Genomic Identification of Significant Targets in Ciliochoroidal Melanoma

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PURPOSE. To identify genomic targets for ciliochoroidal melanoma diagnosis, prognosis, and therapy.

METHODS. Fifty-eight ciliochoroidal melanomas were analyzed by high-resolution, genome-wide, single nucleotide polymorphism (SNP) mapping arrays. The 58 SNP arrays were compared to 48 HapMap normals representing both sexes and assessed with a systematic statistical method, Genomic Identification of Significant Targets in Cancer (GISTIC), to identify significant ciliochoroidal chromosomal abnormalities including chromosome-arm-sized as well as focal events of amplification and deletion. The 58 SNP arrays were also analyzed to assess copy number.

RESULTS. The 58 ciliochoroidal melanomas analyzed by GISTIC showed large regions of chromosome amplification on 6p and 8q in addition to focal amplification peaks on 1q31.3, 4p16.2, 9p23, and 9q33.1. The melanomas also showed large regions of deletion on 1p and all of 3, 6q, 8p, and 16q, as well as focal deletion peaks on 2p12, 2q14.3, 4q26, 5q21.1, 7q21.11, 8p21.3, 9p21.1, 13q21.31, 13q31.3, and 16q23.3. For each large region and focal peak, the statistical significance was computed, and known genes were specified.

CONCLUSIONS. High-resolution analysis of ciliochoroidal melanoma cytogenetic aberration patterns supports the utility of systematic characterization of the cancer genome by corroborating known melanoma-related genomic aberrations and identifying additional melanoma-related genomic abnormalities that can be used to identify potential targets for diagnosis, prognosis and therapy. (ClinicalTrials.gov number, NCT00344799.) (Invest Ophthalmol Vis Sci. 2011;52:3018–3022) DOI:10.1167/iovs.10-5864

C omprehensive knowledge of the genomic alterations responsible for cancer is the foundation for understanding tumor biology, developing diagnostics, assessing prognosis, and formulating targeted therapeutics. Elucidation of the human genome and availability of high-resolution, genome-wide DNA arrays have prompted development of statistical methods to assess chromosomal abnormalities associated with cancer.1–4

Beroukhim et al.3 developed a statistical method for analysis of chromosomal aberrations, called Genomic Identification of Significant Targets in Cancer (GISTIC), that identifies aberrations more likely to promote or drive” cancer pathogenesis. With analysis of a set of high-resolution, whole-genome DNA arrays from a specific type of cancer, GISTIC identifies regions of the genome that are aberrant more often than would be expected by chance, with greater weight given to high-amplitude events (for example, high-level copy number gains or homozygous deletions) that are less likely to be random.

In GISTIC, each genomic aberration is assigned a G-score that considers the amplitude of the aberration and the frequency of occurrence in a set of samples.3–5 False-discovery rate q-values are then calculated for the aberrant regions, and regions with q-values below a user-defined threshold are considered significant.

For each significant region of aberration, a peak region is determined, which is the part of the region with the greatest amplitude and frequency of aberration. Moreover, a wide peak is established by using a leave-one-out algorithm to allow for errors in the boundaries for a single sample. Each significant aberrant region is also tested to determine whether it results primarily from broad events (longer than half a chromosome arm), focal events, or significant levels of both. With this methodology, GISTIC outputs the genomic location and calculated q-value for the aberrant regions, identifies the samples that exhibit each significant amplification or deletion, and lists the known genes located in each wide peak region.3–5

GISTIC has been applied to sets of high-resolution, genome-wide DNA arrays to identify cancer oncogenes and suppressor genes in a range of cancers including breast cancer, colorectal cancer, glioma, B-cell lymphoma, and lung adenocarcinoma. We report the application of GISTIC to identification of cancer-related genes (oncogenes and suppressor genes) in ciliochoroidal melanoma.

MATERIALS AND METHODS

In 58 eyes of 58 patients with a clinical diagnosis of ciliochoroidal melanoma (melanoma arising from the choroid and/or the ciliary body), transscleral fine needle aspiration biopsy (FNAB) was performed immediately before iodine-125 plaque placement (53 eyes) or immediately (<5 minutes) after enucleation (five eyes). Biopsy material was analyzed for cytopathology, cytogenetic characteristics, and gene expression profiles and prepared for cell culture.7–9

This research was approved by the Institutional Review Board of the University of California, Los Angeles (UCLA), and work was in compliance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA). The research also adhered to the tenets of the Declaration of Helsinki. Before treatment, evaluation of each patient included comprehensive ophthalmologic examination, ultrasonography, photography, optical coherence tomography, and fluorescein angiog-
raphy. All patients had systemic evaluation, usually by an oncologist at the Jonsson Comprehensive Cancer Center, which showed no clinical evidence of ciliochoroidal melanoma metastasis or other cancer. In addition, patients were offered psychologic support by a clinical psychologist or social worker with particular expertise in ciliochoroidal melanoma.

Isolation of DNA for Microarray Analysis and Single-Nucleotide Polymorphism Analysis

Pooled FNAB aspirates were stabilized (RNAprotect Cell Reagent; Qiagen, Valencia, CA) and pelleted, and DNA and RNA were simultaneously isolated from the same sample (AllPrep DNA/RNA Mini Kit; Qiagen), per the manufacturer’s instructions. Isolated DNA was quantified (model ND-1000; NanoDrop, Wilmington, DE). No DNA sample was subjected to whole-genome amplification techniques. The DNA copy number was assessed by using high-resolution, genome-wide single nucleotide polymorphism (SNP) gene mapping arrays (250k NSPI; Affymetrix, Santa Clara, CA). Probe preparation, hybridization, and reading were performed by the UCLA DNA Microarray Facility within the Jonsson Comprehensive Cancer Center Gene Expression Shared Resource in accordance with standard manufacturer’s protocols. Copy number variation was computed with allied software (Genotyping Console 4.0; Affymetrix).

GISTIC Analysis

The 58 ciliochoroidal melanomas assessed for chromosomal aberrations by the mapping arrays and quantitated (Genotyping Console 4.0; Affymetrix) were analyzed by using GISTIC to identify significant ciliochoroidal melanoma cytogenetic aberrations, including broad and focal events of amplification and deletion.

Briefly, aberrations were assigned a G-score that considered the amplitude of the aberration as well as the frequency of its occurrence across samples. False-discovery rate q-values were calculated for aberrant regions, and q-values below the default threshold were considered significant. For each significant region, a peak region was identified, which represented part of the aberrant region with greatest amplitude and frequency of alteration. In addition, a wide peak was identified using a leave-one-out algorithm. Each significantly aberrant region was tested to determine whether it resulted primarily from a broad event (longer than half a chromosome arm), a focal event, or significant levels of both. The statistical module reported the genomic locations and calculated q-values for the aberrant regions. In addition, the module identified the samples that exhibited each significant amplification or deletion and listed known genes located in each wide peak and peak region.

For GISTIC analysis, CEL files were analyzed with GenePattern from the Broad Institute (Massachusetts Institute of Technology, Cambridge, MA). First, the CEL files were submitted to SNPFileCreator, where PM/MM Difference Model (dChipSNP) modeling was used. Next, the 58 CEL files were compared to 48 normal HapMap (www.hapmap.org) CEL files with CopyNumberDivideByNormals. The copy number data were then segmented by using GLAD (Gain and Loss Analysis of DNA). Last, segmented copy number data were analyzed with GISTIC by using the Human Genome of May 2004 (build hg17) and default parameters.

In dChipSNP, the arrays were normalized by invariant set normalization, and signal intensities were computed using 48 HapMap samples as a normal reference. The arrays were then genotyped (BRLMM Analysis Tool; Genotyping Console 4.0; Affymetrix). Copy number was inferred by using median smoothing.

**Figure 1.** (A) Global chromosomal gain and loss data of 58 ciliochoroidal melanomas inferred from Log2 ratios by dChipSNP. Red: regions of gain; blue: regions of loss. Log2ratios are inferred by the median-smoothing method in dChipSNP. (B, C) GISTIC analysis of aberrations as well as the frequency of its occurrence across samples. Amplifications are shown in (B) as red peaks and deletions are shown in (C) as blue peaks. False-discovery rate q-values, at the bottom of the figures, are significant for values < 0.25 (represented as a vertical green line). Left: the peak region or significant gene(s) corresponding to the greatest amplitude in each locus. Right: the peak region or significant gene(s) corresponding to the greatest amplitude in each locus. Left: broad and focal regions of amplification and deletion. Chromosomes 1 through 22 are identified on the left and horizontal dotted lines represent the location of the centromeres. G-scores at the top weigh the amplitude of aberration as well as the frequency of its occurrence across samples.
RESULTS

GISTIC analysis of 58 ciliochoroidal melanoma samples showed statistically significant large regions and focal peaks of chromosome amplification and deletion. Broad regions of amplification were identified on 6p and 8q; focal amplification peaks were shown on 1q31.3, 4p16.2, 9p23, and 9q33.1 (Fig. 1B). Genes known to be present in the large regions and the focal peaks of amplification are listed in Table 1. Unknown genes were also likely to be within the regions and peaks of amplification.

Deletions of large regions were identified on 1p, all of 3, 6q, 8p, 16q; focal deletion peaks were shown on 2p12, 2q14.3, 4q26, 5q21.1, 7q21.11, 8p21.3, 9p21.1, 13q21.31, 13q31.3, and 16q23.3 (Fig. 1C). Genes known to be present in the large regions and focal peaks of deletion were identified (Table 2). In all likelihood, unknown genes were also located in the deleted regions and peaks.

DISCUSSION

Somatic mutations in the DNA of tumor cells underlie most cancers and are of fundamental importance in understanding the biology of ciliochoroidal melanoma. Thus, we used high-resolution, genome-wide arrays to characterize DNA in 58 choroidal melanomas and used GISTIC analysis of the 58 samples to elucidate large regions and focal peaks of DNA amplification and deletion. Only with systematic statistical assessment of a reasonably large group of samples is the presence and frequency of recurrent genomic aberrations evident. GISTIC is of particular value in identifying narrow regions and focal peaks of amplification and deletion that are not apparent in study of single samples or small clusters of samples.

In addition to identifying the aberrations, a further challenge is to distinguish between cancer promoter or driver mutations that are functionally important (that is, mutations that confer a biological advantage that enables the tumor to initiate, grow, persist, or metastasize) and passenger mutations that are antecedent or random events that carry no propensity for growth or tumor formation. Although the GISTIC methodology is more likely to identify driver mutations that recur because of affirmative selection during tumor evolution, GISTIC may identify passenger mutations based on biases in DNA mutation or repair processes.3

Emphasizing the tendency of GISTIC to identify driver genes is recognition of deletions of all of 3, and of 1p, 6q, 8p,

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Shown are the parameters of regional amplifications and the genes subject to amplification within the identified regions. The amplification at 9p23 contains no known genes but lists the nearest gene. PTPRD, denoted in brackets.

* The location is according to Human Genome build 17 (2004).
and 16q, together with chromosomal amplification of 6p and 8q. Regional amplifications and deletions have been generally associated with metastasis in our integrative analysis of ciliochoroidal melanomas and identified in other analyses of uveal melanomas as associated with an unfavorable prognosis.9,10 Illustrating this concordance, Trolet et al.18 studied cytogenetic factors associated with uveal melanoma metastasis and showed that the best rate of correlation for uveal melanoma metastasis and showed that the best rate of correlation for uveal melanoma metastasis with a set of five regions combined: gains of 6p and 8q and losses of all of 3 and of 8p and 16q. All five of these chromosomal regions, as well as additional regions and focal peaks, were identified as significant genomic aberrations by GISTIC.

Further illustrating the potential value of GISTIC is focal regions of aberration identified on 8p, 16q, and 16q. During a review of reports that examined chromosomal instability in uveal melanomas, we found that Onken et al.19 had identified LZTS1 through an analysis of 12 SNPs in 8p. They reported the identification of a region of deletion and hypermethylation at 8p12-8p22, encompassing the LZTS1 locus. Against the broad background of 8p loss, our global GISTIC analysis similarly defined a focal region of deletion centered on 8p21.3, which included the LZTS1 locus. Among the 58 samples, the highest frequency of deletion on chromosome 8 occurs at p23.2 in a 258-kb region where no known genes exist. This deletion was identified in 20 (35%) of the samples and encompasses bases 5,834,549-6,092,262.

Two regions of significant focal deletion were identified on chromosome 13 at q21.31 and q31.3. The former occurred in a 10-kb region a containing no known genes and the latter was a specific 28.9-kb deletion centered within the glypican 6 (GPC6) gene. GPC6 is a cell surface heparan sulfate proteoglycan with wide expression in adult tissues. Lau et al.20 identified this gene as a potential tumor suppressor in Chinese sporadic retinoblastoma patients with nonrandom allelic loss of heterozygosity at 13q31.20 Preliminary investigation of the expression of this gene in our ciliochoroidal melanomas has failed to detect its transcription; this warrants further examination.

Perhaps the most intriguing deletions indentified by GISTIC were those found in the telomeric region of 16q. Copy number analysis found that seven samples had near complete loss of the 16q arm. GISTIC corroborated that finding and identified a further nine samples that had focal deletion of the telomorphic 6.38-mb region of 16q. Significantly, the deletion of highest frequency commences at the locus of oxidative stress-induced growth inhibitor 1 (OSGIN1 or, alternatively, OKI38). Three different studies have identified this gene as a negative regulator of cell growth, as having a proapoptotic function, and have shown that its downregulation may lead to tumorigenesis or progression of a number of different carcinomas.21-23 Loss of heterozygosity of the telomeric region of 16q is significant for other genes that have been shown to be involved in the progression of melanocytic lesions including melanocortin-1 receptor (MC1R).

Of distinct relevance is the clinical course of patients who were the sources of the ciliochoroidal melanoma biopsy samples in this report. Within 2 years of primary melanoma treatment, 6 of 58 patients developed clinical evidence of melanoma metastasis. Of those six patients, four had the deletion of 16q identified by GISTIC.

Strengths of this report relate to the cohort of 58 ciliochoroidal melanomas evaluated in a uniform manner with high-
resolution, genome-wide DNA arrays and gene microarrays and application, for the first time to our knowledge, of GISTIC to ciliochoroidal melanoma. Although all samples were obtained through fine-needle aspiration biopsy of primary tumors, heterogeneity was not a relevant factor to GISTIC as used, because GISTIC was performed to identify focal amplifications or deletions on all samples irrespective of the primary chromosomal aberration. Limitations must consider the possible failure of the GISTIC methodology to detect rare cancer-promoter genes and the likelihood that GISTIC methodology selects passenger genes as well as driver genes. Overall, chromosomal copy number aberrations are only one part of the full spectrum of cytogenetic, gene expression and epigenetic events that contribute to choroidal melanoma and melanoma metastasis. Other parts of this complex process must be assessed to advance knowledge regarding ciliochoroidal melanoma.

In summary, we report a series of ciliochoroidal melanoma biopsy samples evaluated for chromosomal aberrations by high-resolution, genome-wide DNA array as well as microarrays and analyzed by the statistical procedures of GISTIC. The analysis identified cytogenetic aberrations of amplification and deletion that warrant study as potential targets for ciliochoroidal melanoma diagnosis, prognosis, and therapy.

References