Tissue-Based Microarray Expression of Genes Predictive of Metastasis in Uveal Melanoma and Differentially Expressed in Metastatic Uveal Melanoma

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Purpose: To screen the microarray expression of CDH1, ECM1, EIF1B, FXR1, HTR2B, ID2, LMCD1, LTA4H, MTUS1, RAB31, ROBO1, and SATB1 genes which are predictive of primary uveal melanoma metastasis, and NFKB2, PTPN18, MTSS1, GADD45B, SNCG, HHIP, IL12B, CDK4, RPLP0, RPS17, RPS12 genes that are differentially expressed in metastatic uveal melanoma in normal whole human blood and tissues prone to metastatic involvement by uveal melanoma.

Methods: We screened the GeneNote and GNF BioGPS databases for microarray analysis of genes predictive of primary uveal melanoma metastasis and those differentially expressed in metastatic uveal melanoma in normal whole blood, liver, lung and skin.

Results: Microarray analysis showed expression of all 22 genes in normal whole blood, liver, lung and skin, which are the most common sites of metastases. In the GNF BioGPS database, data for expression of the HHIP gene in normal whole blood and skin was not complete.

Conclusions: Microarray analysis of genes predicting systemic metastasis of uveal melanoma and genes differentially expressed in metastatic uveal melanoma may not be used as a biomarker for metastasis in whole blood, liver, lung, and skin. Their expression in tissues prone to metastasis may suggest that they play a role in tropism of uveal melanoma metastasis to these tissues.

Keywords: Eye; Uvea; Melanoma; Cancer; Metastasis; Microarray; Gene Expression Profiling

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INTRODUCTION

The most common site for uveal melanoma metastasis is the liver which is involved in more than 90% of patients with metastatic disease, followed by the lung (31%), bone (23%), and skin (17%). Micrometastasis usually occurs before the diagnosis or treatment of uveal melanoma. Tumor doubling time indicates that the majority of tumors metastasize 5 years before the primary choroidal melanoma is diagnosed. Considering the lack of lymphatics in the eye and the fact that the uvea is a highly vascular organ, uveal melanoma cells should have hematogenous metastasis.

Detection of circulating tumor cells in the bloodstream could have an important role in identifying patients at higher risk of developing
systemic metastasis and monitoring the response to adjuvant or systemic therapy. Polymerase chain reaction (PCR) and immunomagnetic cell isolation techniques showed the presence of circulating uveal melanoma cells in uveal melanoma patients. However, controversial results have been reported regarding the association between the presence of circulating uveal melanoma cells and the development of systemic metastasis.

Harbour and coworkers described a gene expression profile predictive of systemic metastasis in uveal melanoma including CDH1, ECM1, EIF1B, FXR1, HTR2B, ID2, LMCD1, LTA4H, MTUS1, RAB31, ROBO1, SATB1, NFKB2, PTPN18, MTSS1, GADD45B, SNCG, HHIP, IL12B, CDK4, RPLP0, RPS17 and RPS12 in normal whole blood, liver, lung, and skin. The gene expression profile of liver metastases was similar to that of the normal liver tissue. It is not known whether this similar gene expression profile contributes to tropism of uveal melanoma metastasis to the liver. The reported correlation between growth factors produced by the liver such as hepatocyte growth factor, epidermal growth factor, insulin-like growth factor-1 and their receptors, and the development of liver metastasis might also implicate homing and survival of metastatic melanoma cells to the liver.

In the current study, we evaluated the microarray expression of genes predictive for metastasis of uveal melanoma and genes differentially expressed in uveal metastases in normal whole blood and in tissues prone to metastatic involvement by uveal melanoma.

RESULTS

Results of microarray screening analysis for each gene in normal whole blood, liver, lung and skin are presented in Figure 1. All predictive genes and differentially expressed genes were expressed 10–fold greater than normalized intensity in all of the above-mentioned tissues except for IL12B expression in the liver and HHIP expression in whole blood and skin. IL12B was expressed around twice the normal values in the liver. In the GNF BioGPS database, no meaningful expression data for the HHIP gene was found in normal whole blood and skin after thresholding and normalization.

DISCUSSION

Uveal melanoma spreads via hematogenous metastasis. Detection of melanoma cells in the
bloodstream might be helpful for classifying the risk of metastasis and monitoring the development of metastasis and also the response to treatment. In uveal melanoma patients, melanoma cells have been detected in the whole blood by using biomicroscopy, immunomagnetic cell sorting and real-time PCR.\(^4\)\(^\text{-}\)\(^15\) Reverse transcription PCR has been used to detect tyrosinase mRNA as a surrogate marker for melanoma cells because it is involved in the synthesis of melanin. Pilot studies showed that patients who were PCR-positive for tyrosinase mRNA in the blood already had or subsequently developed clinically detectable liver metastasis after a mean follow-up of 9 months.\(^5\) However, follow-up studies with larger sample size did not confirm these findings.\(^15\) Later, PCR detection of Melan-A/MART1 and gp100 were found to be inconsistent in detecting uveal melanoma cells in the blood.\(^9\),\(^15\)

Recently, Harbour et al identified 12 predictive genes that categorize uveal melanomas into 3 main classes: 1A, 1B and 2.\(^16\)\(^\text{-}\)\(^19\) Uveal melanoma patients with a tumor class 2 gene expression profile have a 70% risk of developing clinical metastasis over 5 years as compared to a 5% risk in patients with tumor class 1 profile.\(^5\) As seen in our screening of GeneNote and GNF BioGPS databases, these 12 genes are expressed in microarrays of normal whole blood, liver, lung, and skin. Therefore, they cannot be used for microarray detection of circulating melanoma cells or micrometastases in organs susceptible to uveal melanoma metastasis.

Meir et al\(^20\) compared gene expression in liver metastases of uveal melanoma with that of primary uveal melanoma and reported that metastatic melanoma has a distinct gene expression profile. None of the 11 genes differentially expressed in metastatic melanoma was among the 12 predictive genes identified by Harbour and associates.\(^16\)\(^\text{-}\)\(^20\) As seen in our screening of GeneNote and GNF BioGPS databases, all 11 genes except the HHIP gene were expressed in microarray of normal whole blood, liver, lung and skin. Data for the HHIP gene was not complete in the GNF BioGPS database for normal whole blood and skin.

The microenvironment of the end organ and crosstalk between melanoma cells and their microenvironment are important for colonization, survival and growth of the metastatic uveal melanoma cells. This concept is supported by recent studies that have shown
circulating melanoma cells in patients with uveal melanoma independent of tumor size, type of treatment, or length of follow-up. Therefore, circulating melanoma cells are thought to colonize in distant organs, form micrometastases, and remain dormant while sporadically seeding tumor cells into the circulating blood. Although these two sets of genes showed expression of two different tumor microenvironments which were not comparable, microarray analysis for 12 predictive genes described by Harbour and coworkers and 11 differentially expressed genes described by Meir and coworkers are not useful for detecting circulating uveal melanoma cells or tissues which are commonly colonized by them. It remains unclear whether similarity in the gene expression profile of the primary tumor to tissues prone to development of metastasis contributes to the tropism of uveal melanoma for metastasis to the liver.

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Conflicts of Interest
None.

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