JARID1B Protein Expression and Prognostic Implications in Uveal Melanoma

Patrik Radberger,1 Agatha Radberger,1 Vladimir J. N. Bykov,2 Stefan Seregard,3 and Mario A. Economou3

PURPOSE. It has been suggested recently that stem cell marker expression of jumonji AT-rich interactive domain 1B (JARID1B) is required for continuous tumor growth and maintenance in human cutaneous melanoma cells. The aim of this study is to determine whether JARID1B is also expressed in uveal melanoma (UM) and whether JARID1B marks an expanded cancer stem cell pool in poor prognosis UM. Based on the available data, this is the first time JARID1B expression in UM has been studied.

METHODS. A total of 121 consecutive patients diagnosed with UM and who underwent enucleation were included in the study. Immunohistochemical staining with JARID1B antibodies was performed and immunoreactivity was assessed. Correlations of JARID1B expression with established clinicopathological parameters and overall survival (OS) were evaluated in univariate and multivariate analyses.

RESULTS. JARID1B positive expression, as defined by >0% staining, was present in 55% of UM s and invariably in ciliary body epithelium. The correlation between JARID1B negative expression and JARID1B expression >5% inside the tumor tissue and OS was borderline statistically significant based on LogRank test at 5% significance level (P = 0.06). There were significantly more JARID1B positive cells in tumors with extrascleral extension than in tumors with no or minimal intrascleral invasion (P < 0.01, Mann-Whitney test).

CONCLUSIONS. This study demonstrates that JARID1B is expressed by UM cells. Despite that JARID1B was highly expressed in UM, a statistically significant association (P < 0.05) between JARID1B expression and OS could not be obtained. However, a P-value of 0.06 could suggest that high JARID1B expression is correlated with lower survival; thus, a follow up study with a greater patient sample is recommended. In addition, samples of tumors characterized by high invasiveness showed a higher JARID1B expression. Furthermore, this study substantiates the presence of progenitor cells in the ciliary body epithelium. (Invest Ophthalmol Vis Sci. 2012; 53:4442-4449) DOI:10.1167/iovs.11-9296

Uveal Melanoma (UM) is the most frequently diagnosed primary malignant intraocular tumor in adults, with an incidence ranging from 4.9 to 10.9 per million.1–3 Prognosis has not been improved despite new diagnostic and therapeutic modalities that have led to a better eye preservation rate only.3–5 Nearly half of the patients with UM develop fatal metastatic disease, of which 70%–90% of cases involve the liver and an additional 40% of patients also develop extrathoracic metastases involving the lung, bone, and skin.6–12 Once metastases are detected, median life expectancy is reduced to approximately 2 to 9 months, irrespective of treatment.9–15 UM is characterized by constitutive chemoresistance that can only be partially explained by a complex multidrug resistance (MDR) phenotype and several chemoresistance-related antiapoptotic proteins.16 Current therapeutic regimes that predominantly target the rapidly proliferating tumor bulk, such as systemic chemotherapy and dacarbazine (DTIC)-based chemotherapies, have had a limited effect and showed to be for those patients with a metastatic spreading of the disease.17–19 The failure of current chemotherapy strategies targeting the tumor bulk may indicate that UM tumor progression does not follow the stochastic model hypothesis.

An alternative theory on UM tumorigenesis, the cancer stem cell (CSC) model, predicts that only a subset of cells can generate a new tumor, while other cells cannot.20,21 Recent support for this theory has reported expression of CD133 and other acknowledged stem cell markers in uveal melanoma. (Pax6, Musashi, nestin, Sox2, ABCB5, and CD68 expressions) in UM cell lines,22 as well as reported data of a CSC-like subpopulation using single-cell cloning and spheroid culture studies in UM two-cell lines derived from the primary tumor (Mel270) and metastatic liver lesion (Omm2.5) of the same patient.23 Accordingly, the target for curing UM would be cancer stem cells.

However, Roesch et al.24 published in The Cell research using a biomarker, jumonji AT-rich interactive domain 1B (JARID1B), which supports a new understanding of melanoma heterogeneity. Roesch et al. found that JARID1B marks an expanded cancer stem cell pool and is responsible for dynamic tumor maintenance, growth, and metastasis in human cutaneous melanoma. Roesch et al. demonstrated that isolated human melanoma cells expressing JARID1B could initiate and sustain melanoma growth when implanted into mice, whereas JARID1B-negative cells through knockdown by shRNA knock-out of JARID1B could only initiate tumors and led to an initial acceleration of tumor growth followed by exhaustion in serial transplant experiments. These findings suggest that the JARID1B-positive subpopulation is essential for continuous tumor growth and long-term tumor maintenance. Further, as JARID1B negative cells can become positive and even single melanoma cells irrespective of selection are tumorigenic, this research does not support the hierarchical cancer stem cell model in cutaneous melanoma.

JARID1B is a lysine-specific demethylase 5B (KDM5B), or PLU-1, and member of the family of JmjC domain-containing...
Table 1. Patient and Tumor Characteristics (n = 139)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients (%)</th>
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<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>75 (54)</td>
</tr>
<tr>
<td>Women</td>
<td>64 (46)</td>
</tr>
<tr>
<td>Cell type</td>
<td></td>
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<tr>
<td>Spindle</td>
<td>75 (54)</td>
</tr>
<tr>
<td>Mixed</td>
<td>25 (18)</td>
</tr>
<tr>
<td>Epithelioid</td>
<td>10 (7)</td>
</tr>
<tr>
<td>Partially necrotic</td>
<td>29 (21)</td>
</tr>
<tr>
<td>Localization</td>
<td></td>
</tr>
<tr>
<td>Posterior choroid</td>
<td>86 (62)</td>
</tr>
<tr>
<td>Anterior choroid</td>
<td>41 (29)</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>12 (9)</td>
</tr>
<tr>
<td>Invasiveness</td>
<td></td>
</tr>
<tr>
<td>Minimal</td>
<td>74 (53)</td>
</tr>
<tr>
<td>Intrascleral</td>
<td>41 (29)</td>
</tr>
<tr>
<td>Extrascleral</td>
<td>24 (17)</td>
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</table>

Expression and Implications in Melanoma

Table 2. Distribution of JARID1B Expression

<table>
<thead>
<tr>
<th>JARID1B Expression</th>
<th>Negative Staining</th>
<th>0%-5% Staining</th>
<th>Over 5% Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>JARID1B</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Methods

Patient Samples

The study included formaldehyde-fixed and paraffin-embedded primary UM specimens from 139 consecutive patients. All patients underwent enucleation as the first and only treatment modality, which was the standard therapeutic intervention at that time in Sweden. Seventy-five patients were men, 64 patients were women, and the median age at diagnosis was 65 years (range, 23–84 years). Overall survival (OS) was defined as the time elapsed from the date of diagnosis to the date of death from any cause or the date of last follow-up. Survival data was obtained from the Swedish National Causes of Death Registry. OS data was obtained for 121 of the 139 patients. The median follow-up was 7.8 years (range, 0.14–21.7 years). All 139 specimens had been independently analyzed previously by experienced pathologists and were assessed for tumor location within the uveal tract, tumor cell type according to the modified Callender classification, local invasion, and largest tumor diameter. The specimens included 75 spindle cell uveal melanomas, 25 mixed cell uveal melanomas, 10 epithelioid cell uveal melanomas, and 29 partially necrotic uveal melanomas. Eighty-six tumors were located in the posterior choroid, 41 were located in the anterior choroid, and 12 were located in the ciliary body. Local invasion was minimal for 74 tumors, did not exceed the scleral boundaries for 41 tumors, and was extrascleral for 24 tumors. The median largest tumor diameter was 11 mm (range, 5–19 mm). This study conformed to the Declaration of Helsinki and was approved by the Ethics Committee of the Karolinska Institutet. Details regarding patient and tumor characteristics are provided in Table 1.

Immunohistochemistry

Paraffin-embedded UM tissue sections (4 μm) from 121 patients were examined for JARID1B expression with immunohistochemistry (IHC) using JARID1B mouse monoclonal antibodies (1.5 μg/mL, clone 1G10; Abgent, San Diego, CA). Eighteen tissue sections were not included in the JARID1B analysis due to faulty staining or missing tumor tissue. An alkalinephosphatase (AP)-linking antibody conjugate system (Bond Polymer Refine Detection Red; Leica Microsystems, Buffalo Grove, IL) was used as a polymerization method to prepare the polymeric AP-linking antibody. This biotin-free, polymeric AP-linking antibody conjugate system (Leica Microsystems) detects tissue-bound mouse and rabbit IgG and some primary IgM antibodies from mouse. The detection system avoids the use of streptavidin and biotin, thus eliminating nonspecific staining as a result of endogenous biotin.

For immunohistochemical staining with vision surround system BOND-MAX (Leica Microsystems), an in vivo diagnostics red detection system (DS9390 Bond Polymer Refine Red Detection kit; Leica Microsystems) was used containing post-primary AP, AP polymer (secondary antibody), Fast Red, and HTX. Primary antibodies were diluted with diluent (AR9352 Bond Primary Antibody Diluent, Tris-buffered saline, surfactant, protein stabilizer, and 0.35% ProClin 950; Leica Microsystems).

The histology glass slides were first placed on a tray and protected with a covering plate. The automated IHC and ISH staining system (Leica Bond-Max; Leica Microsystems) was then loaded with the slides and reagents. First, there was a deparaffining with (AR9222 Bond Dewax Solution; Leica Microsystems), then a hydrogenation with 99.7% ethanol (Solveco Chemical AB, Täby, Sweden). The slides were heated up to 100°C with an EDTA-based pH 9.0 solution (AR9640 Bond Epitope Retrieval Solution 2, EDTA-based buffer and surfactant; Leica Microsystems), which helps to enhance antigenic activity through unmasking of the antigen. The primary antibody was added and then the post-primary AP (10% animal serum in Tris-buffered saline and 0.09% ProClin 950; SAFC Global, St. Louis, MO) that locates antibodies from mice. The secondary antibody (poly-AP anti-rabbit IgG with 10% animal serum in Tris-buffered saline and 0.09% ProClin 950; SAFC Global) was then added. Between all steps in the staining, slides were washed with a concentrated buffer solution (AR9590 Bond Wash Solution 10X Concentrate, Tris-buffered saline, surfactant and 3.5% ProClin 950; Leica Microsystems). Substrate chromogene Fast Red is
Figure 1. Immunohistochemistry in human uveal melanoma tissues are shown in these photomicrographs. (A) JARID1B expression in tumor tissue. (B) JARID1B expression infiltration of the ciliary body of uveal melanoma tissue sections. Arrows indicate positively stained cells.

composed of four parts: Part A (activator of 0.05% ProClin 950 in solution; SAFC Global), B (medium), Part C (substrate) and Part D (buffer solution containing 0.05% ProClin 950; SAFC Global). These four parts together allowed the antigen with the red chromogene antibodies to be viewed in the light microscope. Finally, glasses were contrast colored with HTX (0.02% hematoxylin) and mounted with coverslips (Menzel GmbH & Co. KG, Braunschweig, Germany). Tonsil tissue and cutaneous melanoma were used as a positive control, and isotype controls for each primary antibody as well as the application of secondary antibody alone were used as negative controls.

Staining Assessment
A modified quantitative adaptation of stereology37-39 was used to evaluate the IHC results. This method yielded approximate data by estimating the percentage of immunoreactive cells irrespective of staining intensity and assessing fields of vision covering the entire tumor area. These fields of vision were manually and randomly selected and the positivity of the cells for JARID1B staining was scored on a percentage point basis. As assessed fields were spread over the whole area, the selection of cells was unbiased and highly reproducible. The slides were evaluated independently by two different readers who were blinded to the clinical data, and intraobserver and interobserver reproducibility levels according to the kappa test were more than substantial (>0.8).

To verify that epitopes would withstand any inadvertently prolonged exposure to formaldehyde, small pieces (approximately 2 mm³) of fresh UM tissue were fixed in 10% formaldehyde for periods of 1, 2, 4, 8, 16, and 32 days, respectively, and paraffin embedded. Sections were then cut from each paraffin block and immunostained for JARID1B as previously described. The tumor cells retained their staining intensity and pattern from days 1 through 32. To verify the staining of tumor cells, a CD68 and JARID1B macrophage control using serial sectioning including seven cases was conducted. Evaluation of adjacent slides by a senior ocular pathologist confirmed that the absolute majority of JARID1B positive cells were tumor cells and not macrophages, but it could not be ruled out that a small proportion of JARID1B positive cells could have been macrophages.

Figure 2. Distribution of JARID1B samples (number of observations versus percent positive cells).

Statistical Analysis
Cumulative survival was calculated using the Kaplan-Meier method and was analyzed using the log-rank test. Univariate and multivariate analyses were based on a Cox proportional hazards regression model. All predictors that had P values <0.15 (cutoff) in the univariate analysis were included in the multivariate analysis. The cumulative proportion survival was calculated for each separate parameter. In order to test whether separate variables led to poor survival, ANOVA, the Bonferroni test, and the Mann-Whitney test were used in follow-up analysis. All analyses were performed using data mining software (Statistica version 7.0; StatSoft Inc., Tulsa, OK) and statistical software (SPSS version 16.0; SPSS Inc., Chicago, IL) at a significance level of 5% (P < 0.05).

Results
Expression of JARID1B in Primary UM
The expression of JARID1B, defined as >0% positive cells (Fig. 1A) within the tumor tissue, was present in 66 patients (55%). Samples gathered using the stereological assessment model (see “Staining Assessment”) and measured in percentage points of JARID1B expression resulted in a left skewed logistic distribution (Fig. 2). The samples were thus grouped into 55 slides with negative JARID1B expression (45% of all slides), 54 slides with 0%-5% JARID1B expression (45%), and 12 slides with >5% JARID1B expression (10%; n = 121) (Table 2). Interestingly, this study also substantiated the presence of progenitor cells in the ciliary body epithelium as JARID1B was invariably expressed in the ciliary body (Fig. 1B).

Table 3. Univariate JARID1B Proportional Analysis of OS Incorporating Established Clinicopathologic Factors for All Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wald</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.06</td>
<td>0.94 (0.57-1.55)</td>
<td>0.81</td>
</tr>
<tr>
<td>Age (y)</td>
<td>2.24</td>
<td>1.02 (0.99-1.04)</td>
<td>0.13</td>
</tr>
<tr>
<td>Tumor diameter, mm</td>
<td>14.19</td>
<td>1.14 (1.06-1.22)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Maximal tumor height, mm</td>
<td>2.70</td>
<td>1.07 (0.99-1.17)</td>
<td>0.10</td>
</tr>
<tr>
<td>Cell type: mixed, epithelioid, partially necrotic, spindle</td>
<td>2.10</td>
<td>1.15 (0.95-1.39)</td>
<td>0.15</td>
</tr>
<tr>
<td>Localization: anterior choroid, ciliary body, posterior choroid</td>
<td>4.72</td>
<td>1.51 (1.04-2.19)</td>
<td>0.03</td>
</tr>
<tr>
<td>Invasiveness: intrascleral, extrascleral, minimal</td>
<td>1.52</td>
<td>1.24 (0.88-1.73)</td>
<td>0.22</td>
</tr>
<tr>
<td>JARID1B score &gt;5%, 0%-5% and negative expression (n = 121)</td>
<td>0.79</td>
<td>1.04 (0.96-1.12)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval.
TABLE 4. Univariate JARID1B Proportional Analysis Corrected for JARID1B Expression

<table>
<thead>
<tr>
<th>Variable</th>
<th>JARID1B Expression, Negative Staining, n = 55</th>
<th>JARID1B Expression, 0%–5% Staining, n = 54</th>
<th>JARID1B Expression, &gt;5% n = 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Wald 0.10, HR (95% CI) 0.87 (0.38–2.02) 0.75</td>
<td>Wald 0.54, HR (95% CI) 1.37 (0.60–3.12) 0.46</td>
<td>Wald 0.04, HR (95% CI) 0.82 (0.10–6.77) 0.85</td>
</tr>
<tr>
<td>Age (y)</td>
<td>Wald 0.35, HR (95% CI) 1.01 (0.97–1.06) 0.55</td>
<td>Wald 1.25, HR (95% CI) 1.02 (0.99–1.05) 0.26</td>
<td>Wald 1.39, HR (95% CI) 1.05 (0.97–1.14) 0.24</td>
</tr>
<tr>
<td>Tumor diameter (mm)</td>
<td>Wald 0.85, HR (95% CI) 1.08 (0.92–1.26) 0.36</td>
<td>Wald 6.37, HR (95% CI) 1.20 (1.04–1.39) 0.01</td>
<td>Wald 2.37, HR (95% CI) 1.39 (0.91–2.11) 0.12</td>
</tr>
<tr>
<td>Maximal tumor height (mm)</td>
<td>Wald 0.97, HR (95% CI) 1.07 (0.93–1.25) 0.32</td>
<td>Wald 1.18, HR (95% CI) 1.07 (0.94–1.22) 0.28</td>
<td>Wald 0.17, HR (95% CI) 0.95 (0.73–1.24) 0.68</td>
</tr>
<tr>
<td>Cell type: mixed, epithelioid, partially necrotic, spindle</td>
<td>Wald 0.74, HR (95% CI) 1.14 (0.84–1.55) 0.39</td>
<td>Wald 6.33, HR (95% CI) 1.55 (1.10–2.12) 0.01</td>
<td>Wald 0.24, HR (95% CI) 0.87 (0.49–1.53) 0.63</td>
</tr>
<tr>
<td>Localization: anterior choroid, ciliary body, posterior choroid</td>
<td>Wald 0.87, HR (95% CI) 1.39 (0.70–2.76) 0.35</td>
<td>Wald 0.02, HR (95% CI) 1.05 (0.52–2.11) 0.89</td>
<td>Wald 0.74, HR (95% CI) 1.87 (0.45–7.73) 0.39</td>
</tr>
<tr>
<td>Invasiveness: intrascleral, extrascleral, minimal</td>
<td>Wald 0.11, HR (95% CI) 1.12 (0.57–2.19) 0.74</td>
<td>Wald 1.24, HR (95% CI) 1.33 (0.80–2.21) 0.27</td>
<td>Wald 0.50, HR (95% CI) 0.72 (0.29–1.78) 0.48</td>
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JARID1B Expression May Be Associated with Survival Prediction

In univariate analysis, the already established prognostic markers of tumor diameter and localization had significant prognostic value for OS (Table 3). In consideration of potential divergence within the microenvironment caused by JARID1B expression, univariate analysis was performed on the JARID1B >5%, 0%–5%, and negative expression subgroups. In the analysis of the 0%–5%, JARID1B expression subgroup, tumor diameter showed prognostic relevance for OS (P = 0.01) and cell type was associated significantly (P = 0.01) with OS. The 0% expression JARID1B subgroup did not show any prognostic relevance for any of the independent prognostic factors (Table 4).

When patients were stratified and compared based on JARID1B positive (>0%) versus JARID1B negative expression groups, univariate analysis showed statistical significance for tumor diameter (P < 0.01) and cell type (P = 0.06) variables. However, there were no further statistically significant findings (Table 5). The results for JARID1B in the Cox proportional hazards models were elaborated in Kaplan-Meier survival analyses (see Fig. 3). When the JARID1B expression was divided into the >5%, 0%–5%, and negative expression subgroups, LogRank test found a borderline significant association with OS was shown between 0% and >5% JARID1B expression groups, P = 0.06.

Multivariate Survival Analysis

The clinicopathologic covariates of the univariate analysis (age, tumor diameter, maximal tumor height, cell type, localization, and JARID1B expression) were also included in the multivariate analysis. The regression model indicated that tumor diameter had statistically significant impact on survival, similar to univariate analysis in which tumor diameter was also found to be a prognostic factor for OS (P < 0.01; Table 6). No significant correlation between parameters was observed.

Separate Parameter Analysis: Differences in JARID1B Expression Divided by Subgroup

In the attempt to find whether cell type, localization, invasiveness, or JARID1B expression led to poor survival, ANOVA tests were used to compare individual differences between subgroups and JARID1B expression. For cell type, differences in JARID1B expression between mixed epithelioid, partially necrotic, and spindle cells did not reach any statistical significance (P = 0.54; Fig. 4). For localization, ANOVA showed that differences between posterior choroid, anterior choroid, and ciliary body location did not reach any statistical significance (P = 0.45; Fig. 5). Invasion subgroups showed a significant difference in JARID1B levels between different locations, minimal, intrascleral, and extrascleral (P = 0.045; Fig. 6). There were significantly more JARID1B positive cells in tumors with extrascleral extension than in tumors with no or minimal intrascleral invasion (P < 0.01, Mann-Whitney test).

DISCUSSION

UM is a heterogeneous, poorly genetically understood, and highly aggressive malignancy. It is the most common primary ocular malignancy in adults and has a poor prognosis, with the average death occurring within 10 years of diagnosis.1–5 After development of metastatic disease, median survival is approximately 6 months, with an estimated survival of 15%–20% at one year and 10% at 2 years, irrespective of treatment.14 In
order to help provide new insights in the development of UM, the concept of CSCs has gained momentum over the last decade in many excellent reviews. These studies emphasize the important implications of the CSC model for understanding tumor biology and cancer treatment. In particular, it is thought that cancer treatments, such as chemotherapy, target rapidly dividing tumor cells, and consequently, in order to develop effective ways to suppress one or more steps in the processes of tumor progression and metastatic spread, it is essential to be able to characterize CSCs and the signaling pathways controlling them. However, as UM remains to be characterized by constitutive chemoresistance, and has shown resistance to traditional debulking chemotherapies, the analysis of new possible genes in uveal melanoma continues to be of upmost importance in better understanding the cellular processes involved in UM development and progression.

Recent findings by Roesch et al. using stem cell marker JARID1B supports a new understanding of melanoma heterogeneity finding tumor maintenance a dynamic process mediated by a temporarily distinct subpopulation. JARID1B has even been shown to be overexpressed in cutaneous melanoma in two additional studies, as well as in several other malignancies. For instance, JARID1B has been found to be upregulated in many breast cancer cell lines and tumors, which suggests that JARID1B has tumor promoting activities. Similarly, JARID1B is overexpressed in bladder and lung tumors and stimulates the proliferation of respective tumor cells. Using both in vitro and in vivo models, Roesch et al. found that JARID1B expression in malignant melanoma has been shown to have immediate antiproliferative effects, but later has a role in continuous tumor growth and maintenance.

Although cutaneous melanoma and UM behave in different ways when it comes to metastasis (cutaneous spreads through the lymphatic system while uveal hematogenously), organ preference (liver almost in every case for uveal melanoma)
and very rarely for cutaneous melanoma); and response to chemotherapy (cutaneous melanoma more sensitive than the uveal), the two kinds of melanoma share several qualities as previously discussed in the introduction. These similarities and differences between uveal and cutaneous melanoma, in combination with the recent findings concerning the dual role of JARID1B in cutaneous melanoma growth and maintenance,\textsuperscript{24} mandated the further investigation of JARID1B and its role in uveal melanoma. Based on available data, this is the first time JARID1B in UM has been studied.

In the current study, which was performed on a relatively large sample of 139 serially enucleated UM tumors of which 121 consecutive patients were examined for JARID1B expression without preceding preselection, JARID1B was tested as an independent predictive factor for survival. JARID1B positive staining were detected in approximately 55% of the UM samples. Tumor size was significantly correlated with JARID1B expression. One could hypothesize that larger tumors are older, and due to the plasticity characteristics of JARID1B in that negative cells can become positive as mentioned in Roesch et al.,\textsuperscript{24} one would expect a greater proportion of JARID1B positive cells in the tumor after a longer period of time. Similarly, it was found that samples of tumors characterized by high invasiveness showed a higher JARID1B expression, suggesting that JARID1B expression proportionally correlated to the aggressiveness of the tumor.

Positive JARID1B staining was found within the tumor tissue and also invariably in the ciliary body epithelium. Interestingly, all 121 samples had positive JARID1B staining in the ciliary body epithelium. It has been suggested in the literature that progenitor cells do exist in the epithelium of the ciliary body,\textsuperscript{49–52} which this study substantiates as JARID1B is thought to be a marker for stem/progenitor cells.\textsuperscript{24}

With regard to the recent study by Roesch et al. that studied JARID1B expression in cutaneous melanoma,\textsuperscript{24} study data did not support the hypothesis that JARID1B is an independent predictive factor for UM survival. One would have expected that JARID1B expression should have been significantly correlated with overall survival if JARID1B had a maintaining and proliferative role in UM. However, despite the fact that JARID1B expression did not show a statistically significant effect ($P < 0.05$) on survival rates, study data suggests borderline significant results in OS between the JARID1B negative expression and $>5\%$ JARID1B expression groups ($P = 0.065$). This borderline result indicates that it would be advisable to repeat the study with a greater sample size, as the $>5\%$ JARID1B expression group only had 12 samples, thus increasing the probability that the results would become statistically significant at the $5\%$ significance level.

Future research of JARID1B expression in UM is recommended as it may help in understanding the role of new possible genes in uveal melanoma, thus providing new insights in development of UM, which could explain the heterogeneity of this malignancy as well as its elusiveness in terms of chemoresistance and failure of current therapies. In order to address the question whether JARID1B is a prognostic factor for OS, both in vitro and in vivo models could be used, such as transfection of stable tumor cells with JARID1B expression vector and then running several tests such as resistance to therapy or on cell migration. As a continuation, such cells could be introduced in immunodeficient mice and the growth and spread of JARID1B positive tumors could be compared with negative tumors. This would specifically address the question of whether JARID1B indeed has a direct effect on tumor proliferation and survival in UM.

In conclusion, the major novelty of this work is that it is the first study in which JARID1B expression is studied in UM. It was found that JARID1B is expressed in a majority of UM cells and it was found that samples of tumors characterized by high invasiveness showed a higher JARID1B expression. Further, the existence of progenitor cells in the ciliary body epithelium was affirmed. Although a definite relationship between JARID1B expression and OS was not established, the current study’s borderline significant results with a $P$ value of 0.063 suggest that JARID1B may mark an expanded cancer stem cell pool in poor prognosis UM. These findings mandate further investigation in a greater sample of patients.

**Acknowledgments**

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**References**


