Insulin-Like Growth Factor-1 as a Predictive Biomarker for Metastatic Uveal Melanoma in Humans

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Purpose. High expression levels of insulin-like growth factor-1 (IGF-1) receptor were associated with metastatic uveal melanoma (UM). The purpose of this study was to examine the potential of serum IGF-1 in early detection of liver metastasis.

Methods. IGF-1 serum levels were analyzed using enzyme-linked immunosorbent assay for 118 subjects in three different groups: 55 disease-free (DF) UM patients who did not develop metastasis within 10 years of diagnosis; 22 metastatic patients; and 41 healthy subjects. Matched pairs univariate analysis was performed for sera of 19 metastatic patients 12 and 6 months before the diagnosis of metastasis and on the day of diagnosis, both as time groups and normalized levels per patient. IGF-1 levels were compared among groups by analysis of variance and Student *t*-test.

Results. Mean ± SD IGF-1 serum levels for the control, DF, and metastatic groups were 152.48 ± 49.76, 119.92 ± 60.66, and 96.99 ± 56.91 ng/mL, respectively ($P < 0.001$). Normalized changes in IGF-1 per metastatic patient from 6 months prior to the diagnosis of metastases compared to the day of diagnosis of metastases showed a decreasing trend.

Conclusions. IGF-1 levels in 10-years' disease-free UM patients were significantly lower than those in healthy subjects and were even lower in metastatic patients. IGF-1 levels decreased toward the diagnosis of metastases. Therefore, serum IGF-1 level may be used as a predictive biomarker for metastatic UM when measured repeatedly. (*Invest Ophthalmol Vis Sci.* 2013; 54:490–493) DOI:10.1167/iovs.12-10228

Uveal melanoma (UM) is the most common primary intraocular malignant tumor in adults,[1] albeit a rare tumor with a mean age-adjusted incidence in the United States of 5.1 per million, representing only 3.1% of all recorded cases of melanoma.[2] The liver is the predominant metastatic organ, and in the collaborative ocular melanoma study (COMS), the liver was involved in 89% of cases.[3]

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Brachytherapy and external irradiation (proton beam, stereotactic radiosurgery, or gamma knife) are the most common treatment options for small- to medium-sized tumors, with a success rate of approximately 90%, while enucleation remains the common treatment for large tumors. Despite the high success rate in treating the primary tumors, the 5- and 10-year cumulative rates for developing metastases are 25% and 34%, respectively, with 80% of the metastatic patients dying within 1 year and 92% within 2 years of the diagnosis of metastases.[4] However, metastases have been diagnosed even more than 40 years from enucleation.[5] These findings led to the hypothesis that micrometastases had already been seeded at the time of diagnosis and developed over time as a result of unknown factors until metastatic disease was diagnosed.[6]

Currently there is no adjuvant chemotherapy to eliminate micrometastases, and treatments for overt metastatic disease are of limited value except for a subgroup of patients who are diagnosed early enough to allow complete surgical resection of liver metastases.[7,8] Liver resection may increase the survival after diagnosis of metastases by 3.7-fold.[7] Therefore, effort is being made both to classify patients at diagnosis, in order to identify patients at high risk for the development of metastases, and to find early detection methods for the metastases.

The current method of detecting metastases includes abdominal imaging directed to the liver (usually by ultrasonography) twice yearly, combined with blood tests. We have recently shown that serum biomarkers are superior to the liver function test in the diagnosis of metastases.[9] However, in a considerable number of cases, liver ultrasonography detects the metastases when it is too late for liver surgery, and currently available biomarkers do not provide a long enough lead time compared to that of ultrasonography. Thus, the search for a reliable diagnostic serum biomarker that would give an earlier diagnosis is still on.

Insulin-like growth factor 1 (IGF-1) is a soluble protein produced by the liver that binds to a transmembrane receptor called IGF-1 receptor (IGF-1R). IGF-1R is expressed in the entire body but mainly in the cartilage, bone, liver, kidneys, lung, and central nervous system. It has important roles in cell cycle, anaerobic breathing, growth (in children), and aging.[10–13] IGF-1R has also been found to have an important role in the development of metastatic tumors and their malignant phenotype and in cell proliferation and prevention of apoptosis.[11]

In the last decade, several studies have shown the importance of IGF-1R in UM.[14–17] It was shown that high expression levels of IGF-1R in primary tumors correlate significantly with lower survival rates.[18] Moreover, blocking the activity of IGF-1R with picropodophyllin (PPP) caused tumor regression and reduced the incidence of liver metastasis in xenografted mice.[19] Oral PPP also caused total growth inhibition and decreased VEGF expression in UM in mice.[14] These findings indicate that IGF-1R blockage is a possible new treatment modality for metastases that may also play a role as neoadjuvant therapy in preventing the development of

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metastatic disease. The Swedish group also assumed that the high expression levels of the IGF-1R in the liver, the production site of the IGF-1 ligand, may have a chemoattractant role in the seeding of UM in the liver.[14]

We speculated that since the receptor expresses on the tumor cell membranes, the formation and enlargement of liver metastases would increase the number of available receptors, and upon binding, would reduce the amount of the soluble ligand (IGF-1). The purpose of this study was to examine the potential of IGF-1 as a predictive factor in the early detection of liver metastasis in patients with UM.

Methods

Patients and Serum Samples

This was a prospective study of patients diagnosed with UM and were examined twice yearly in the Ocular Oncology Service of Hadassah-Hebrew University Medical Center. In order to detect metastasis, the evaluation included abdominal ultrasonography and blood samples for liver function tests and serum biomarkers. Upon detection of a suspicious mass on abdominal ultrasonography, patients underwent triphasic computed tomography (CT) scanning for confirmation, followed by fine needle aspiration biopsy.

Patients' blood samples were centrifuged for 10 minutes at 1200 rpm, and serum was stored at −20°C until analyzed. Levels of serum IGF-1 were analyzed using enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. All samples were analyzed together within two sequential days.

Use of patients' serum was approved by the patients (who signed an informed consent) and by Hadassah-Hebrew University Medical Center Institutional Review Board. Control subjects were healthy volunteers who donated their sera for biomarker studies.

Analysis Sets

Two sets of analyses were performed. The first set tested levels of serum IGF-1 in 118 subjects in three different groups: 55 disease-free (DF) UM patients who had not developed metastasis for at least 10 years from diagnosis; 22 metastatic patients; and 41 healthy subjects. Serum levels of metastatic patients were obtained on the day of diagnosis of metastasis. Levels of the biomarker were compared among the control group, the DF group, and the metastatic UM patients' group by using analysis of variance (ANOVA) and Student *t*-test.

In the second set, we performed matched-pairs univariate analysis for 19 metastatic patients' sera for whom we had sera at three different time points: 12 and 6 months prior to the diagnosis of metastasis and on the day of diagnosis. Levels were compared twice: once as time groups and once as individual personal trends per patient along time when levels were normalized for each patient to the 12 months prior to diagnosis time point. In order to examine the individual trends, changes in IGF-1 levels were normalized per patient as follows: levels at 12 months prior to diagnosis of metastases were considered baseline. The percentage of change from the previous examination was calculated per patient.

Statistical Analysis

Statistical analysis including matched pairs analysis and Student *t*-test, and ANOVA was performed using JMP Statistical Discovery software version 7.0 (SAS Institute, Cary, NC). The overall significance level was set to an alpha value of 0.05. Sample size estimation was based on the expected difference of the biomarker levels among the three study groups. We assumed a medium effect level ($F = 0.25$) with an alpha value of 0.05. To achieve a power of 80%, we calculated the required number of subjects needed for each group to be 40 controls, 55 DF patients, and 22 metastatic patients (Power and Precision, version 4 software; Biostat, Englewood, NJ).

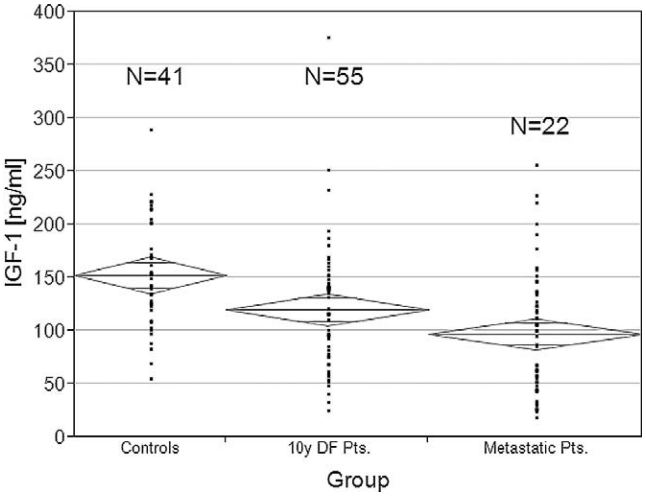

Figure 1. Differences in IGF-1 levels among control, DF, and metastatic groups (ANOVA, $P < 0.001$).

Results

Differences in IGF-1 Levels Among Control, DF, and Metastatic Groups

Mean ± SD levels for the control, the DF, and the metastatic groups were 152.48 ± 49.76, 119.93 ± 60.66, and 96.32 ± 56.64 ng/mL, respectively ($P < 0.001$, $R^2 = 12\%$, Fig. 1). IGF-1 serum levels were statistically significantly higher in the control subjects than in both the DF patients (Dunnett $P = 0.012$) and metastatic UM patients (Dunnett $P < 0.001$). IGF-1 serum levels were not significantly higher in DF patients than in those who developed metastases (Tukey-Kramer honestly significant difference [HSD] $P = 0.224$). Since IGF-1 levels decrease with age and our youngest metastatic patient was 57 years old, we repeated this analysis only for subjects over 50 years of age. Mean ± SD levels for the control, DF, and metastatic groups were 133.80 ± 27.25, 111.83 ± 45.80, and 96.32 ± 56.64 ng/mL, respectively ($P = 0.235$, $R^2 = 4\%$).

Differences in IGF-1 Levels in the Year Before Diagnosis of Metastases

Due to interpatient variability in IGF-1 levels, we normalized each patient's three time points of serum levels to the 12 months prior to diagnosis time point, and evaluated the change from visit to visit as would be done in a clinical setting (Fig. 2). For this small group of 19 metastatic patients, we found a 45% mean increase in IGF-1 level from 12 months prior to the diagnosis of metastasis to 6 months prior to the diagnosis, followed by a 5% mean decrease to the day of diagnosis of metastasis. When testing the change per patient, 70.6% of the patients had an increase in IGF-1 level from 12 months to 6 months prior to diagnosis (Fig. 3A) followed by a decrease in IGF-1 levels in 68.4% of the patients from 6 months to the day of diagnosis (Fig. 3B).

Discussion

Egan et al.[20] presented a similar work as a poster at the annual meeting of the Association for Research in Vision and

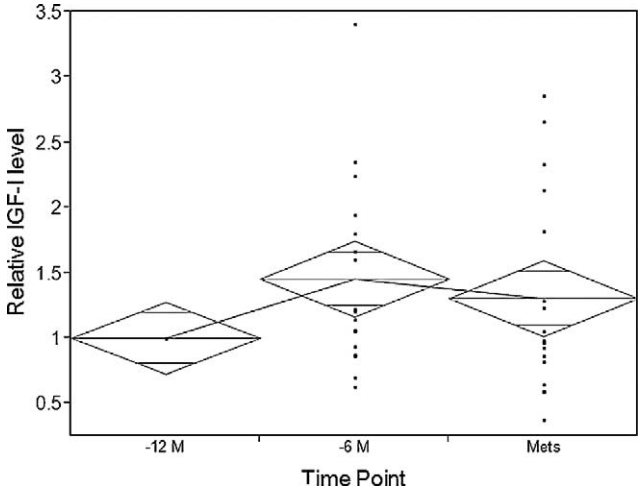

Figure 2. ANOVA of IGF-1 serum levels by time group when every patient's IGF-1 level was normalized to the same patient's at −12-month time point (−12-month time-point was considered baseline) and plotting the change in IGF-1 level from the −12-month visit.

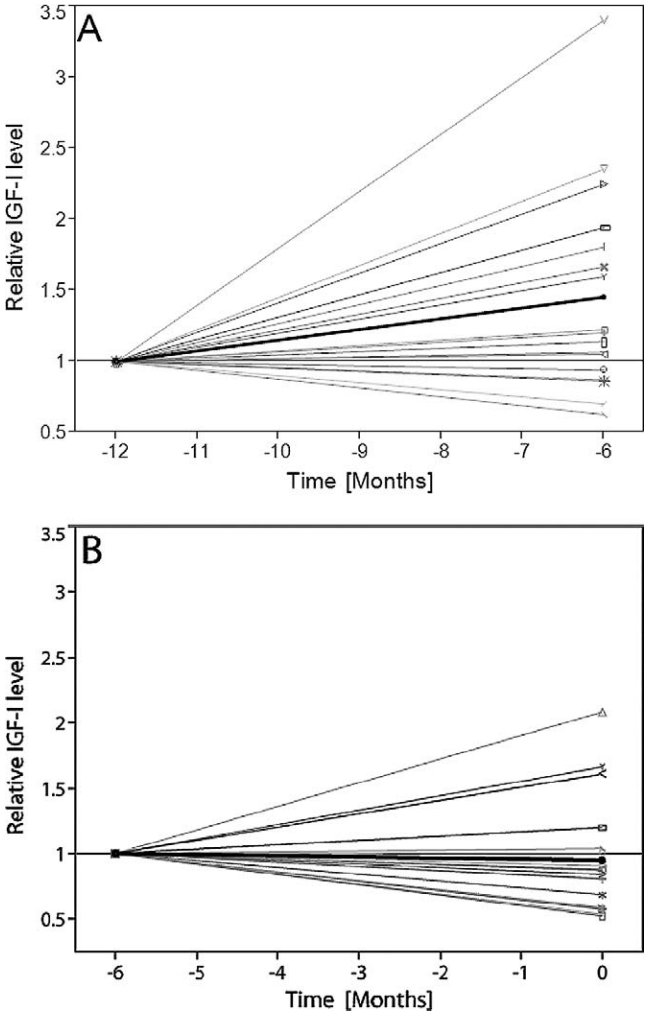

Figure 3. Individual changes in normalized IGF-1 levels. Pairwise comparison of IGF-1 serum levels from one visit to the next. Previous examination findings were set as baseline, and a ratio was calculated using the previous and current visits' examination values. (**A**) Change in IGF-1 serum level from 12 months prior to the diagnosis of metastasis to 6 months prior to the diagnosis of metastasis. (**B**) Change in IGF-1 serum level from 6 months prior to the diagnosis of metastasis to the day of diagnosis. *Thick black line* represents the mean change over all patients.

Ophthalmology. According to their abstract, they found no statistically significant difference between the mean serum levels of IGF-1 for patients with and without metastases (155.2 ng/mL and 158.4 ng/mL, respectively). We found lower mean serum IGF-1 level in metastatic patients, which was not statistically significantly different from the 10-year DF patients. This difference diminished when excluding patients younger than 50 years of age. However, we have shown that over the course of time leading to the diagnosis of metastasis, serum IGF-1 levels decreased in the 6 months prior to the diagnosis. Egan et al.[20] also tested the pretreatment serum levels of patients who later developed metastases and found higher levels of IGF-1 than in patients who had not developed metastases (211.8 vs. 163.2 ng/mL, respectively; $P = 0.011$). This is the opposite of findings by Fuchs et al.[21] in colorectal cancer, where higher baseline plasma levels of IGF-1 predicted a longer time to tumor progression and better overall survival. We have not tested the baseline IGF-1 serum level in UM patients, and we have started collecting data to evaluate this point prospectively.

The trend in which serum IGF-1 levels decrease in patients who developed metastasis prior to the diagnosis of metastasis contrasts with the previously tested serum biomarkers in uveal melanoma. Serum levels of S100β,[22] osteopontin,[22] melanoma inhibitory antigen,[22] VEGF, [23] and DJ-1,[24] have all been shown to increase upon development of metastatic disease. Although it is uncommon for serum biomarkers to decrease with disease progression, a similar decrease upon development of metastasis was reported in other cancers, for example, vascular adhesion protein-1 (VAP-1) in colorectal cancer.[25] We did not have enough data to compare IFG-I levels in our patients with other serum biomarker levels, but we hope to do so in a larger prospective study, which is currently underway.

IGFs are generally tightly complexed with IGF-binding proteins (IGFBPs), a family of at least six members, which determine the bioavailability of the IGFs and modulate their biological activities.[26] However, free IGF-1 can be measured in the serum. Without a direct measurement of IGF-1 binding protein levels in the sera of our patients it is difficult to correctly evaluate whether the decrease in IGF-1 serum levels upon development of metastasis that was found in our work results from binding of the soluble IGF-1 to the IGF-1R expressed on the cell membrane of the metastatic cells (our initial hypothesis), or from changes in the binding affinity with the IGFBP, or changes in IGFBP levels. We plan to answer this question in the future. However, whatever the answer to this question is, the data presented here point to the importance of measuring IGF-1 serum levels in UM patients as a biomarker for metastasis.

Patients with advanced cancer often suffer from a metabolic imbalance, which may result in involuntary weight loss that has been associated with decreased production of IGF-1 by the liver.[27] It is important to state that UM patients are usually completely asymptomatic at the time of diagnosis of metastasis and do not suffer from a metabolic imbalance at that stage, let alone involuntary weight loss. Thus, the reduction in IGF-1 serum levels observed here is not related to metabolic changes.

At our center, we follow patients via a routine biannual biomarker and abdominal ultrasonography to detect metastases, and send patients for triphasic CT scanning or magnetic resonance imaging (MRI) only upon suspicious findings. It may

be that centers which use triphasic CT scanning or MRI for their routine evaluation may find metastases in their imaging at an earlier stage than with ultrasonography findings. However, most centers prefer to minimize the use of CT due to the high dose of irradiation and risk to the kidneys from repeated contrast material injection, and in most institutions MRI is too expensive for routine follow-up. Therefore, we believe that most centers will be able to detect metastases at an earlier stage if they add IGF-1 serum level screening to their follow-up routine. Last, we would like to point out that as with other potential biomarkers,[23] interpatient variability in the biomarker's serum level should mandate a personalized approach in which levels are compared from visit to visit for each patient, without setting a general cutoff level for all patients.

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