Tumor Classification Based on Gene Expression Profiling Shows That Uveal Melanomas with and without Monosomy 3 Represent Two Distinct Entities

Frank Tschentscher, Johannes Hüsing, Tanja Hölter, Elisabeth Kruse, Irina Gana Dresen, Karl-Heinz Jöckel, Gerasimos Anastassiou, Harald Schilling, Norbert Bornfeld, Bernhard Horsthemke, Dietmar Rudolf Lohmann, and Michael Zeschnigk


ABSTRACT

Uveal melanoma is the most common intraocular malignancy. About 50% of patients die of metastases, which almost exclusively originate from primary tumors that have lost one chromosome 3 (monosomy 3). To gain insight into the biological mechanisms that underlie the various metastasizing potential of uveal melanoma, we have determined gene expression levels in 20 primary tumors using oligonucleotide microarrays containing 12,500 probe sets. The expression measurements of those 7902 genes that were expressed in more than 10% of tumors were analyzed using two different statistical approaches. We used a modified Wilcoxon rank-sum test to identify genes differentially expressed between tumors with and without monosomy 3. Seven genes showed complete loss of expression in tumors with monosomy 3 but were expressed in tumors with disomy 3. Two of them, CHL1 and βf485, are located within or close to the uveal melanoma susceptibility locus UVM2 at 3p25. However, mutation analysis of both genes in eight tumors with monosomy 3 did not reveal structural or epigenetic alteration. To identify tumor classes, we performed unsupervised hierarchical cluster analysis; this approach separated uveal melanomas into two groups. We found that this classification is strikingly robust because, when tested by "resampling," the same grouping is obtained from 47 of 50 subsamples of genes. In clusterings of the three remaining subsamples, the grouping of only one tumor does not conform with the original classification. Excluding this tumor, cluster analyses of subsamples containing as few as 300 randomly chosen genes consistently result in the same classification, thus indicating that the difference between the two tumor classes is pervasive. Interestingly, all of the tumors in one of the groups have disomy 3, whereas all of the others have monosomy 3. Our findings suggest that there are two distinct entities of uveal melanoma that were previously unrecognized because they are not obviously distinguishable by clinicopathological features.

INTRODUCTION

Uveal melanoma is the most common intraocular malignancy (1). The incidence of this tumor increases with age and reaches a maximum between the 6th and 7th decade of life. Approximately 50% of patients die of metastases, a proportion that, despite all efforts to improve treatment, has remained constant during the last century. The average life expectancy after diagnosis of metastases is 7 months (2–4).

Cytogenetic analyses and comparative genomic hybridization have revealed recurrent chromosomal aberrations, including chromosomes 3, 6, and 8 (5, 6). Loss of an entire chromosome 3, which is an early event in tumorigenesis, is detected in ~50% of tumors (7, 8). Long-term studies have shown that ~70% of patients with monosomy 3 in the primary tumor have died of metastases within 4 years after the initial diagnosis, whereas tumors with normal chromosome 3 status (disomy 3) rarely gave rise to metastatic disease (9). Consequently, monosomy 3 is a highly specific prognostic marker for poor prognosis. The prognostic significance of parameters other than monosomy 3, e.g., vascular patterns (10), cell type, tumor diameter or location, is still under discussion.

It has been proposed, that the loss of one chromosome 3 is part of a two-step mutation mechanism typical for the inactivation of tumor suppressor genes. In support of this hypothesis, we identified two regions of deletion overlap on chromosome 3 (designated UVM1 and UVM2; OMIM3 606661; Ref. 11). These loci provide information on the location of putative suppressor genes in uveal melanoma. Assuming that inactivated genes show reduced transcript levels, putative suppressor genes might be detected by global expression analysis if including the positional information contributed by known regions of deletion overlap.

We have performed expression analysis in 20 uveal melanomas using oligonucleotide microarrays containing more than 12,500 probe sets. We used the data to identify genes differentially expressed between uveal melanomas with and without monosomy 3. Expression data were also used for tumor class discovery by unsupervised hierarchical cluster analysis. We applied bootstrap analysis and clusterings of small subsamples of expressed genes to test the reliability of the observed tumor classification.

MATERIALS AND METHODS

Patients and Tumor Specimens. All of the patients were given diagnoses according to current ophthalmologic criteria. Informed consent of the patients was obtained before tumor sampling. Vital tumor samples were obtained from patients treated by primary enucleation without prior radiation or chemotherapy. Tumor (snap-frozen in liquid N2) and peripheral blood samples were obtained at the time of surgery and were stored at −80 and −20°C, respectively.

Histological Examination and Genotyping. Each tumor was classified according to cell type by conventional histology using the modified Callender system (12). Its location and degree of extension were noted. For the evaluation of vascular patterns, conventional periodic acid Schiff staining without hematoxylin counterstaining after bleaching for strong pigmented sections was performed. The vascular patterns were visible with a conventional microscope using a dark green filter and were classified according to the guidelines of Folberg et al. (10). DNA extraction from blood and tumor samples and microsatellite analysis, which was used for the identification of alterations of chromosomes 3, 6, and 8, were performed as described previously (13). Chromosome 6p alterations were not determined in three samples because of shortage of DNA.

Microarray Analysis. Analysis was performed on RNA derived from 20 tumors that, in microsatellite analysis of informative chromosome 3 loci, showed either complete loss of the signal of one allele (10 tumors) or no allelic imbalance (10 tumors). Total RNA was isolated from primary tumor samples using a column-based method (Qiagen RNeasy; Qiagen, Hilden, Germany). Approximately 20 μg of RNA were obtained per sample. Biotinylated “cRNA targets” were prepared according to the manufacturer’s instructions (Affymetrix Expression Analysis Technical Manual; Affymetrix, Santa Clara, CA). Briefly, 6 μg of RNA were transcribed to double-stranded cDNA using the...
Custom SuperScript ds-cDNA Synthesis kit (Life Technologies, Inc., Karlsruhe, Germany). Included in the reaction was a T7-(dT)$_{24}$ primer containing a T7 RNA polymerase promoter site (primer sequence: 5′-GGCCAGTG-GAGTTGATAATCGACTTCATATGGAGGGCGG-3′). cDNA was transcribed to ~60 µg of biotinylated cRNA with the BioArray HighYield RNA Transcription Labeling Kit (Enzo Diagnostics, Farmingdale, NY). The cRNA samples were hybridized to HG-U95Av2 oligonucleotide arrays containing ~12,500 probe sets. Hybridization, washing, staining, and scanning was performed following standard Affymetrix protocols (Technical Manual).

To enable the comparison of all of the arrays, the average intensity of all of the genes was set to 1000 before analyses. Expression values (average difference) for each gene were calculated by use of the Affymetrix Microarray Suite 4.0 analysis software. For data analysis, each probe set was considered as a separate gene.

Data Analysis. For additional statistical analyses, all of the average difference values and gene information were exported to SAS 8.2, and expression values below 50 were set to 50. For subsequent analyses, genes that were called “present” or “marginal” by the Affymetrix software in more than 10% of the tumors were included in the dataset and are referred to as “expressed genes.” We used the Wilcoxon rank-sum statistic (14) as the central means to identify genes differentially expressed between tumors with monosomy 3 and disomy 3. This way, a consistently differentially expressed gene would show up regardless of its absolute expression level. To take single exceptions into account, we recalculated Wilcoxon rank sum for all of the 20 subsets in which one tumor in turn was excluded, and we maximized overall subsets. This analysis is referred to as “leave-one-out Wilcoxon” in the “Result” section. Fold change was calculated by dividing the median of the average difference values of all of the tumors with disomy 3 by the median of the average difference values of all of the tumors with monosomy 3.

Before hierarchical clustering, all of the expression values were divided by the median of a respective gene across all of the tumors, followed by log2 transformation. We applied an average linkage hierarchical clustering algorithm to group tumors according to similar expression patterns (15). Spearman rank correlation was used as a nonparametric distance function. We used bootstrapping to obtain a statistical estimate of the reliability of a grouping. We generated 50 subsamples of the same size with replacement so that any data point could be sampled multiple times or not at all (16), and we performed cluster analysis based on those subsamples. To assess how many genes were needed to find stable grouping, we performed cluster analysis with small samples generated without replacement. This process was iterated 50 times for each sample size.

Real-Time QRT-PCR. Single-stranded cDNA was generated from 1 µg of total RNA by the use of random hexamers in a 25-µl reaction using the GeneAmp RNA PCR kit (Applied Biosystems (ABI), Foster City, CA). QRT-PCR was performed in a 20-µl reaction volume containing 40 ng of cDNA, 300 nm each primer, 250 nm TaqMan probe and 1× TaqMan Master Mix (ABI) according to the manufacturer’s instructions. Reaction was monitored in an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). To amplify CHL1 (GenBank accession no. GI-5729766), fls485 (GenBank accession no. GI-7705707), and HTR2B cDNA sequences (GenBank accession no. GI-4504538) specific primers and probes were designed using the Primer Express Software (PE Applied Biosystems). Primers and TaqMan probes (see Table 1 in supplementary data) were chosen to the effect that genomic DNA was not detectable. To correct for the amount of cDNA added to any individual reaction, PCR was performed in triplicate. The expression of the gene of interest was calculated relative to the β actin (ACTB) mRNA, which was detected with the “Pre-developed TaqMan assay β actin” (ABI). Relative expression values were calculated as described in the “User Bulletin no. 2: ABI PRISM 7700 Sequence Detection System.”

Sequence Analysis. All of the coding of the exons of the CHL1 (exons 3–28) and fls485 (exons 1–122) were PCR amplified and sequenced directly by the use of a standard cycling sequencing protocol provided by the manufacturer on an ABI automated sequencer (ABI PRISM 3100 Genetic Analyzer). Primers (see supplemental material)3 that were used for amplification and sequence analysis were derived from published sequences (NCBI Accession: NT_005927).

MSP. Bisulfite treatment of 5 µg of tumor DNA was performed as described elsewhere (17). PCR was performed in a 25-µl reaction volume containing 2 µl of bisulfite-treated DNA, 10 nm Tris-HCl (pH 8.3), 50 mm KCl, 1.5 mm MgCl$_2$, 200 µM each dNTP, 1 unit AmpliTaq (Applied Biosystems), and 1 µM each primer (CHL1met5’ and CHL1met3’ for the amplification of the methylated allele and CHL unmets’ and CHL unmets3’ for the amplification of the unmethylated allele, respectively). After initial denaturation at 95°C for 2 min, 35 cycles (denaturation at 95°C for 15 s, annealing at 64°C for 15 s and extension at 72°C for 30 s, were performed, followed by a final extension at 72°C for 5 min. MSP products were separated by electrophoresis on 2% agarose gels and were visualized by ethidium bromide staining. The MSP was controlled by the use of methylated control DNA as a template. Primer sequences were as follows: CHL1met5’ 5′-CAGCAAAACCCCGCCAAAGCGACACG-3′; CHL1met3’ 5′-CAGCGGAGGGACGCGGCGGAGGTTCGCC-3′; CHL unmets’ 5′-CAACAAAAACCCCCCACAACACACAACA-3′; CHL unmets3’ 5′-TGGAGGGAGGAGTGGGAGGTGTGTGTGT-3′.

Competitive PCR. Assays were developed to identify possible homozygous deletions of CHL1 or fls485. For each gene, two duplex PCRs were established, each containing primers to amplify a transcribed region and for a control region on chromosome 3 (fls485 exon1 (30 µM) + D3S2386 (100 µM); fls485 exon11 (5 µM) + W916 (100 µM); CHL1 exon27 (100 µM) + GATA78702 (50 µM); CHL1 exon1 (100 µM) + WI1780 (20 µM)). For primer sequences see supplemental material4 and public database UniSTS of the National Center for Biotechnology Information, Bethesda, Maryland.5 PCR was performed in a final volume of 25 µl containing 10 nm Tris-HCl (pH 8.3), 50 mm KCl, 1.5 mm MgCl$_2$, 1 unit AmpliTaq (Applied Biosystems), 50 µM each dNTP, and 50 ng of tumor or blood DNA (primer concentration see above). After initial denaturation at 95°C for 2 min, 35 cycles of amplification were performed as follows: 15 s at 95°C, 15 s at 58°C, and 30 s at 72°C, followed by 72°C for 10 min. PCR products were analyzed on 2% agarose gels followed by ethidium bromide staining. The intensity of bands was inspected visually.

Supplementary Data and Material. Ancillary data including primary expression data and primer sequences can be obtained online.4

RESULTS

We analyzed the expression patterns of ~12,500 genes in 20 primary uveal melanomas by hybridizing labeled cRNA samples onto oligonucleotide microarrays (for all average difference values see Table 3, supplementary data).4 The average difference values of 7902 genes, which were found to be expressed in more than 10% of the tumors (Table 4, supplementary data),4 were analyzed by two different statistical methods.

To identify genes differentially expressed between uveal melanomas with disomy 3 and monosomy 3, we applied the Wilcoxon rank-sum test using the chromosome 3 status of a tumor as the preset parameter. We identified 36 genes with the lowest possible rank sum of 55. These genes show lower expression in all tumors with monosomy 3 compared with tumors with disomy 3. The highest possible rank sum of 155 was found for six genes that are expressed at higher levels in all tumors with monosomy 3. To account for possible biological variability, we also performed a “leave-one-out Wilcoxon” analysis. This approach, which permits one tumor in turn to be in exception to the perfect separation, identified an additional 119 and 38 genes with the lowest and the highest possible rank sum, respectively.

For each of the 201 genes identified by Wilcoxon analysis, we determined the fold change (Table 2, supplementary data).3 Fig. 1 lists all of the 34 genes (2 genes are represented by two probe sets) with a fold change of at least 3 and all of the 8 genes with transcripts absent in at least 9 of 10 tumors in one of either group. Seven of the eight genes showed loss of expression in tumors with monosomy 3. Interestingly, two of these genes, CHL1 and fls485, map within or close to

---

1 The abbreviations used are: QRT-PCR, (real-time) quantitative reverse transcription-PCR; MSP, methylation-specific PCR; MPP, matrix metalloproteinase; TIMP, tissue inhibitor of MMP; GO, gene ontology; OMIM, Online Mendelion Inheritance in Man.
2 Internet address: http://www.uni-essen.de/humangenetik/download.

---
the uveal melanoma susceptibility locus UVM2 at 3p25. We used an insertion/deletion polymorphism in intron 7 of fls485 and single nucleotide polymorphisms in exons 3, 9, and 13 of the CHL1 gene, to detect allele losses of both genes in tumors that define the UVM2 region at 3p25 (tumors M13789 and M16397 (see Ref. 11). We found loss of heterozygosity for the intronic fls485 polymorphism in both tumors thus proving that this gene is located within this region (data not shown). Heterozygosity of polymorphic sites within CHL1 was lost in tumor M16397 but was retained in tumor M13789, thus indicating that CHL1 is located outside UVM2.

To assess the transcript levels of CHL1 and fls485 by an independent method, we performed QRT-PCR in 10 tumors randomly chosen from the series used for microarray analysis. The serotonin receptor HTR2B, which is one of the six differentially expressed genes with a rank sum of 155, was included in QRT-PCR analyses. As shown in Fig. 2a, the average difference values as determined by microarray analysis were in agreement with the relative transcript quantities as measured by QRT-PCR. To analyze whether differentially expression of CHL1, fls485, and HTR2B is replicated in another set of tumors, we performed QRT-PCR analysis on an additional 20 uveal melanomas: 11 tumors with monosomy 3 and 9 tumors with disomy 3. In 17 of 20 tumors, the relative expression levels of CHL1, fls485, and HTR2B correspond to the findings in the set of tumors analyzed by microarray hybridization. In tumors M18410 and M17373 with monosomy 3, the expression of HTR2B was rather low. The latter tumor also showed clearly detectable transcript levels of CHL1 and fls485. In tumor M18025 with disomy 3, the expression levels of CHL1, fls485, and HTR2B conformed with the pattern typical for tumors with monosomy 3 (Fig. 2b).
Because microarray and QRT-PCR results showed an absence of CHL1 and fls485 transcripts in almost all of the tumors with monosomy 3, we searched for mutations that might account for this. We identified no homozygous deletion in CHL1 and fls485 in 45 and 36 tumors with monosomy 3, respectively. Sequence analysis of all of the coding exons of CHL1 and fls485 in eight tumors with monosomy 3 showed polymorphic variants but no mutation. We also investigated methylation of the CHL1 gene promoter by MSP. We detected no methylated allele in any of the 5 tumors with disomy 3 or 12 tumors with monosomy 3 that were tested (data not shown). Because there is no CpG island in the S′ region of the fls485 gene, transcriptional inactivation by promoter methylation is unlikely for this gene (18).

Next, we used the data set of the 7902 expressed genes for unsupervised hierarchical cluster analysis using Spearman rank correlation as similarity measure (15). This algorithm allows the separation of tumor samples into groups based on the similarity of their gene expression patterns without prior knowledge of sample identity. In the resulting tree, two discrete clusters were formed (Fig. 3a). We tested whether the two groups identified by this classification are associated with clinicopathological features or genetic alteration. Interestingly, all nine of the tumors in one of the two clusters showed disomy 3, whereas in the other group, all but one tumor had monosomy 3 (P < 0.0001, Fisher’s exact test). The two tumor classes showed no significant association with any other clinicopathological features or genetic alteration (Fig. 3a). Specifically, neither vascular patterns nor tumor diameter (data not shown) are specific features of either of the clusters (Ps greater than 5%). Only chromosome 8q alterations (P = 0.065) and ciliary body involvement (P = 0.07) came close to statistical significance.

To assess whether the observed tumor classification is a simple consequence of reduced dosage of chromosome 3 genes in tumors having one chromosome 3 only, we excluded those 251 expressed genes from the dataset that were found to be located on chromosome 3 (see supplementary data).4 Cluster analysis of the expression data of the remaining 7651 genes generated the same two distinct clusters of tumors (Fig. 3b). To assess the robustness of the observed clustering results, we applied bootstrapping on the original dataset of 7902 and on the dataset without chromosome 3 gene (19). From each dataset, we generated 50 bootstrap samples and performed cluster analysis. For both datasets, 47 of 50 bootstrap samples resulted in the same two distinct clusters of tumors (Fig. 3, a and b). In each of the 3 various samples only one tumor, M18672, did not maintain class assignment.

We also performed cluster analysis with subsamples of randomly chosen genes. We excluded tumor M18672 from this analysis, because class assignment of this tumor was found to be unstable in bootstrap analysis. All 50 subsamples containing 2000 genes resulted in the original classification that was obtained with all 7902 genes. When decreasing the size of the random subsamples, this classification remained predominant even when clustering as few as 300 genes (Fig. 4).

We also tested whether the expression profiles of functionally related genes contribute more or less to the dissimilarity of the two tumor classes. We used the assignment of genes to functionally related groups as provided by the GO consortium (20). We compared the
and differentiation of neuronal cells. The function of the \(\beta\delta485\) gene, which was identified in a cDNA library prepared from fetal liver mRNA, is not elucidated yet. We cannot exclude that, despite drastically reduced transcript levels, there is still enough protein for normal function. Typically, loss of expression of tumor suppressor genes is a consequence of allele loss and mutations or epigenetic alterations in the remaining allele but we did not identify any structural or epigenetic alterations in \(CHL1\) and \(\beta\delta485\) in DNA from 8 tumors with monosomy 3 that could account for the absence of their transcripts. However, recent data indicate that inactivation of suppressor genes in tumors can also result from other mechanisms. In metastatic cutaneous melanoma, tumors with allele loss in the region of the \(Apaf-1\) gene also show loss of expression of \(Apaf-1\), although no structural alteration or promoter methylation was identified in the remaining allele (23). Interestingly, in cell lines derived from these tumors, expression of \(Apaf-1\) was restored after incubation with 5-aza-\(2'-\text{deoxycytidine}, a\) nucleotide analogue that cannot be methylated, thus indicating that transcriptional silencing depends on methylation of DNA outside the promoter region of this gene. This shows that inactivation of suppressor genes is not necessarily dependent on mutations or epigenetic alterations within the gene and points to the possible role of distant cis-regulatory elements.

Several genes show highly significant differential expression between tumors with and without monosomy 3 (Fig. 1) and some of them have been implicated in progression of tumors other than uveal melanoma. One of these genes is osteopontin (\(SPP1\)), which codes for a cytokine that can bind to several integrins and various isoforms of CD44 and can also function as a cell adhesion protein. In various cancers, expression of osteopontin was found to be a marker for metastatic disease (for review see 24, 25). Recently, gene expression profiling in colon cancers of multiple stages has shown that osteopontin expression is correlated with advanced tumor stages (26). In uveal melanoma, however, high transcript levels of osteopontin were only present in tumors with disomy 3, which rarely give rise to metastatic disease. This indicates that high levels of osteopontin are not a marker for metastatic disease in all tumor types. Another differentially expressed gene is \(TIMP-3\). Tissue inhibitors of metalloproteinases (TIMPs) negatively regulate the activity of MMPs, which are important for remodeling of the extracellular matrix (27). In several tumors, increased activity of MMPs has been associated with malignant progression (for review see 28). Although, recent data indicate that the role of TIMPs is more complex and that some TIMPs may also promote tumorigenesis by growth stimulatory and antiapoptotic ef-
fects (28), no such activities have been reported for TIMP-3 thus far. Therefore, low to absent expression of TIMP-3, as observed in 9 of 10 uveal melanomas with chromosome 3 loss, might significantly contribute to the high metastatic potential of these tumors. Whereas osteopontin and TIMPs were found to be involved in the progression toward metastatic disease (25), no such role has been reported for HTR2B thus far. We found high transcript levels of this gene in all uveal melanomas with monosomy 3 compared with low expression in all tumors with disomy 3. As monosomy 3 is associated with metastatic disease, HTR2B might serve as a marker to identify patients with poor prognosis. This might be done by using immune histochemistry analysis on primary tumor samples, a methodology that is straightforward compared with cytogenetic or molecular evaluation of chromosome 3 loss. Moreover, expression of this proposed marker may be used to detect disseminated tumor cells in blood or other readily accessible biological samples. This would permit prognostic stratification in many patients from whom, because of conservative therapy, no tumor material is available.

Unsupervised hierarchical cluster analysis of the expression data separated the 20 uveal melanomas into two groups. The two classes of tumors identified by these clusters are, barring one tumor, completely associated with the status of chromosome 3. However, this classification is not a simple consequence of gene dosage-related expression changes, because the structure of the tree is essentially unchanged after exclusion of expression data of genes known to be located on chromosome 3. The classification is also robust because clusterings of 47 of 50 bootstrap samples resulted in the same two distinct classes of tumors. In the three discordant bootstrap trees, only one tumor does not maintain class assignment. Interestingly, this is also the tumor not classified consistent with its chromosome 3 status. Possibly, this tumor would become part of a third, smaller-sized cluster, if expression data from a substantially larger set of tumors were available. If excluding this tumor, the majority of clusterings obtained from random representations containing as few as 300 genes establish the same classification as obtained with all 7902 of the expressed genes. This shows that the expression patterns of a large proportion of genes contribute to the classification and indicates a high degree of molecular dissimilarity between the tumors assigned to these classes. In a further analysis we compared Wilcoxon rank-sum distributions of subsets of functionally related genes to identify whether some subsets contribute more or less to the dissimilarity between tumor classes. Only the subset of genes involved in protein synthesis showed a clearly distinct distribution, which was characterized by coordinate down-regulation of genes for ribosomal proteins but not of aminoacyl-tRNA-synthetases in tumors with monosomy 3. This suggests that the expression of these functionally related components of the translation machinery is unbalanced in uveal melanomas with monosomy 3. It remains to be tested whether or not this finding is correlated with the variation of nucleolus size that has been found to be a prognostic marker in uveal melanoma (12, 29). The distribution of Wilcoxon rank sums of the other nine subsets of functionally related genes is almost identical to that obtained with all 7902 expressed genes. This indicates that the dissimilarity between the two classes of uveal melanomas is based on the expression patterns of genes assigned to diverse biological pathways.

The two classes of uveal melanoma identified by unsupervised cluster analysis are distinguished by a pervasive difference in their gene expression patterns and, therefore, may be regarded as two distinct tumor entities. Alterations of chromosome 8 and 6 are found in tumors of both classes, and, therefore, the two presumed entities possibly share some molecular mechanisms of transformation. However, monosomy 3, which is an early event in the development of uveal melanoma, is confined to only one of these classes. Consequently, the dissimilarity of gene expression patterns might result from distinct initiation pathways of tumors with or without chromosome 3 loss. Alternatively, a dissimilar gene expression pattern could also result if the two entities originate from different cells. Uveal melanoma arises from melanocytes in the uveal tract, which, like their cutaneous counterparts, are derived from the neural crest. Despite the common embryonal origin of their precursor cells, clinical behavior and molecular biology of uveal and cutaneous melanoma are distinct, thus indicating the influence of the tissue from which the tumors have originated. However, the uveal tract is not a uniform tissue but is composed of morphologically and functionally distinct parts including the iris, ciliary body, and choroid. Therefore, it is not unreasonable to assume that uveal tissues are populated by different melanocytes that give rise to melanomas with distinct expression profiles. This hypothesis would also account for the variation of chromosome 3 status between tumors located in the ciliar and posterior part of the eye, which is also present in our data (30).

In the present study, we have investigated tumors from patients with recent diagnoses of uveal melanoma, and, therefore, data on metastasis formation are not yet available. However, it is well established that uveal melanomas with disomy 3 rarely give rise to metastatic disease (9). This suggests that the genetic program of tumors with disomy 3 is unlikely to acquire the genetic changes required for metastasis formation. This corresponds to our classification data, because numerous mutations may be required to reprogram these tumors and obtain the widely different gene expression pattern observed in uveal melanomas with monosomy 3. It will be interesting to learn of the fate of the patient whose tumor, M18672, showed no loss of chromosome 3 but was grouped with monosomy 3 tumors in cluster analysis.

ACKNOWLEDGMENTS

We thank Bianca Beyer for technical assistance. We also thank Karl Worm for assistance with real-time PCR experiments and Knud Nierhaus for helpful discussion.

REFERENCES

TWO DISTINCT ENTITIES OF UVEAL MELANOMA


Tumor Classification Based on Gene Expression Profiling Shows That Uveal Melanomas with and without Monosomy 3 Represent Two Distinct Entities

Frank Tschentscher, Johannes Hüsing, Tanja Höltter, et al.


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/63/10/2578

Cited articles This article cites 28 articles, 5 of which you can access for free at: http://cancerres.aacrjournals.org/content/63/10/2578.full#ref-list-1

Citing articles This article has been cited by 37 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/63/10/2578.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/63/10/2578. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.