Molecular Characteristics of Liver Metastases from Uveal Melanoma

Tal Meir,1,2 Rinit Dror,1,2 Xueping Yu,3 Jiang Qian,5 Itamar Simon,4 Jacob Pe’er,1 and Itay Chowers1

PURPOSE. The liver is the most common site of systemic metastases from uveal melanoma (UM). Such metastases usually continue to develop despite the application of current treatment modalities. This study was conducted to obtain insight into the molecular pathways that underlie the development of UM metastasis and thus to identify potential novel therapeutic pathways for this disease.

METHODS. Microarray analysis of seven primary UM s and seven liver metastases from UM s was performed by using oligonucleotide microarrays containing 35,035 features. Bioinformatics was applied to identify expression patterns associated with metastases. Results were validated with real-time quantitative RT-PCR (QPCR) and immunohistochemistry (IHC).

RESULTS. Metastasis-associated expression was detected for 193 genes at the false discovery rate (FDR) level of 0%. QPCR confirmed microarray results for all 11 genes that were evaluated (r² = 0.9, P = 0.0001), and IHC validated microarray data for the two proteins (NFKB2 and CDK4) that were assessed. The gene expression pattern of UM liver metastases demonstrated a resemblance to normal liver tissue. Bioinformatics facilitate identification of transcription factors, among them NFKB, which potentially regulate expression of several metastasis-associated genes.

CONCLUSIONS. Liver metastases from UM s have a distinct gene expression pattern compared with the primary tumor while sharing similarities with gene expression patterns of normal liver. Several candidate genes for involvement in UM metastasis have been identified—among them several in the NFKB pathway. (Invest Ophthalmol Vis Sci. 2007;48:4890–4896) DOI:10.1167/iovs.07-0215

Uveal melanoma (UM), the most common primary ocular cancer in adults, is associated with the development of systemic metastases in approximately 50% of the cases.1–3 Via pathways that are poorly understood, such metastases demonstrate remarkable tropism to the liver.2–6 Unfortunately, despite the application of current therapies, the average survival time after the diagnosis of metastatic UM is only 6 to 8 months.2,7,8

Several histologic, genetic, and demographic factors have been associated with metastases in UM. Among these factors, identification of vasculogenic mimicry patterns in tumor sections, loss of one copy of chromosome 3 (monosomy 3), and specific gene expression patterns appear to be the most accurate predictors of death from metastasis in patients with UM.9–14

Conceivably, micrometastases are present in many patients with UM years before their diagnosis. This possibility is suggested by the fact that metastases are often found years after the enucleation of the affected eye with a negative systemic screening for metastasis at the time of surgery.15,16 The fact that metastasis might precede initial treatment in many cases of UM was also suggested by Eskelin et al.1 and colleagues based on tumor doubling time calculation. Thus, to achieve prolonged survival, novel therapies for UM should be directed at suppressing metastasis from the primary tumor, rather than at prevention of its occurrence.

A major obstacle in developing such novel treatments is the limited understanding of pathways that are involved in UM metastasis. Although significant insight has recently been obtained into the gene expression characteristics of primary UM,1,11,18,19 and of the cell lines obtained from UM metastases,13,20,21 there is limited knowledge on the molecular pathways that underlie the metastasis.

To that end, we have taken a high-throughput gene-expression profiling approach to compare gene expression patterns between primary UM and its liver metastases. We performed bioinformatic analyses that enabled identification of several candidate genes and pathways for involvement in the development of UM metastases.

METHODS

Tissues and Patients

Samples from 14 patients with UM (seven primary tumors and seven liver metastases) were included in the study. All patients were treated at the Hadassah-Hebrew University Medical Center (Jerusalem, Israel). The study was approved by the institutional committee and adhered to the guidelines in the Declaration of Helsinki. Patient’s age ranged from 27 to 90 years of age; seven were women and seven were men. Additional demographic data are included in Supplementary Table S1 (supplementary tables are online at http://www iovs org/cgi/content/ full/48/11/4890/DC1). Fresh tissue from each primary or metastatic lesion was processed for histology, RNA extraction, and immunohistochemistry (IHC). For RNA extraction, the metastases were identified by their pigmented appearance, and the adjacent tissue was processed for histology, to confirm the absence of normal liver tissue in the area. Cell type was assessed in formalin-fixed, paraffin-embedded sections stained with hematoxylin and eosin. Vasculogenic mimicry patterns were assessed in PAS-stained sections, as previously described.22

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Microarray Analysis

Microarray experiments were performed as previously described, with minor modifications. Briefly, RNA was extracted (Tri Reagent) according to the manufacturer’s protocol (Sigma-Aldrich Corp., St. Louis, MO), and 40 μg of total RNA was then treated with DNase (DNasefree; Ambion, Austin, TX), followed by RNA purification (RNeasy MiniElute Cleanup kit; Qiagen, Valencia, CA). Quantification by spectrophotometer and assessment of quality on a gel were then performed. Indirect fluorescent labeling method was applied using 20 μg of purified RNA as template for cDNA synthesis with reverse transcriptase (SuperScript II; Invitrogen, Karlsruhe, Germany), and incorporation of aminomethyl-UTP (Sigma-Aldrich Corp.) during first-strand cDNA synthesis followed by coupling nonradioactive cys or cys 5 fluorescent dye (GE Healthcare, Piscataway, NJ). The total amount of dye incorporation (measured in picomoles of dye per probe) and the ratio of unlabelled to fluorescent-labeled nucleotide in the probe were assessed by measuring probe absorbance at 260, 550, and 650 nm to assess DNA, Cy3, and Cy5 concentration, respectively.

A human array generated from an oligonucleotide set by Operon Biotechnologies Inc. (Cologne, Germany), version 3.0, which contains 35,035 oligonucleotide probes representing approximately 25,100 unique genes was used for all experiments. The fluorescent probe was placed on the slide followed by incubation at 42°C for 16 to 22 hours. Posthybridization washes were performed, followed by scanning (scanner with the GenePix Pro 4.1 Microarray Image Analysis software). A common reference sample experimental design was used in which every donor tumor RNA was hybridized along with a standard RNA reference. The reference RNA was composed of a mixture of RNA extracted from HeLa cell line, ARPE 19 cell line, human tonsils, and human white blood cells. Tumor sample RNA was labeled with Cy3 and reference sample RNA with Cy5 in each experiment.

Data normalization and analysis were preformed using the TIGR MeV software version 3.124 followed by lowess normalization with TIGR Microarray Data Analysis System (MIDAS) and identification of differential expression patterns between primary and metastasis tissues by applying the significance analysis for microarray (SAM) algorithm.25

Bioinformatics

Functional annotation of genes represented on the array was performed with Web-based software (http://info.bioinfo.vanderbilt.edu/gotm, http://fatigo.bioinfo.cipf.es).26-27 Potential enrichment of specific functional classes among genes with significantly high or low expression was evaluated using Fisher’s exact test.

Similarities of gene expression across UM liver metastases and primary tumor tissues were evaluated using an average linkage hierarchical clustering analysis (Hierarchical Clustering caffe; IncRNA.org; St. Louis, MO), and 40 μg of total RNA was then treated with DNase (DNasefree; Ambion, Austin, TX), followed by RNA purification (RNeasy MiniElute Cleanup kit; Qiagen, Valencia, CA). Quantification by spectrophotometer and assessment of quality on a gel were then performed. Indirect fluorescent labeling method was applied using 20 μg of purified RNA as template for cDNA synthesis with reverse transcriptase (SuperScript II; Invitrogen, Karlsruhe, Germany), and incorporation of aminomethyl-UTP (Sigma-Aldrich Corp.) during first-strand cDNA synthesis followed by coupling nonradioactive cys or cys 5 fluorescent dye (GE Healthcare, Piscataway, NJ). The total amount of dye incorporation (measured in picomoles of dye per probe) and the ratio of unlabelled to fluorescent-labeled nucleotide in the probe were assessed by measuring probe absorbance at 260, 550, and 650 nm to assess DNA, Cy3, and Cy5 concentration, respectively.

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(QPCR) was applied for validation of microarray results. The following 11 genes that demonstrated metastasis-associated expression pattern in microarray analysis were evaluated with QPCR: RPS12, RPS17, RPLP0, CDK4, MTSS1, IL12, GADD45B, PTEN, NCG, NFKB2, and HHIP. QPCR was performed on RNA samples from the seven primary UM and seven metastases samples, which were also assessed by microarrays. From each sample 1 μg of RNA was reverse transcribed and used as a template for QPCR. Reactions were performed in triplicate with a SYBR Green PCR kit and primer assay (QuantiTect; Qiagen, Valencia, CA) on a PCR system (Prism 7000 Applied Biosystems, Inc. [ABI] Foster City, CA). The results were quantified using the system software (SDS Prism 7000; ABI). Measurements of TBP were used for normalization of expression levels across samples.

Semiquantitative PCR

Semiquantitative PCR was performed for the potential presence of normal liver tissue in the metastases sample. cDNA from metastases was amplified using primers for ASGR1, a hepatocyte-specific gene (forward primer: 5’-ACGTGAAGAGCTTCGTGTGTC-3’, reverse primer: 5’-AGGTGAGGGCATTGAAAGA-3’). GAPDH mRNA levels were used as an internal control (forward primer: 5’-GGGGGAGGCGAAAAAGGCTAT-3’, reverse primer: 5’-GCCCAACGTCAAGGTTGAG-3’). PCR was performed as follows: 94°C for 5 minutes, 40 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 30 seconds, and a final elongation of 72°C for 5 minutes. Ten microliters of each sample were run on 2.5% agar gel, the gel was photographed, and the band intensity was quantified (BIS 305 PC software: DNR Bio-Image Systems, Jerusalem, Israel) with normalization to GAPDH levels.

Immunohistochemical Analysis

IHC was performed on 6-μm-thick frozen sections from each primary tumor and metastasis tissue sample. The mouse monoclonal anti-CDK4 antibody (BioSource-Invitrogen, Carlsbad, CA), at a dilution of 1:100, and the rabbit polyclonal anti-NFκB p100/p52 antibody (Abcam, Cambridge UK), at a dilution of 1:50, were used for IHC. Sections were incubated for 30 minutes with H2O2 followed by a 30-minute incubation with the primary antibody and washes. The sections were then incubated for 30 minutes with polymer horseradish peroxidase (HRP) anti-mouse, or polymer HRP anti-rabbit (both from DakoCytomation, Glostrup, Denmark). All incubations were performed at room temperature in a humidified chamber. The slides were then washed and incubated in 3-amino-9-ethylcarbazole (AEC; DakoCytomation) for 20 minutes followed by counterstained with hematoxylin. A tissue specimen of mouse skin and mouse spleen served as positive controls for CDK4 and NFKB2, respectively. For negative control, nonimmune buffer was substituted for the primary antibody. Stained sections were evaluated under high magnification (×400), and the mean positive cell count in 10 random high-power fields was calculated. Mean positive
cell counts in primary and metastasis sections were then compared by $t$-test.

RESULTS

Identification of Differential Gene Expression in Liver Metastases

Microarray analysis identified 193 differently expressed genes in liver metastases from UM compared with the primary tumor at an FDR of 0%. One hundred eighty-four of these genes showed increased mRNA levels in metastases, whereas only nine were increased in primary tumors. At a less-stringent FDR level of 5% there were 481 differently expressed genes, of which 393 showed increased mRNA levels in metastases, whereas 88 were increased in primary tumors. Hierarchical clustering using the expression profile of the 193 differentially expressed genes according to the SAM algorithm distinguished well between the primary UM and liver metastasis samples (Fig. 1), demonstrating the validity of the SAM results with an alternative algorithm (clustering).

Microarray results were validated with QPCR measurements of the mRNA level of 11 of the 193 differentially expressed genes at an FDR of 0% (Table 1). All 14 tumor samples that were included in the microarray analysis were tested with QPCR. Seven of the 11 genes were upregulated in metastases according to the microarray results and the remaining four genes showed increased expression in primary UM. QPCR results confirmed microarray findings for each of these 11 genes and demonstrated significant correlation with microarray data ($R^2 = 0.9, P = 0.0001$, Table 1, Fig. 2).

Microarray results were also validated at the protein level by IHC with two proteins: NFKB2 and CDK4. These proteins were selected for IHC, as they were differentially expressed between primary tumors and metastases according to both microarray and QPCR analyses and because both proteins are known to be involved in the pathogenesis of a variety of tumors.32,33 NFKB2 mRNA was increased in metastases, whereas CDK4 was increased in primary tumors. IHC demonstrated protein expression patterns that correlated with the mRNA levels. IHC of NFKB2 was more intense in metastases compared with primary UM (mean positive cell count of 20.3 $\pm$ 3.5 and 3.6 $\pm$ 3.7, respectively, $P = 0.002$, $t$-test, Fig. 3). IHC of CDK4 was more intense in primary tumors than in metastases, with a mean positive cell count of 7.4 $\pm$ 6.9 and 16.8 $\pm$ 7.5, respectively ($P = 0.01$, $t$-test, Fig. 3).

Comparing Gene Expression across Liver Metastases, Other Malignancies, and Normal Tissues

The average change ($n$-fold) in genes with increased expression in liver metastases from UM at FDR 5 was calculated in a variety of microarray datasets as a measure of similarity of gene expression across other tissues (see the Methods section). This analysis demonstrated marked increased expression of UM metastasis-associated genes according to microarray analysis (FDR 5) in normal liver tissue (average change = 3.1-fold). Such increased expression was not identified in other normal tissues (Fig. 4A). Increased expression was also detected in several malignancies—among them, leukemia (average = 2.5-fold) and skin melanoma (average = 2-fold)—but not in other malignancies, including colon and gastric carcinomas (Fig. 4B). Such increased expression was not detected in metastases from variety of malignancies (data not shown). None of the genes with metastasis-associated expression in our study were found to be differentially expressed between primary UM with high or low risk for development of metastases, respectively, according to Tschentscher et al.12 and Onken et al.13

Several facts suggest that the metastasis tissue which was included in this study did not contained liver tissue. UM metastases were identified by gross appearance of pigmented tissues which was clearly distinct from the surrounding non-pigmented liver. This gross distinction between metastasis and normal liver tissue was validated by histology. Typical histology of UM metastasis was demonstrated in the pigmented area with
TABLE 1. Comparison of Microarray and QPCR Results

<table>
<thead>
<tr>
<th>Gene Symbol (HUGO ID)</th>
<th>Full Name</th>
<th>EMBL ID</th>
<th>Microarray</th>
<th>QPCR</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFKB2</td>
<td>Nuclear factor of kappa light polypeptide gene enhancer in B-cells</td>
<td>U20816</td>
<td>1.5</td>
<td>3.4</td>
<td>Immune response, apoptosis, and cell growth</td>
</tr>
<tr>
<td>PTNP18</td>
<td>Protein tyrosine phosphatase, nonreceptor type 18</td>
<td>X79568</td>
<td>1.6</td>
<td>19.8</td>
<td>Tyrosine phosphatase family</td>
</tr>
<tr>
<td>MTSS1</td>
<td>Metastasis suppressor 1</td>
<td>AF116674</td>
<td>1.8</td>
<td>5.2</td>
<td>Cytoskeletal organization, related to cancer progression in variety of organs</td>
</tr>
<tr>
<td>GADD45B</td>
<td>Growth arrest and DNA damage inducible, beta</td>
<td>AC005624</td>
<td>1.9</td>
<td>24.7</td>
<td>Regulation of cell growth, apoptosis, and response to stress</td>
</tr>
<tr>
<td>SNCG</td>
<td>Synuclein, gamma (breast cancer specific protein 1)</td>
<td>AF219257</td>
<td>1.9</td>
<td>4.0</td>
<td>Synuclein family, identified in advanced breast carcinomas</td>
</tr>
<tr>
<td>HHIP</td>
<td>Hedgehog-interacting protein precursor</td>
<td>BC025311</td>
<td>2.2</td>
<td>33.7</td>
<td>Regulatory component of hedgehog signaling pathway</td>
</tr>
<tr>
<td>IL12B</td>
<td>Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)</td>
<td>Q9P1E1</td>
<td>2.5</td>
<td>45.4</td>
<td>Cytokine, subunit of interleukin 12</td>
</tr>
<tr>
<td>CDK4</td>
<td>Cyclin-dependent kinase 4</td>
<td>BC015669</td>
<td>−2.1</td>
<td>−3.2</td>
<td>Ser/Thr protein kinase family, cell cycle progression</td>
</tr>
<tr>
<td>RPLP0</td>
<td>Ribosomal protein, large, P0</td>
<td>AB007187</td>
<td>−2.7</td>
<td>−17.5</td>
<td>Component of ribosome 60S subunit</td>
</tr>
<tr>
<td>RPS17</td>
<td>Ribosomal protein S17</td>
<td>BC022570</td>
<td>−2.7</td>
<td>−2.8</td>
<td>Component of ribosome 40S subunit</td>
</tr>
<tr>
<td>RPS12</td>
<td>Ribosomal protein S12</td>
<td>AB007153</td>
<td>−3.2</td>
<td>−7.5</td>
<td>Component of ribosome 40S subunit</td>
</tr>
</tbody>
</table>

EMBL ID. European Molecular Biology Laboratory identification; (−) genes that were downregulated in primary UM compared with liver metastases; microarray/QPCR, fold expression level difference between liver metastases and primary UM according to microarray analysis and quantitative real-time RT-PCR, respectively.

a distinct border from the nonpigmented area composed of normal liver tissue (Fig. 5).

Bioinformatics further excluded potential bias that might be introduced by normal liver tissue. Our analysis showed that 14 genes that were represented on the array and which normally show liver enriched expression pattern (http://expression.gnf.org/cgi-bin/index.cgi? Q/ Novartix GNF) including APOF, MPST, CFHL3, CYP2D6, SARDH, IQCE, SLC6A12, SLC25A20, CCL16, CYP4F11, PGM1, IQCE, KIAA0841, and SEC14L2 did not manifest increased expression levels in UM liver metastasis. Finally, expression of ASGR1, a liver hepatocyte-specific gene, as measured by semiquantitative PCR, demonstrated expression levels ranging between 0 and 3.7 OD/mm² in the metastases samples that were used for microarray analysis, compared with 177 OD/mm² in normal liver sample. Four of the six metastasis samples that were used for microarray analysis, compared with 177 OD/mm² in normal liver sample. Four of the six metastasis samples that were tested demonstrated no expression of ASGR1. These results demonstrate that only a negligible quantity of liver tissue was present in a minority of the metastasis samples.

Bioinformatic Analyses for Chromosomal Location and Functional Classes

Genes represented on the array were mapped on the genome followed by assessment for enrichment of genes with metastasis-associated expression patterns at FDR 0 on particular chromosomal location. We found that differently expressed genes are significantly enriched on chromosome 10 where the genes WDFY4, SNCG, NFKB2, and NEURL are located (P = 0.013, Fisher exact test.), as well as on chromosome 21 where the genes CRYZL1, DONSON, CBS, and FTCD are located (P = 0.0001, Fisher exact test) (Supplementary Fig. S1; supplementary figures are online at http://www iovs.org/cgi/content/full/48/11/4890/DC1).

To assess enrichment of particular functional classes among the metastasis-associated genes we annotated all genes with known function that were represented on the array according to the biological, molecular, and cellular processes in which they have a role. Several functional classes were significantly enriched among the differentially expressed genes at FDR 5, including ribosome, ribonucleoprotein complex and small ribosomal subunit belonging to cellular processes, protease inhibitor activity, RNA binding, and oxidoreductase activity belonging to the molecular process, cell homeostasis, ion homeostasis, and regulation of transferase activity processes belonging to biological processes (Supplementary Fig. S2).

FIGURE 2. Correlation between measurements of mRNA levels in liver metastases and primary UM according to microarray and QPCR, respectively. Expression of 11 genes was evaluated; results are presented on a logarithmic scale (log base 2). \( R^2 = 0.9 \) (P = 0.0001).
Cellular localization of all differently expressed genes was analyzed to identify secreted proteins that may serve as markers for development of metastases. Nine such secreted proteins were identified, eight of them with increased expression in metastases (at FDR 0) (Supplementary Table S2).

**Transcription Factor Analysis**

Transcription factors that may underlie altered expression of genes in UM metastases were identified by applying two different bioinformatic algorithms (see the Methods section). According to the Expander algorithm the most significant transcription factors that are known to regulate the genes with metastasis-associated expression (FDR 5) were: Crx (\(P = 0.007\)), Egr1 (\(P = 0.005\)), MTF1 (\(P = 0.008\)), and NFKB (\(P = 0.003\)). NFKB was found to affect the following genes which demonstrated metastasis-associated expression: \(\text{CACNA2D4}, \text{HHIP}, \text{OTP}, \text{NFKB2}, \text{GADD45B}\), and \(\text{DCX}\). As showed in Table 1, microarray findings of altered expression of \(\text{NFKB2}, \text{GADD45B}\), and \(\text{HHIP}\) were also validated by QPCR.

According to the second analysis based on an algorithm developed by Yu et al.,\(^{31}\) several transcription factors may regulate genes with metastasis-associated expression (with FDR 0) including USF2, USF1, Arnt, Max, c-Myc, AP-4, AP-2\(^\gamma\),

**Figure 3.** IHC analysis of NFKB2 and CDK4 expression in primary UM and in liver metastases from UM. Smaller number of NFKB2-positive cells are evident in primary tumor (A) compared with liver metastases (B), the mean positive cell count per high-power field (MPCC/HPF) was significantly higher in metastases (m) (C) \((P = 0.002, \text{t-test})\). By contrast, a larger number of CDK4-positive cells was evident in primary tumor (p) (D), compared with liver metastases (E) and was also demonstrated by assessment of MPCC/HPF \((P = 0.01, \text{t-test})\) (F).

**Figure 4.** Assessment of relative expression levels of UM metastasis-associated genes in a variety of normal (A) and malignant (B) tissues. Histograms represents the median change (in log base 2) of UM metastasis-associated genes in each microarray dataset. Only tissues in which at least five different microarray datasets were available for analysis were included in the graph.

**Figure 5.** Histologic section from UM liver metastasis demonstrating the typical UM metastasis histology (black arrowhead) in distinction from liver tissue histology (white arrowhead). A clear distinction between metastatic and normal liver tissue is readily evident.
CUTL1, HEB, PPAR-α, and RXR-α. Several transcription factors including USF2, Arnt, Max, c-Myc, AP-4, and AP-2γ overlapped between the two analysis approaches (Supplementary Table S3). None of these transcription factors is mapped to a chromosomal area in which aberrations tend to occur in UM except of c-Myc which is positioned on the long arm of chromosome 8. Trisomy of this chromosomal arm is often found in UM.54

**Discussion**

According to our microarray analysis, which was validated by complementary experimental approaches and bioinformatics, liver metastases from uveal melanoma have a distinct gene expression pattern that is significantly different from that of the primary eye tumor. Many of the genes with UM metastases-associated expression which were identified in this study are candidates for involvement in metastasis based on their known function and involvement in other malignancies. For example, NFκB2 is known to influence both apoptosis and cell cycle,55 and CDK4 is known for its crucial role in cell cycle progression56; both genes are involved in several malignancies.57,58 Therefore, alterations in the expression of these genes may contribute to UM metastasis through modulation of cell cycle regulation and apoptosis.59

UM metastases demonstrate remarkable tropism to the liver; approximately 80% to 95% of UM metastases involve this organ.2,5 and in most cases the liver is the sole site of metastasis.2,6 Analysis of gene expression patterns in liver metastases from UM compared with expression patterns from a variety of normal and malignant tissues suggest that genes with increased expression in UM liver metastases also show relatively increased expression in liver tissue.59 Whether gene expression similarities between normal liver and UM metastases are primary, and whether it contributes to the tropism of UM metastasis to the liver or such similar gene expression patterns stem from exposure of UM metastasis to the microenvironment of the liver is unclear. Studies of UM metastases from other organs may facilitate further insight into this issue. However, in accordance with our findings, recent studies have suggested the existence of similarities between gene expression patterns in cancer tissue and its target organs for development of metastases.38-40 Conceivably, such similarity between metastasis and the host organ may facilitate the development and growth of metastases.

UM liver metastases also showed gene expression similarities with other malignant tissues—in particular, with skin melanoma and leukemia. Such similarities may reflect activation of common pathways in malignant tissues of different origins. For example, the NFκB pathway is also known to be involved in different malignancies—among them, leukemia42 melanoma,43 and liver malignancies.57 Functional analysis enabled identification of several secreted proteins that are expressed at higher levels in metastases. The potential of such proteins as serum markers for metastasis should be evaluated in patients with UM. If confirmed, such markers may be used in combination with other serum markers for UM metastasis that were recently described.43,44

Several of the genes that demonstrated metastasis-associated expression in our study are known to be regulated by a few transcription factors including c-Myc and NFκB. c-Myc has been implicated in the pathogenesis of several malignancies,9,45,46 and its expression in UM is well documented.47 Whereas amplification of this gene has been described and associated with tumor size in primary UM, expression of c-Myc has also been associated with favorable prognosis of patients with UM.58 NFκB is known to regulate the expression of several of the UM metastasis-associated genes which were identified in our study, including CACNA2D4, HHIP, OTP, GADD45B, and DCX. NFκB2 was itself upregulated in liver metastases, supporting its potential contribution for increased expression of its downstream genes, including genes involved in cancer progression such as GADD45B and HHIP.49,50 These data suggest that the NFκB pathway may be important in the development of UM metastases.

This study provides several candidate genes and pathways for involvement in the development of liver metastases from UM. The data also provide first insight into similarities between gene expression patterns of UM metastases and normal liver. Microarray analysis enables a look at a mass effect of gene expression patterns in a tissue. Therefore, further research should demonstrate the potential functional significance of the candidate genes that we have identified as involved in UM metastasis. Future studies are also needed, to identify the underlying causes of similarities in gene expression between UM metastasis and its host organ.

**References**
