ME20-S as a Potential Biomarker for the Evaluation of Uveal Melanoma

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Purpose. We previously identified the presence of the melanocyte-specific secreted (ME20-S) glycoprotein in secretomes of uveal melanoma (UM) cultures. The aim of this study was to test for the presence and levels of ME20S in the serum of patients with choroidal nevi and UM and correlate these levels with individual clinical data.

Methods. Serum ME20-S levels were determined by ELISA in 111 patients distributed into four categories (55 choroidal nevi, 30 untreated UM, 11 10-year disease-free [DF] UM, 17 hepatic metastatic UM) and 32 age- and sex-matched controls. ME20-S levels were correlated with individual clinical data.

Results. The UM and the metastatic groups showed significantly higher levels of serum ME20-S than the other groups (P < 0.001). ME20-S levels in the DF patients did not differ from those in the control group. In addition, log-transformed serum ME20-S levels showed a positive correlation with the thickness of the lesion mass in UM patients (regression coefficient 0.0689, 95% confidence interval 0.0689–0.1123, R2 = 27.1%).

Conclusions. Elevated ME20-S serum levels are associated with tumor size and advanced stages of UM while low levels are characteristic of DF patients. ME20-S might be a promising serum marker for UM and useful for monitoring metastatic disease.

Keywords: melanocyte protein PMEL, M20-S, uveal melanoma, choroidal nevi, circulating biomarker

As the most common primary malignant intraocular tumor, uveal melanoma (UM) is also the main intraocular disease that can be fatal in adults. Its incidence in the general population is 5.3 to 10.9 cases per million people per year.1,2 Uveal melanoma disseminates mainly through the bloodstream and preferentially metastasizes to the liver.3 Even with successful treatment of primary UM tumors, patients remain at risk of developing metastases for more than 20 years after initial diagnosis.4 In the Collaborative Ocular Melanoma Study, Kaplan-Meier analysis estimated 2-, 5-, and 10-year metastasis rates of 10%, 25%, and 34%, respectively. However, only 0.24% of the patients exhibited detectable metastases at the time of diagnosis.5 In this regard, the metastatic rate has been related to the tumor height.6

Poor prognosis is associated with various clinical and molecular factors of the primary UM, such as tumor height,6 presence of monosomy 3, and gain of chromosome 8.7,8 More recently, UM research has evolved toward finding genetic prognostic markers to identify patients at risk for developing metastatic disease. In particular, tumor-specific mutations have been found in the GNAQ, GNA11, and BAP1 genes.9–11 In addition, gene expression profiling from fine-needle biopsies has emerged as a powerful tool for molecular prognostication in UM, able to discern low- and high-risk patients.12,13 However, the risk of understimating the prognostic probability of metastasis and metastasis death by fine-needle aspiration biopsy has to be considered.14 Under this scenario, the identification of noninvasive blood biomarkers could have a crucial impact in the management of UM. Ideally, these prognostic markers would be effective for assessment of metastatic risk and guiding follow-up as well as facilitating adjuvant therapy decisions.

We previously applied proteomics technology to detect UM tumor-specific proteins released into the extracellular surroundings and presumably to the blood circulation.15 We identified several potential UM biomarkers, including the 95-kDa premelanosome protein (PMEL), also known as glycoprotein 100 (gp100) and melanoma-associated ME20 (ME20M), and the oncoprotein PARK7/DJ-1. Both proteins, DJ-1 and the ME20M soluble form (ME20-S), were detected in the serum of patients with UM.16–17 Thereafter, a larger survey enabled us to describe for the first time that elevated serum levels of DJ-1 are associated with choroidal nevi transformation risk factors.18 ME20M is thought to be an oncofetal self-antigen that is normally expressed at low levels in quiescent adult melanocytes, but is overexpressed by proliferating neonatal melanocytes and during tumor growth.19 Because it is considered a tumor-associated antigen that is specific to patients with cutaneous melanoma,20 monoclonal antibodies against this
protein are routinely used in melanoma diagnosis.\textsuperscript{21} ME20M has a central role in melanosome biogenesis, mediating the maturation of melanosomes from stage I to stage II.\textsuperscript{20} Moreover, the secretion of the soluble form, ME20-S, has been suggested to protect melanoma cells from antibody-mediated immunity.\textsuperscript{22} Taking into account the melanoma-specific nature of this molecule, we hypothesize that it could be a good UM biomarker candidate.

The purpose of the present study was to test for the presence and assay the levels of circulating ME20-S in patients with choroidal nevi and UM and correlate this with individual clinical data to evaluate its potential as an individual prognostic factor.

METHODS

Patients

This study was based on serum samples from 111 patients at the Ocular Oncology Unit at the Complexo Hospitalario Universitario de Santiago (Spain) and at the Catalan Institute of Oncology (Barcelona, Spain) collected between January 2009 and May 2015. The study groups were classified as choroidal nevi group (\( n = 55 \)), UM group comprising patients with untreated UM (\( n = 30 \)), disease-free UM (DFUM) group comprising disease-free UM patients who were previously treated by brachytherapy and/or enucleation and who did not develop metastasis for at least 10 years from diagnosis (\( n = 11 \)), and patients who developed hepatic metastasis after local treatment (\( n = 17 \)). Patients comprising the untreated UM group were classified as stage I or II, stage III (A, B), and stage IIIC according to American Joint Committee on Cancer (AJCC) staging\textsuperscript{3}; serum samples in this group of patients were always extracted prior to treatment. Clinical diagnosis was made on the basis of complete ophthalmic examination and standardized oculard ultrasoundography.

Inclusion criteria for the nevi group were the presence of one nevus up to 2.9 mm in thickness and a large basal diameter (LBD) less than 12 mm based on oculard ultrasoundography or fundus retinography. Clinical signs of prognostic value for nevi transformation were evaluated in all cases. These included the following: presence of ophthalmic symptoms directly related to the tumor (floaters, photopsias, and visual acuity diminution), drusen affecting the overlying retina, presence of orange pigment, and tumor margin within 3 mm of the optic disc.\textsuperscript{24,25} Treatment was not indicated for any choroidal nevus, as none exhibited sufficient evidence of risk. Thickness measurement was determined by ultrasonographic study, except in nine cases in which the thickness was not sufficiently large to be detected by the ultrasonic signal. In these nine cases, thickness was assumed not to exceed 0.5 mm.\textsuperscript{26}

In the DFUM group of patients, eight patients were treated with brachytherapy using I-125 and three patients by primary enucleation. These patients were treated at a minimum of 10 years before blood collection. In order to detect metastasis, the evaluation included abdominal ultrasonography and blood samples for liver function tests and serum biomarkers (alkaline phosphatase [AP], aspartate aminotransferase [AST/GOT], alanine aminotransferase [ALT/GPT], lactate dehydrogenase [LDH], and gamma-glutamyl transpeptidase [GGT]). Upon detection of a suspicious mass on abdominal ultrasonography, patients underwent magnetic resonance imaging (MRI) for confirmation. Exclusion criteria were prior history of any type of cancer and the presence of suspicious cutaneous melanocytic lesions.

As a control group, 32 healthy volunteers were selected from among individuals undergoing routine ophthalmologic examinations. This group was matched in age and sex distribution and had the same exclusion criteria as the patient groups. Individuals from all groups were exposed to identical clinical procedures. All participants provided written informed consent, according to the Declaration of Helsinki, prior to participation in the study, which was approved by the Comité Etico de Investigación Clínica de Galicia (Spain).

Clinical Procedures

Standardized ultrasonography was performed using the 13-ABD System (Innovative Imaging, Inc., Sacramento, CA, USA) with a 10-MHz probe. After the instillation of anesthetic drops, topographic, quantitative, and kinetic ultrasound\textsuperscript{27} was performed directly over the eye using a coupling gel (Viscotears, carbomer gel 2%; Novartis, Barcelona, Spain). The same physician performed this examination in all patients. The following acoustic parameters were studied: tumor location, LBD and thickness, and the presence of acoustic hollowness. The existence of subretinal fluid related to the tumor was examined by Cirrus SD-OCT (Carl Zeiss Meditec, Inc., Jena, Germany).

Blood samples for ME20-S serum detection were collected in STT II Advance Vacutainer tubes (Ref. 586967; Becton-Dickinson, Franklin Lakes, NJ, USA). Samples were allowed to clot for 30 minutes. They were then centrifuged for 15 minutes at 1500 \( \text{g} \) to separate the serum. Serum samples were immediately aliquoted, coded, and frozen at \(-80^\circ\text{C}\) until processing. Clinical information and data collected from the patient sera were double masked.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum levels of ME20-S were quantified with the Melanoma-Associated ME20 kit (USCN Life Science, Inc., Wuhan, PR China) according to the manufacturer’s instructions. Absorbance from each sample was measured in duplicate with a spectrophotometric microplate reader at 450 nm (VersaMax Microplate Reader; Molecular Devices, CA, USA).

Statistical Analysis

Differences in ME20-S values between patient groups were analyzed by nonparametric Kruskal-Wallis and Mann-Whitney tests due to the nonnormal data distribution. Holm’s method was used to adjust for multiple comparisons.\textsuperscript{28} Relationships of LBD and thickness measurements with ME20-S levels (as the dependent variable) were analyzed by additive regression models (AMs), which were used to avoid imposing arbitrary parametric effects.\textsuperscript{29} Both relationships were linear; therefore, a linear regression model was fitted, and the ME20-S levels were log transformed to achieve normality. Regression results are expressed in terms of the regression coefficient (RC), 95% confidence interval (95% CI), and proportion of the variability explained (\( R^2 \)). All statistical analyses were performed in the R software package (version 2.15.1).\textsuperscript{30} Additive regression models were fitted with the mgcv package.\textsuperscript{31} A \( P \) value \( < 0.05 \) was considered statistically significant in all tests.

RESULTS

Clinical Findings

The subjects comprised 111 Caucasian patients with a mean age of 65 years (range, 42–81). Among these patients, 41 were diagnosed with UM with a mean age of 67 years (range, 35–87), 53 with choroidal nevus with a mean age of 65 years (range, 23–91) and 17 with hepatic metastases of UM with a mean age of 66 years (range, 41–83). The control group comprised 32 individuals with a mean age of 66 years (range, 26–98). There
FIGURE 1. Logarithmic scale representation of serum ME20-S levels in five groups of patients (control, choroidal nevi, untreated UM, 10-year disease-free [DFUM], hepatic metastatic UM). Vertical lines indicate the range, and horizontal boundaries of the boxes represent the first and third quartiles. Significance level: ***P < 0.001.

were not statistically significant differences in the age or sex distribution between groups.

Within the measurable nevi group (n = 38, height > 0.50 mm), the median thickness was 1.50 mm (interquartile range [IQR] = [0.85; 1.82]) and median LBD was 5.61 mm (IQR = [4.79; 8.32]). Median tumor thickness of the untreated UM group was 7.51 mm (IQR = [4.81; 10.82]) and median LBD was 11.46 mm (IQR = [10.35; 13.17]). In the DFUM group, those patients previously treated by brachytherapy showed a median thickness of 4.67 mm (IQR = [3.85; 6.82]) and a median LBD of 10.9 mm (IQR = [8.50; 11.6]).

With respect to patients treated with enucleation, they showed a median height of 11.13 mm (IQR = [8.32; 14.23]) and a median LBD of 12.9 mm (IQR = [8.50; 13.6]). Median primary tumor thickness of the metastatic group was 10.00 mm (IQR = [6.87; 13.02]), and median LBD was 11.46 mm (IQR = [11.00; 16.94]).

ME20-S Serum Levels

Serum levels of ME20-S differed significantly between groups (Kruskal-Wallis, P < 0.001; Fig. 1). Specifically, serum ME20-S levels diverged significantly between the choroidal nevi group (0.73 ng/mL, IQR = [0.43; 1.36]) and the UM group (6.92 ng/mL, IQR = [3.44; 11.7]). Mann-Whitney test, P < 0.001). Moreover, serum ME20-S levels in the DFUM patient group (0.37 ng/mL, IQR = [0.31; 0.49]) were lower than those in the UM group (Mann-Whitney test, P < 0.001) and than those in the choroidal nevi group (Mann-Whitney test, P = 0.027).

Although serum ME20-S levels in the DFUM patients did not differ from those in the control group (0.70 ng/mL, IQR = [0.38; 1.05]) (Mann-Whitney test, P = 0.052), ME20-S levels in the control group were significantly different from those in the UM group (Mann-Whitney test, P < 0.001) and the choroidal nevi group (Mann-Whitney test, P < 0.001) (Fig. 1). The most striking result was that there was significant difference between DFUM patients and those presenting with metastasis (4.21 ng/mL, IQR = [2.83; 4.96]; Mann-Whitney test, P < 0.001). Both the UM and the metastatic group showed significantly greater levels of circulating ME20-S compared to the rest of the groups (Table).

In the choroidal nevi group, no statistically significant differences were detected in ME20-S serum levels between groups defined by the presence of known risk factors of malignant transformation (orange pigment, choroidal excavation, fluid, or the absence of drusen) (Supplementary Table S1).

When specifically studying the group of untreated UM patients, we found that there were differences in the concentration of ME20-S among the different stages of the disease as classified by the AJCC staging. Stages I and II showed a concentration of 3.01 ng [1.88; 3.66], while those in stages III (A, B) and IIC revealed higher levels, 7.74 ng [5.03; 9.62] and 16.00 ng [12.60; 35.38], respectively (P = 0.005). In addition, we found that serum ME20-S levels were associated with untreated UM tumor size. Log-transformed serum ME20-S levels were positively associated with UM thickness (RC 0.0689, 95% CI 0.0251–0.1123, $R^2 = 27.1\%$, $P = 0.003$), although the relationship was weak (Fig. 2). A deeper study considering the clinical characteristics of untreated UM patients detected higher concentrations of ME20-S in patients with extrascleral extension and in those with pigmented melanomas compared to amelanotic ($P < 0.05$). No differences were observed based on the cell type (fusiform, mixed, and epithelial), location, or the presence of associated retinal detachment.

TABLE. Comparisons of ME20-S for Each Patient Group

<table>
<thead>
<tr>
<th>Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Choroidal nevi</td>
<td>0.3764</td>
</tr>
<tr>
<td>UM</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DFUM</td>
<td>0.0517</td>
</tr>
<tr>
<td>Metastasis</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Choroidal nevi</td>
<td></td>
</tr>
<tr>
<td>UM</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DFUM</td>
<td>0.0271</td>
</tr>
<tr>
<td>Metastasis</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uveal melanoma</td>
<td></td>
</tr>
<tr>
<td>DFUM</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metastasis</td>
<td>0.0240</td>
</tr>
<tr>
<td>DFUM</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

P values are based on nonparametric Mann-Whitney test (corrected by Holm method).

FIGURE 2. Relationship between log-transformed ME20-S values and tumor thickness. Linear regression estimation (solid line) of the relationship between ME20-S levels and UM thickness together with point-wise 95% confidence intervals (dotted line).
DISCUSSION

Our study demonstrates for the first time a positive correlation between serum ME20-S protein levels and UM tumor thickness. While those patients with treated UM (DFUM) showed no significant differences compared to healthy individuals, in the present work we show that increased serum ME20-S levels are positively associated with the presence of nontreated UM and the existence of UM metastatic disease.

Despite significant advances in UM diagnosis and treatment, the prognosis for this type of tumor remains poor. Metastatic liver disease is the leading cause of death in patients with UM and can develop after a long disease-free interval, suggesting the presence of hidden micrometastases seeded prior to local treatment.\(^3\) Thus, the ability to monitor tumor progression by assaying malignancy indicators would be very valuable. The discovery of tumor-specific biomarkers has been a challenge for cancer research for decades. Unfortunately, very few markers have been found to be useful in a routine clinical setting, stressing the need for new clinically relevant sources such as proteomics.\(^33\) Uveal melanoma tumor cells secrete proteins into the tumor environment that subsequently spread through the surrounding vascular networks and into the bloodstream.\(^16\) Therefore, proteins secreted by tumor cells, which could be detected by a simple blood test, could be potential biomarkers for disease diagnosis and/or prognosis. Serum biomarkers have an advantage over histopathologic biomarkers in that it is not necessary to obtain tissue samples from the primary tumor or metastasis. Therefore, serum markers may be used to monitor patients with UM. The identification and characterization of ME20M protein and its soluble form ME20-S in UM have the advantage that it is a structural protein and specific for melanoma as compared to other nonspecific markers investigated previously.\(^34\)\(^\text{-}46\) In support of this hypothesis, it is important to note that ME20M has been recently used as a therapeutic target for drug conjugate therapy in cutaneous melanoma.\(^57\)

We previously demonstrated that UM cells secrete ME20-S into the extracellular environment and into the bloodstream.\(^16\) Protein ME20M (gp100) is specific to normal and malignant melanocyte lineage cells, and its presence is routinely assessed in the diagnosis of malignant cutaneous and UM, as it is a tumor antigen expressed by more than 75% of human melanomas.\(^34\)\(^\text{-}38\) Little attention, however, has been paid to the soluble form (ME20-S) of this protein as a circulating biomarker. Because the melanocyte protein ME20M is a structural protein found in the membrane of melanocytes, it is reasonable to hypothesize that serum ME20-S values could correlate with the number of cells comprising the tumor mass and the existence of UM metastases.\(^39\) Although the precise mechanism responsible for ME20-S shedding is yet unclear, it was recently postulated that ME20-S is released by ectodomain shedding through regulated proteolysis not only at the cell surface but also via intracellular compartments such as exosomes.\(^34\)

In light of the small sample size used in the current study, caution must be taken in interpreting the results. Further analyses using larger sample sizes and during follow-up are essential for determining the use of the ME20-S level as a biomarker. Unfortunately, our Retinal Oncology Unit was essential for determining the use of the ME20-S level as a biomarker. Unfortunately, our Retinal Oncology Unit was not able to perform gene expression or chromosome analysis in the early stages of the study. In the future, it would be valuable to increase the number of patients in the sample and to include the above-mentioned variables.

To our knowledge, this is the first report detecting the soluble form of melanocyte protein ME20-S in the circulation of UM patients. The most significant finding from this study is that elevated ME20-S serum levels were associated with tumor thickness and advanced stages of the disease while low levels are characteristic of patients free of disease. Taken together, these results suggest that serum ME20-S determination might be useful as a potential serologic biomarker for UM.

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References

ME20-S as a Potential Biomarker to Evaluate UM


