Chromosomal rearrangements in uveal melanoma: Chromothripsis

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Abstract
Uveal melanoma (UM) is the most common primary intraocular malignancy in the Western world. Recurrent mutations in GNAQ, GNA11, CYSLTR2, PLCB4, BAP1, EIF1AX, and SF3B1 are described as well as non-random chromosomal aberrations. Chromothripsis is a rare event in which chromosomes are shattered and rearranged and has been reported in a variety of cancers including UM. SNP arrays of 249 UM from patients who underwent enucleation, biopsy or endoresection were reviewed for the presence of chromothripsis. Chromothripsis was defined as ten or more breakpoints per chromosome involved. Genetic analysis of GNAQ, GNA11, BAP1, SF3B1, and EIF1AX was conducted using Sanger and next-generation sequencing. In addition, immunohistochemistry for BAP1 was performed. Chromothripsis was detected in 7 out of 249 tumors and the affected chromosomes were chromosomes 3, 5, 6, 8, 12, and 13. The mean total of fragments per chromosome was 39.8 (range 12-116). In 1 UM, chromothripsis was present in 2 different chromosomes. GNAQ, GNA11 or CYSLTR2 mutations were present in 6 of these tumors and 5 tumors harbored a BAP1 mutation and/or lacked BAP1 protein expression by immunohistochemistry. Four of these tumors metastasized and for the fifth only short follow-up data are available. One of these metastatic tumors harbored an SF3B1 mutation. No EIF1AX mutations were detected in any of the tumors. To conclude, chromothripsis is a rare event in UM, occurring in 2.8% of samples and without significant association with mutations in any of the common UM driver genes.

KEYWORDS
cancer, chromothripsis, genetics, ophthalmology, uveal melanoma

1 | INTRODUCTION

Uveal melanoma (UM) is a relative rare disease and has a high mortality rate due to metastasis in about half of all patients within 15 y after diagnosis. It is the most common primary intraocular malignancy in adults in the Western world. UM specific mutations in the alpha subunit genes GNAQ and GNA11 are described as well as mutations in BAP1, SF3B1, and EIF1AX. Mutations in the latter 3 genes are found in ~75% of all UM and are useful for prognostication of patients. BAP1-mutated UM gives rise to early-onset metastasis whereas SF3B1-mutated UM hardly metastasizes. Mutations in PLCB4 and CYSLTR2 are described in UM in a mutually exclusive manner to GNAQ or GNA11 mutations but so far have not been associated with prognosis. Copy number alterations in chromosomes 1, 3, 6, and 8 are correlated with prognosis of the UM patient. UM with EIF1AX, SF3B1 and BAP1 mutations are associated with unique chromosomal patterns, suggesting distinct UM subclasses. BAP1-mutated UM harbors entire chromosome copy number variations (CNVs) and entire chromosome arm CNV anomalies (isochromosomes). UM with an SF3B1 mutation is characterized by many structural variants, often affecting the terminal ends of chromosomes and thus not entirely...
chromosomes or chromosome arms. Besides these recurrent CNVs, also other cytogenetic patterns are described such as polyplody of the genome, which occurs in ~10%-15% of all UM. Another chromosomal anomaly described in UM is chromothripsis. This is a phenomenon in which many genomic rearrangements occur in a single chromosome or chromosome arm. It has been described in congenital abnormalities, UM and a variety of other cancers such as bone cancer, lung cancer, myelodysplastic syndrome (MDS), colorectal cancer, breast cancer and neuroblastoma. Chromothripsis predicts a poor outcome in skin melanoma and occur in high risk neuroblastoma, breast cancer and MDS. A positive correlation between chromothripsis and progression free survival was observed in metastatic colorectal cancer. The clinical consequence of this phenomenon in UM remains unclear. In this case series we report on chromothripsis in 7/249 UM.

The mechanism of chromothripsis remains elusive but several hypotheses are described such as the formation of micronuclei, premature chromosome compaction (PCC), TP53 mutations and breakage-fusion-bridge cycles or irradiation. The formation of chromothripsis involving telomere regions and 1 chromosome arm is described and supports the hypothesis that events during the cell cycle are involved in the formation of these chromosomal rearrangements. It is hypothesized that chromothripsis occurs through the formation of micronuclei that arise from lagging chromosomes or chromatid fragments during mitosis. Moreover, these micronuclei are more prone to DNA damage, with subsequently DNA nuclease repair by non-homologous end joining (NHEJ), which could explain the chromosome reshuffling.

2 | MATERIALS AND METHODS

2.1 | Inclusion

Patients with UM that underwent enucleation, enubersection or tumor biopsy at the Erasmus University Medical Center (Rotterdam, The Netherlands) or The Rotterdam Eye Hospital (Rotterdam, The Netherlands) between 1992 and 2017 were selected. SNP (single nucleotide polymorphism) array data of the tumor were available from 249 patients. Chromothripsis was defined as 10 or more breakpoints per chromosome detected with SNP array. A breakpoint is present between 2 fragments with different copy number states in a chromosome. This study was approved by the local ethics committee and followed the tenets of the Declaration of Helsinki. Informed consent was obtained prior to treatment.

2.2 | SNP array

DNA was extracted from fresh tumor samples using the Qiamp DNA mini kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. SNP array was performed using 200 ng of DNA as input for whole-genome analysis (Illumina, San Diego, CA). The data were analyzed with Nexus Copy Number 9.0 software (BioDiscovery Incorporated, El Segundo, CA). The amount of copy number gains and losses was used to determine the number of fragments. The total fragments were counted including copy number neutral fragments as separate fragments.

2.3 | Mutation detection

Mutation analysis of GNAQ, GNA11, EIF1AX, SF3B1, and BAP1 was performed with Sanger sequencing and Ion Torrent next-generation sequencing (NGS; Thermo Fisher Scientific, Waltham, MA) as described before. UM without a GNAQ or GNA11 mutation were sequenced for PLCB4 and CYSLTR2. If the tumor did not harbor a mutation in EIF1AX, SF3B1 or BAP1, mutation analysis for SRSF2 was performed. SeqScape Software 3 (Applied Biosystems, Foster City, CA) and Integrative Genomics Viewer (IGV) Version 2.3.68 (Broad Institute, Cambridge, MA) was used to analyze the data. BAP1 immunohistochemistry (IHC) was scored for the presence of nuclear BAP1 expression and performed as described previously.

3 | RESULTS

3.1 | Patient characteristics

Chromothripsis was detected in the UM of 7 patients. These comprised 5 women and 2 men with a mean age at diagnosis of 57.4 y (range 46.2–73.4 y). Six patients underwent enucleation as primary treatment. In 1 patient, primary treatment was followed by external beam radiotherapy because of unclear surgical margins. One patient underwent brachytherapy as primary treatment, followed by enucleation almost 3 y later due to tumor recurrence. Metastasis developed in 5 patients after 31.9–78.7 mo. In 1 patient, a metastasis was located subcutaneously in abdominal skin followed by a local relapse in the orbit. Three years later metastases in the liver and bone were detected. One patient developed metastasis in the bone, lung and paramediastinal nodes. Metastases in the liver were present in 2 patients, together with cutaneous, muscular and retroperitoneal nodal metastases in one of them. In 1 patient the location of metastases is unknown. The mean disease free survival (DFS) was 51.5 mo (range 15.5–99.0 mo). In Table 1 an overview of patient characteristics is listed. For none of these patients was a family history including UM or other related cancers documented.

3.2 | Tumor characteristics

Six tumors were located in the posterior choroid whereas 1 UM originated from the ciliary body. Mean largest tumor diameter was 13.5 mm (range 9.5–19 mm) and mean tumor thickness 7.5 mm (range 2–12 mm; Table 1). Three UM contained epithelioid cells and 4 were classified as spindle cell type. Closed vascular loops were present in 2 of the 7 UM and extra-ocular extensions were found in 2 cases. Inflammatory infiltrate was insignificant in 2 tumors and present in 3 tumors, of which extensively in one. Correlations of chromothripsis with patient and tumor characteristics were not performed due to the limited number of cases.

BAP1 expression was present in 3 cases and absent in 4 cases. Mutation analysis was performed in all 7 tumors (Figure 1). A mutation in GNAQ c.626A>T:p.(Gln209Pro), was detected in 2 tumors. A GNA11 c.626A>T:p.(Gln209Leu) mutation was detected in the UM of 3 patients. The 2 UM without a GNAQ or GNA11 mutation did not harbor a mutation in PLCB4 but in 1 tumor a c.386T>A:p(Leu129Gln) in
CYSLTR2 was detected (UM 6). One c.1873C>T:p.(Arg625Cys) mutation in SF3B1 was found (UM 1) but all tumors were wildtype for EIF1AX. BAP1 mutations were detected in 4 patients: a c.89A>Gp. (Glu31Gly; UM 2), a c.172_173insA.(Ser58Phefs*10) (UM 6), a c.206_207insA.(Thr69Asnfs*5; UM 7) and a mutation 2 base pairs after exon 5 (c.375+2T>C; UM 4) resulting in alternative splicing with a premature stop before the next predicted splice site (prediction in Alamut Visual, Interactive Biosoftware, Rouen, France). Three of these 4 BAP1-mutated UM had an absent BAP1 expression. In 1 tumor a BAP1 mutation was not detected with NGS, although IHC revealed lack of BAP1 expression. The 2 UM without a mutation in EIF1AX, SF3B1 and BAP1 were wildtype for SRSF2 as well. Polyploidy occurred in 2 out of 7 UM. See Figure 1 for an overview of mutation status and BAP1 IHC.

### 3.3 Chromothripsis

Eight chromosomes showed chromothripsis (Figure 2). One tumor harbored chromothripsis in 2 separate chromosomes (UM 5; Figure 3). UM 7 (chromothripsis of chromosome 13) showed 8 fragments in chromosome 16 as well. However, since this did not meet our criteria of ten fragments, this chromosome was not included for further analysis. Chromosome 3 and 6 were affected in 2 UM. Regarding chromosome 3, the breakpoints were not present in the BAP1 gene. Other affected chromosomes were chromosome 5, 8, 12, and 13. The mean of the total fragments per chromosome was 39.8 (range 12–116, Figure 1). In 4 of the 8 chromosomes, the B-allele frequencies indicates more than 2 copy number states of the separate chromosome fragments (Figures 2A and 3). In 5 cases (UM 2, UM 3, UM 4, UM 5, and UM 6) DNA from blood was available for germline analysis using SNP array. No chromothripsis was observed in these samples.

### 4 DISCUSSION

Recurrent chromosomal aberrations have been described in detail in UM, which are strongly correlated to the mutation status. In this paper, another chromosomal aberration, called chromothripsis, is described. Chromothripsis is characterized by ten to hundreds of chromosome fragments that are shattered and randomly rearranged. This is found in several malignancies with a mean pan-cancer prevalence of 1%-2%. Similar to other malignancies, chromothripsis is also rare in UM. In one study, chromothripsis was observed in 2/25 UM. We detected chromothripsis in 2.8% of the UM which is in line with the low frequency rate as previously described.

A relation between prognosis and chromothripsis has been reported in several studies on different malignancies. In high risk neuroblastoma, breast cancer and MDS, chromothripsis is correlated with a poor outcome while in metastatic colorectal cancer a better progression free-survival has been described. Probably metastases with chromothripsis respond better to therapy while the metastatic rate is higher in cancers harboring chromothripsis. This might be true in UM as well; however, no standardized treatment for metastatic UM is available yet. When such treatment is available it might be interesting to compare the response to therapy in UM with and without
Metastatic disease was present in 5 out of 7 patients in this report. Four of the metastasizing tumors harbored a BAP1 mutation and/or lacked BAP1 expression and in 1 tumor an SF3B1 mutation was present. One of the 2 patients without metastatic disease did not harbor a BAP1 or SF3B1 mutation in the tumor and the IHC showed a positive BAP1 expression while from the other patient (harboring a BAP1 mutation in the tumor) only short follow-up data were available (16 mo). The overall poor prognosis of this cohort could be explained by the mutations in BAP1 and SF3B1 since it is known that mutations in these genes are correlated with a high risk of metastasis.8,10 Therefore, there is no indication that chromothripsis itself causes metastatic disease, but it is possible that the rate of SF3B1 and BAP1 mutations is higher in UM with chromothripsis.

Other features of UM and the relation to chromothripsis could have a clinical impact. In this cohort there were no UM with a hypermutable status, features of microsatellite instability or an indication of germline mutations causing UM. Consequently, the relation between chromothripsis and these tumor characteristics cannot be determined. In about half of the UM, inflammation was present. The tumor with extensive inflammation was the UM with a BAP1 mutation but without metastatic disease. No conclusions about the immunogenicity can be drawn because of the small numbers of UM with inflammation. Further studies are needed to elucidate these relations and the outcome of patients with chromothriptic UM. There are several risk factors known for chromothripsis such as irradiation.23 In one case, brachytherapy was followed by enucleation. Therefore, the chromothripsis in this UM
could be an irradiation effect. Other factors correlated with chromothripsis formation are hyper- and polyploidization\textsuperscript{36,37}. For a long time it was assumed that chromothriptic chromosomes only have 2 copy number states\textsuperscript{17,30,31}. However, an observation was made in a subtype of acute lymphoblastic leukemia, in which more copy number states were found in chromothriptic chromosomes\textsuperscript{38}. In this study, 2 of the 7 UM (29\%) were polyploid. Since polyploidy occurs in only 11\% of all large UM\textsuperscript{16} and chromothripsis is a rare event, this could explain the co-occurrence of polyploid UM with chromothripsis. In addition, in our cohort, 7 out of 8 chromothriptic chromosomes harbored more than 2 chromosomes. This observation was also made in the only other study that described 2 cases of UM with chromothripsis\textsuperscript{14}. This suggests that chromothripsis occurs in already duplicated chromosomes. Altered chromosomes might even be more susceptible to chromosome lagging, as 50\% of the chromosomes with chromothripsis in this study have more than 2 copy number states\textsuperscript{38}. Furthermore, chromothripsis can occur in more than 1 chromosome in the same tumor\textsuperscript{17}. In our cohort, more than 1 chromosome was affected in 1 tumor. It is noteworthy that the affected chromosomes in this study included chromosomes 3, 6, and 8, since copy number variations in these chromosomes are correlated with mutation status in UM\textsuperscript{15}. This is in line with other studies in which chromothripsis occurs among known cancer driver genes\textsuperscript{25,39}. Nevertheless, chromothripsis-like patterns across different tumor types showed a limited preference according to chromosome size. However, chromosome 17 was most frequently affected and to a lesser degree chromosomes 8, 11, and 12 in another study\textsuperscript{26}. This could be explained by the fact that chromosome 17 also harbors TP53, an important cancer associated gene, which is correlated to chromothripsis as well\textsuperscript{25}.

**FIGURE 3** A case with 2 chromothriptic chromosomes. UM 5 showing chromothripsis of (A) chromosome 6 and (B) chromosome 8. Note the 3 copy number states and a general gain of the entire chromosomes[Color figure can be viewed at wileyonlinelibrary.com]
To conclude, chromothripsis is a complex event that occurs in a variety of cancers.\textsuperscript{14,18,20,25,26,40} This study shows chromothripsis in almost 3\% of UM affecting different chromosomes. Limitation of this study was the small number of cases with chromothripsis. Although a large patient cohort was investigated, the rare occurrence of chromothripsis prohibited proper statistical analyses. Further studies are needed to investigate the evolutionary advantage of this complex chromosomal aberration.

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