers. A search of our institutional pathology files identified 50 consecutive cases of uveal melanoma with additional material utilized for retrospective IDH1-R132H immunohistochemical testing. The demographics of these patients included similar ages, gender distributions, and other clinical characteristics as described in previous studies. Similarly, histological subtype distributions and the presence of high risk pathologic features were consistent with other reports. All 50 of the uveal melanoma cases demonstrated negativity for IDH1-R132H by immunohistochemistry. This rate is unlike that of non-uveal melanoma and further supports their distinct molecular oncogenic profile.

1. Introduction

Uveal melanomas are the most common primary malignant intraocular tumors and are associated with high morbidity and mortality (Schoenfield, 2014). Uveal melanomas comprise approximately 2.9% of all melanomas, with a reported incidence of 4.3 cases per million persons in the United States (Singh and Topham, 2003). There is slight male predominance (4.9 per million) for increased risk when compared to female patients (3.7 per million) (Singh and Topham, 2003). The mean age at diagnosis is around 61.4 years, with spindle cell histology tumors occurring more frequently in younger patients (mean age 60 years) than those with epithelioid cell variants (mean age 65 years) (Andreoli et al., 2015). Treatment varies amongst cases, however most management strategies for primary uveal melanoma incorporate conservative plaque radiotherapy and/or enucleation, depending upon several clinical characteristics, including maximal tumor size (Shields and Shields, 2015). In some centers, external beam radiotherapy is substituted for plaque radiotherapy as a means for ocular conservation therapy (Andreoli et al., 2015). Systemic therapy may be offered to patients who demonstrate high risk clinical characteristics or genetic features *via* testing of Fine Needle Aspiration (FNA) biopsy or enucleation surgical samples (Shields and Shields, 2015). Several prognostic factors have been investigated over the years, however prognosis is strongly predicted by the classification and staging system set forth by the American Joint Committee on Cancer Classification (AJCC) for uveal melanoma, which is based upon tumor characteristics such as size, ciliary body involvement, and extrascleral extension (Andreoli et al., 2015; Bagger et al., 2015; Force, 2015; Shields et al., 2015).

In conjunction with the clinical and pathological based AJCC classification, molecular genetic diagnostics are increasingly being incorporated to provide important prognostic information. Gene expression profiles can segregate tumors into highly prognostic categorical groups, the Class 1 (low metastatic risk) and Class 2 (high metastatic risk) tumors (Correa and Augsburger, 2016; Harbour, 2012, 2014; Onken et al., 2004; van Gils et al., 2008; Worley et al., 2007). The poor prognostic class 2 tumors correspond to those cases with the cytogenetic abnormality of monosomy chromosome 1, which occurs in approximately half of all uveal melanoma cases (Schoenfield, 2014; Tschentscher et al., 2003). At the gene level, mutations in the *BRCA-Associated Protein 1* (*BAP1*) gene located on chromosome 3p21.1 have been frequently found and are strongly associated with the class 2 tumors (Harbour, 2014; Harbour et al., 2010). *BAP1* mutational status can also be detected by immunohistochemical staining of surgical samples, which can serve as an adjunct prognostic tool in uveal melanoma (Kalirai et al., 2014;

☆ Grant Numbers/Source of Support: This work was supported with funds provided by the University of Washington Departments of Ophthalmology and Pathology.
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http://dx.doi.org/10.1016/j.yexmp.2016.05.002
0014-4800/

Koopmans et al., 2014; van Essen et al., 2014). While somatic *BAP1* mutations are frequently identified in sporadic primary and metastated uveal melanomas, persons with germline *BAP1* mutations are at increased risk for multiple cancers, including uveal melanoma (Gupta et al., 2015; Harbour et al., 2010; Klebe et al., 2015; Testa et al., 2011; Wiesner et al., 2011). In addition to *BAP1*, other mutated recurrent genes associated with uveal melanoma include *PLCB4*, *GNAQ, GNA11*, *EIF1AX*, and *SF3B1* (Harbour et al., 2013; Johansson et al., 2015; Martin et al., 2013; Van Raamsdonk et al., 2009, 2010). Although recently there have been significant advances in our knowledge of the molecular drivers involved in uveal melanoma oncogenesis, the molecular pathogenesis and profile of these tumors is not completely understood, especially of uveal melanomas with disomy chromosome 3 and/or wild type *BAP1* gene status.

In a 2011 series of non-uveal melanoma, four cases were identified to have mutations in the *isocitrate dehydrogenase* (*IDH*) 1 and 2 genes, including the mutation encoding for the mutant IDH1-R132H protein (Shibata et al., 2011). More recently, a large scale genomic study by The Cancer Genome Atlas (TCGA) reported *IDH1* as being mutated in 6.2% of cutaneous melanoma cases (Cancer Genome Atlas, 2015). Furthermore, mutations of *IDH1* in these TCGA cases of cutaneous melanoma are associated with a CpG island methylator phenotype (CIMP) as in other cancers. Additionally, experimental models have shown that mutant *IDH1* confers growth advantage of melanoma cells *in vitro* and IDH2 expression influences tumor-free survival in *in vivo* zebrafish animal models (Lian et al., 2012; Shibata et al., 2011). The status of *IDH* mutations in uveal melanoma, however, has not yet been described. *IDH1*, and less frequently *IDH2*, is commonly mutated early the oncogenesis of diffuse gliomas, where it has strong prognostic and therapeutic implications (Bleeker et al., 2010; Cancer Genome Atlas Research et al., 2015; Eckel-Passow et al., 2015; Gorovets et al., 2012; Houillier et al., 2010; Jha et al., 2011; Metellus et al., 2010; Olar et al., 2015; Reuss et al., 2015a; Turcan et al., 2012; Yan et al., 2009). The most common mutation of *IDH* seen in gliomas, IDH1-R132H, is easily and routinely tested for in clinical neuropathology practice by immunohistochemistry (Capper et al., 2010a, 2010b; Horbinski et al., 2009; Reuss et al., 2015b). *IDH* mutational status has been shown in some studies to impart prognostic and predictive value in a small subset of acute myeloid leukemia (Emadi et al., 2015; Im et al., 2014; Patel et al., 2012). In a large clinical series of 2119 cases of myeloid neoplasms, *IDH1* mutational status was found to have the opposite prognostic value of that of gliomas, as a subset *IDH1* mutant leukemias is associated with a decreased overall survival, especially in cases with early (ancestral) gene mutations (Molenaar et al., 2015). In addition to glioma, acute myeloid leukemia, and melanoma, other cancers are emerging to also demonstrate *IDH* mutations, including angioblastic T-cell lymphoma, chondrosarcoma, chondroma, and thyroid carcinoma (Amary et al., 2011; Cairns et al., 2012; Murugan et al., 2010). With the discovery of *IDH* mutations in a small subset of non-uveal melanoma, along with the strong clinical implications of *IDH* mutations in gliomas and other cancers, we sought to determine if uveal melanoma, like cutaneous melanoma, harbors *IDH1* mutations.

2. Material and methods

2.1. Tissue and case selection

Approval for the use of human subject material was granted by the Institutional Review Board for the University of Washington. A search of our institutional files identified a total of 51 consecutive enucleation specimens containing ocular melanoma spanning the years 2009 to 2012. Hematoxylin and eosin (H&E) stains performed during routine clinical work up were reviewed when available, and the remaining information was assessed from finalized pathology reports. Of these 51 patients, 50 had additional material to be used for IDH1-R132H immunohistochemistry. Chart review was performed to obtain demographics and pertinent clinical information when possible.

2.2. IDH1-R132H immunohistochemistry

Formalin-fixed paraffin-embedded (FFPE) tissue slides were prepared and immunohistochemistry (IHC) was performed by a CAP/CLIA-certified pathology core IHC lab within the University of Washington Department of Pathology. The primary antibody used was the anti-IDH1-R132H mouse monoclonal antibody clone H09 at a dilution of 1:100 (Dianova, Hamburg, Germany). Staining was performed using a Leica Bond III Fully Automated IHC and ISH Staining System (Leica Biosystems Inc., IL, USA). Antigen retrieval was performed using citric acid at pH 6.0. The final step included the Bond Polymer Refine Red Detection Kit (catalog #DS9390). Oligodendroglioma samples were used as positive external immunostaining controls. IDH1-R132H IHC stained slides were reviewed and interpreted independently by two experienced neuropathologists, PJC and CDK.

2.3. Public database query

Possible somatic variants in *IDH1* and *IDH2* genes were queried in published datasets from the Catalog Of Somatic Mutations In Cancer (COSMIC); accessed on January 18, 2016 (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) (Forbes et al., 2015). Using the Cancer Browser tool, 'eye' tissue was selected with the subtissue selection of 'uveal tract'. Then 'malignant melanoma' was chosen from the histology selection with 'all' subhistology cases chosen. Both *IDH1* and *IDH2* genes were then searched for somatic variants.

2.4. Statistics

Comparisons made between groups were performed using the *R* statistics package (*R*, version 3.2.5, *R*Project for Statistical Computing, http://www.r-project.org/). *P*-values were determined by the use of the binomial test; binom.test(0,106).

3. Results

3.1. Demographics and clinical parameters

Here we report the clinical and pathologic findings for 50 consecutive cases of uveal melanoma enucleation specimens (findings summarized in Table 1). Patient age varied widely from 23 to 94 years (average age of 60.9 years). A slight male predominance in distribution was observed with 60% (30 of 50) occurring in male and 40% (20 of 50) in female patients. The laterality of melanoma cases was nearly equally represented, with involvement of the right globe in 46% (23 of 50) and the involvement of the left globe in 54% (27 of 50) of cases. The basal tumor width ranged from 0.6 to 3.0 cm in greatest dimension, with an average width of 1.4 cm. The tumor height, or thickness, ranged from 0.1 to 2.1 cm in greatest dimension, with an average height of 0.8 cm. Nineteen of the patients had long term follow up documented. Seven of these 19 patients demonstrated distant metastases at an average of 28.3 months (range of 7–60 months) post-enucleation.

3.2. Pathology and IDH1 mutational status

Gross and microscopic pathologic evaluation was performed for all 50 enucleation specimens (Table 1). A majority of tumors were centered in the choroid (Figs. 1A, B, 2A, and B), with 34% (17 of 50) involving the ciliary body (Figs. 1C and 2C). Hematoxylin and eosin (H&E)-stained sections showed that cytologically, a 58% (29 of 50) majority of the melanomas exhibited mixed epithelioid and spindle cytomorphologies. Predominantly epithelioid or predominantly spindle morphologies were seen less frequently, at rates of 18 (9 of 50) and 24% (12 of 50),