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Genomic Aberrations Frequently Alter Chromatin Regulatory Genes in Chordoma

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Abstract

Chordoma is a rare primary bone neoplasm that is resistant to standard chemotherapies. Despite aggressive surgical management, local recurrence and metastasis is not uncommon. To identify the specific genetic aberrations that play key roles in chordoma pathogenesis, we utilized a genome-wide high-resolution SNP-array and next generation sequencing (NGS)-based molecular profiling platform to study 24 patient samples with typical histopathologic features of chordoma. Matching normal tissues were available for 16 samples. SNP-array analysis revealed nonrandom copy number losses across the genome, frequently involving 3, 9p, 1p, 14, 10, and 13. In contrast, copy number gain is uncommon in chordomas. Two minimum deleted regions were observed on 3p within a ~8 Mb segment at 3p21.1–p21.31, which overlaps *SETD2*, *BAP1* and *PBRM1*. The minimum deleted region on 9p was mapped to *CDKN2A* locus at 9p21.3, and homozygous deletion of *CDKN2A* was detected in 5/22 chordomas (~23%). NGS-based molecular profiling demonstrated an extremely low level of mutation rate in chordomas, with an average of 0.5 mutations per sample for the 16 cases with matched normal. When the mutated genes were grouped based on molecular functions, many of the mutation events (~40%) were found in chromatin regulatory genes. The combined copy number and mutation profiling revealed that *SETD2* is the single gene affected most frequently in chordomas, either by deletion or by mutations. Our study demonstrated that chordoma belongs to the C-class (copy number changes) tumors whose oncogenic signature is non-random multiple copy number losses across the genome and genomic aberrations frequently alter chromatin regulatory genes.

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Additional Supporting Information may be found in the online version of this article.

INTRODUCTION

Chordomas are rare and occur predominantly in older adults (50–60 years) with a male preponderance. As a primary bone neoplasm of axial skeleton, it occurs in equal distribution at skull base (32%) and mobile spine (32.8%), and is also commonly seen in sacrum (29.2%) (McMaster et al., 2001). Less than 5% of chordomas occur in children and clivus is the most common site for this age group. Chordomas are histologically classified as conventional, chondroid and dedifferentiated and the classic cell type seen in chordomas is the so-called “physaliferous cell” (Greek for “bubble-appearing”) which are distributed in groups and cords in an extracellular matrix rich in mucin and glycogen (WHO-4). En-bloc surgical resection has remained the treatment of choice since the 1970s and recently high dose proton beam therapy has been used in combination with surgery. Because chordomas are slow growing, standard cytotoxic chemotherapy agents that kill rapidly growing cells are generally ineffective. Despite aggressive surgical measures, there is frequent local recurrence and eventual metastasis (up to 40%) with no effective therapy available at the present time (Casali et al., 2007; Chugh et al., 2007; Yakkioui et al., 2014).

While a notochordal origin for this tumor has been hypothesized for many years, evidence supporting it was finally brought about by the discovery of the transcription factor, *T-brachyury* which is found to be expressed in embryonic notochord and also in chordomas (Cleaver et al., 2001; Vujovic et al., 2006; Presneau et al., 2011; Nibu et al., 2013). Moreover, unique duplications in 6q27 containing only the *T-brachyury* gene were seen in tumor samples of patients with familial chordoma further strengthening the role of *T-brachyury* as an important biological marker of this tumor (Kelley et al., 2001; Yang et al., 2005; Yang et al., 2009). While *T-brachyury* has been shown to regulate several stem cell-related genes and has been implicated in epithelial–mesenchymal transition (EMT) in carcinoma, the pathogenic role of this biomarker in chordomas is unknown and the genetic mechanisms underlying the development of chordomas, in particular sporadic chordomas, have not been fully characterized.

For a typically chemo-resistant tumor such as chordoma, the identification of genetic aberrations for which there exists a therapeutic strategy can trigger the development of novel drugs for targeted therapies. To define the genetics of apparently sporadic chordomas, we utilized a genome-wide high-resolution SNP-array and next generation sequencing (NGS)-based molecular profiling platform to analyze copy number changes, allelic imbalances as well as somatic gene mutations. Furthermore, we correlated the genetic aberrations identified to disease outcomes for each patient to evaluate the prognostic significance of recurrent genetic aberrations.

MATERIALS AND METHODS

Patient Data and Tumor Specimens

This study was conducted with the approval of the Memorial Sloan Kettering Cancer Center (MSKCC) Institutional Review Board. Upon IRB approval, 24 patient samples with typical histopathologic features of conventional chordoma and adequate tumor content, majority >70% (confirmed by reviewing the frozen section slides and FFPE slides), including 23

fresh frozen (FF) specimens and 1 formalin-fixed and paraffin-embedded (FFPE) tumor, were selected for SNP-array and NGS-targeted sequencing analysis. Matching normal tissues were available for 16 samples. Clinical data were obtained through electronic medical record review.

SNP-Array Analysis of the Tumor Genome

Genomic DNA was extracted from FF and FFPE tumor tissues using Qiagen DNeasy Tissue and Blood kit. Genome-wide DNA copy number alterations and allelic imbalances were analyzed by SNP-array using Affymetrix OncoScan Assay (Affymetrix, CA). We used 80 ng of genomic DNA for each sample. Processing of samples was performed according to the manufacturer's guidelines (Affymetrix). OncoScan SNP-array data were analyzed by the software couple of OncoScan Console (Affymetrix) and Nexus Express (BioDiscovery, CA) using Affymetrix TuScan algorithm. All array data were also manually reviewed for subtle alterations not automatically called by the software.

NGS-targeted Sequencing

The tumor DNA samples used for SNP-array analysis were also screened for gene mutations in 341 key cancer-associated genes using solution-phase exon capture and next generation sequencing (MSK-IMPACT, MSK-Integrated Mutation Profiling of Actionable Cancer Targets) (Cheng et al., 2015). Briefly, barcoded sequences are prepared and captured by hybridization with custom biotinylated DNA probes for all exons and selected introns of 341 oncogenes and tumor suppressor genes using 100–250 ng of input DNA. Captured libraries are sequenced on an Illumina HiSeq (2 × 100 bp paired-end reads). Bioinformatics analysis included alignment of reads to the human genome (hg19) using BWA-MEM; duplicate read removal, base recalibration and indel realignment using GATK (v 2.6-5) following best practices; variant calling using MuTect (v 1.1.4) for single nucleotide variants and Somatic Indel Detector (GATK 2.3-9) for indels. Annovar was used to annotate the variants for cDNA and amino acid changes as well as presence in dbSNP database (v137) and COSMIC database (v68) and 1000 Genomes minor allele frequencies.

Immunohistochemical Staining

Immunohistochemical detection of H3K36me3 was performed at Molecular Cytology Core Facility of Memorial Sloan-Kettering Cancer Center using Discovery XT processor (Ventana Medical Systems). The tissue sections were deparaffinized with EZPrep buffer (Ventana Medical Systems), antigen retrieval was performed with CC1 buffer (Ventana Medical Systems), sections were blocked for 30 min with Background Buster solution (Innovex), followed by blocking with avidin/biotin block for 8 min. Sections were incubated with H3K36me3 antibody (Abcam, cat#ab9050, 1 µg ml⁻¹) for 5h, followed by 60-min incubation with biotinylated goat anti-rabbit IgG (Vector labs, cat#PK6101) at 1:200 dilution. The detection was performed with DAB detection kit (Ventana Medical Systems) according to manufacturer instruction, followed by counterstaining with hematoxylin (Ventana Medical Systems) and coverslipped with Permount (Fisher Scientific).

RESULTS

Clinical and Pathologic Features

Tumor sites of the 24 chordoma cases included sacrum (18), spine (3), pelvic recurrences (3). All patients were adults with a median age of 55 (range of 30–80 years). There were fifteen males and nine females. Radiation therapy was given for 16 patients, of whom 5 patients received the radiation therapy prior to surgical operation, 8 patients received it after surgical operation, and 3 received it prior and after surgery. At the time of last follow up, 11 patients were alive and no evidence of disease (A/NED), 3 alive with disease (AWD) and 10 had died of disease (DOD). Clinical and histopathologic information of all patients recruited in this study was summarized in Table 1.

Copy Number and Allelic Imbalance Analysis by SNP-array

Genome-wide DNA copy number and allelic imbalance data were obtained from 22 conventional chordoma samples. DNA samples were not available for SNP-array analysis for CD17 and CD27. SNP-array analysis showed predominantly copy number losses across the genome in chordoma samples, and large deletions involving whole chromosomes or chromosome arms were common. The aggregated DNA copy number and allelic imbalance analyses (Fig. 1a) demonstrated the frequency of recurrent unbalanced genomic aberrations. The common recurrent (frequency >30%) changes included losses involving 3, 9p, 1p, 14, 10, and 13, and the observed alteration frequencies (allelic imbalance with and without copy number loss) in this study were summarized in Table 2. In contrast, copy number gain is uncommon in chordomas, and the only common recurrent gain involved chromosome 7 and was observed in ~41% of the tumors. Amplification of *T-brachyury* was detected in one sample only (CD10), and gain of one copy of the gene was detected in CD9 and CD13. The latter was associated with a trisomy of chromosome 6.

In addition to the demonstration of losses involving 3 and 9p as most frequent aberrations in chordomas, we also delineated commonly deleted segments on 3p and 9p. Among the 17 typical chordomas with genetic alteration of chromosome 3, copy number loss at 3p, either through entire chromosome 3 loss or segmental deletion on 3p, was observed in 14 samples. As for the remaining three samples, one showed copy neutral loss of heterozygosity (CN-LOH) of the entire chromosome 3 and two had loss of 3q only. On 3p, two minimum deleted regions (MDRs) were observed within a ~8Mb segment at 3p21.1–p21.31, each was about 3–4 Mb in size. The telomeric one containing *SETD2* gene was detected in 14/22 cases; the centromeric one containing *BAP1* and *PBRM1* genes was detected in 13/22 cases (Fig. 1b). Overall, our SNP-array analysis demonstrated that *SETD2*, *BAP1*, and *PBRM1* were altered, primarily by deletion/LOH, in over 60% chordomas.

The nature of 9p loss across the samples was illustrated in Figure 1c. The minimum deleted region on 9p was mapped to *CDKN2A/2B* gene loci at 9p21.3. There was one sample bearing a 26 Kb deletion involving *CDKN2A* gene only. Of note, homozygous deletion of *CDKN2A* was detected in 5/22 chordomas in this study set (~23%).

Another recurrent genomic abnormality revealed in this study is chromothripsis-like aberrations. Chromothripsis was a phenomenon first described by Stephens et al. in 2011 as

tens to hundreds of chromosomal rearrangements involving localized genomic regions that can be acquired in an apparently one-off cellular catastrophe (Stephens et al., 2011). Over the past few years, the occurrence of chromothripsis in human cancers has been discussed in a number of studies, some of which used array-based technologies and different criteria for classifying complex genomic rearrangements as chromothripsis (Kloosterman et al., 2014). Technically, only NGS-based whole genome sequencing (WGS) is potentially fully capable to “diagnose” chromothripsis (Korbel et al., 2013). Being aware of the limitation of SNP-array in the classification of chromothripsis, we used the term “chromothripsis-like” aberration in our study to describe a group of complex abnormalities, i.e. more than 10 breakpoints along the length of a chromosome arm or chromosome segment with copy number changes between two and three stages (loss, normal $\pm$ gain). By this criteria, chromothripsis-like aberration was observed in 4/22 (~18%) of the samples (Table 3), and different chromosomes were involved in different samples, with 1 to 2 chromosomes affected in each sample. Chromothripsis-like aberration affecting 16q was observed in two samples (CD16 and CD19), and a representative copy number profile demonstrating chromothripsis-like aberration as described above is illustrated in Figure 1d.

Mutation Screening by NGS-targeted Sequencing

Among the 24 chordoma tumor samples, 23 had sufficient DNA for NGS-targeted sequencing. 16 out of 23 cases also had matching normal tissues for sequencing. For the remaining 7 chordoma samples, a mixture of pooled normal DNA was used as unmatched normal for mutation calling.

Screening for mutations in coding exons of 341 key cancer-associated genes by MSK-IMPACT revealed very low mutation rate in chordomas with an average of 0.5 mutations per sample for the 16 cases with matched normal and 2.7 per sample for the 7 cases without a matched normal (the higher figure in the latter likely reflects private germ-line SNPs not filtered due to the absence of a matched normal). MSK-IMPACT did not detect any aberrations in nine chordoma samples, all with matching normal tissues (9/16, 56%). The identified mutations are listed alongside patient characteristics in Table 1, and more details of all mutations is available in the Supporting Information Table. Recurrent somatic mutations or structural aberrations that could be potentially used for characterizing chordomas were not identified in this study. However, when the mutated genes were grouped based on molecular functions, many of the mutation events (~40%) affected chromatin regulatory genes (Supporting Information figure), most commonly *SETD2* and *PBRM1*, but also *ARID1B* and *SMARCB1*, among others (Brown et al., 2012; Masliah et al., 2015). Focusing on the selected chromatin regulatory genes found to be mutated, we integrated the copy number and mutation status of these genes across all tumor samples (Fig. 2). *SETD2* and *PBRM1* come to light in the list as they are also commonly affected by deletions on 3p. As deletion of *CDKN2A/2B* locus is another most common aberration observed in chordomas, we viewed the possible co-occurrence of these two types of alterations across the study set. As illustrated in Figure 2, co-occurrence of alterations in both chromatin regulatory pathway genes and *CDKN2A/2B* is fairly common; however, no significant difference in the prevalence of *CDKN2A/2B* deletion between the groups with and without mutations in chromatin regulatory genes.

Immunohistochemical Staining of H3K36me3

The chromatin regulatory gene, *SETD2* at 3p21, was conspicuous in that (1) deletion/LOH of the gene was detected in 68% of the samples by SNP-array analysis, and (2) mutations of the gene were identified in two samples by MSK-IMPACT targeted NGS. *SETD2* encodes the histone methyltransferase that is non-redundantly responsible for trimethylation of Lys-36 of histone H3 (H3K36me3) (Edmunds et al., 2007); therefore, the H3K36me3 immunohistochemical (IHC) staining can be used as a surrogate marker for *SETD2* loss-of-function. H3K36me3 IHC staining was performed on 10 tumor samples with sufficient tumor materials available, including the two samples (CD5 and CD19) with biallelic alterations of *SETD2*, i.e. one by deletion and the other by mutations/indels. Both CD5 and CD19 had loss of 3p which resulted in the deletion of one copy of *SETD2*, however, the mutation identified in CD19 was an indel that resulted in frame shift and a premature stop codon; in contrast, the mutation in CD5 was an in-frame indel for which the pathogenic significance could not be determined by sequencing alone. Among the remaining eight samples, six had hemizygous deletion of *SETD2*, one (CD13) had copy neutral loss of heterozygosity of *SETD2* gene locus, and one (CD16) had two intact copies of the gene that was used as a positive control for the H3K36me3 IHC staining. As expected, positive staining of H3K36me3 was observed in CD16 (Fig. 3a), and H3K36me3 staining was lost completely in CD19 (Fig. 3b). All six samples with *SETD2* haploinsufficiency showed positive staining of H3K36me3 with similar intensity as that of CD16. CD5 showed weak signal intensity compared to the control sample as well as focally loss of expression of H3K36me3 (Fig. 3c).

DISCUSSION

Chordoma is a rare primary bone neoplasm accounting for 1–4% of all bone malignancies. Although the morphology and immune-profile of chordoma is well recognized, the genetic mechanisms underlying its development have not been fully characterized. To delineate the genetics of sporadic chordomas, we have utilized a genome-wide high-resolution MIP-technology SNP-array and a hybrid capture-based targeted NGS molecular profiling platform (MSK-IMPACT) to analyze copy number changes, allelic imbalances as well as somatic gene mutations in 24 conventional chordoma samples.

Our findings of DNA copy number changes in chordomas are consistent with data reported by other groups using array-CGH or SNP 6.0 array genome-wide analysis (Le et al., 2011; Rinner et al., 2013; Choy et al., 2014; Gulluoglu et al., 2016). Compared to SNP 6.0 array which consists of probes generally tiling evenly along the genome, OncoScan is a “cancer gene” centered SNP-array platform with high probe density and high detection resolution (50–125 Kb) in ~900 cancer-associated genes, whereas the probe-coverage for the remaining genome is relatively low with an average detection resolution at 300–400 Kb. We have demonstrated that high-quality SNP-array data can be generated with OncoScan from both fresh-frozen and FFPE DNAs with an input of only 80 ng DNA (Wang et al., 2014). In addition, according to our experience, OncoScan is adequate in the detection of both large-size copy number changes and small-size, even intragenic, aberrations affecting cancer genes. It is worth noting that in the previous study using SNP6.0 or array-CGH, the vast

majority aberrations reported were either large-size abnormalities or aberrations affecting common cancer genes. As such, our data from OncoScan is technically comparable with that generated using whole-genome tiling arrays. Similar to previous findings, chordomas are characterized by large copy number losses, typically involving chromosomes/chromosomal arms 1p, 3, 9p, 10, 13, and 14. Copy number gains were much less frequent than losses, and amplification of common oncogenes was not seen in this study set.

A novel observation in the present study was the delineation of the two minimum deleted regions (MRDs) on chromosome 3, within a ~8 Mb segment at 3p21.1–p21.31. Three cancer associated chromatin remodeling and putative tumor suppressor genes, *SETD2*, *BAP1*, and *PBRM1*, are located in the two MRDs, respectively. The alteration of chromatin remodeling genes in chordoma was a noticeable finding in this study as mutation screening by the targeted NGS platform (MSK-IMPACT) also highlighted chromatin remodeling genes.

MSK-IMPACT provides a comprehensive genome-wide survey of point mutations and indels of 341 cancer-associated genes and selected structural rearrangements in 14 genes. In our study set, MSK-IMPACT revealed a very low mutation rate in chordomas (0.5 mutations/sample with matched normal), which is in contrast to our experience in epithelial malignancies (~5 mutations/sample with matched normal). Previous mutation screening studies in chordoma have been more limited in genomic scope. Choy et al studied 45 chordoma samples for 865 “hotspot” mutations in 111 oncogenes using the mass spectrum Sequenom iPLEX genotyping platform (Choy et al., 2014) and Fischer et al screened for mutations in 48 cancer genes on 9 chordoma samples using an amplicon-based targeted next-generation sequencing platform (Fischer et al., 2015). A few mutations were reported, however, the alteration frequency in their study set was extremely low, typically presenting in <2 samples in each study cohort.

The present study using the captured-based NGS platform (MSK-IMPACT) represents the most extensive mutation profiling of chordomas performed to date. With the mutation screening of 341 cancer-associated genes, we further demonstrated that chordoma typically has very low mutation load. Integrating all mutations identified in previous studies and ours, it is clear that there are no recurrent oncogene mutations in chordoma; however, it is worth noting that mutations in *SMARCB1*, a gene participating in chromatin remodeling, were detected both in one case in our study set and in one patient by Choy et al. More intriguing, our data demonstrated that among the total of 27 mutations identified across 14 chordoma samples, 40% mutations affected genes that encode chromatin regulators. Furthermore, the combined copy number and mutation profiling analyses in our study revealed that the histone-modifying enzyme gene *SETD2* is the single gene affected most frequently in chordoma (68%). This aspect of the molecular profile of chordoma was not detected in previous studies may be due to the limited coverage of chromatin regulators in their screening panels.

Chromatin regulatory factors consist of enzymes that modify histones covalently or remodel chromatin by ATP-dependent mechanisms. Together, they function to control packaging and unpackaging of the chromatin fiber, as the cis-elements in DNA (enhancers, promoters, replication origins) must be exposed in a regulated manner to properly execute various

processes, such as gene transcription, DNA replication, DNA repair, and DNA recombination. Therefore, chromatin structure not only provides a packaging solution, but also a venue for gene regulation (Nair et al., 2012; Papamichos-Chronakis et al., 2013). Recently, chromatin regulators have emerged as potential gatekeepers and signaling coordinators for the maintenance of genome integrity. Mutations in particular subunits of a chromatin remodeling complex can lead to specific types of cancers, suggesting that certain subunits contribute to distinct transcriptional programs that regulate cell-type specific growth or differentiation (Dalglish et al., 2010; Gui et al., 2011; Varela et al., 2011; Fujimoto et al., 2012; Zang et al., 2012; Zhang et al., 2012; Gonzalez-Perez et al., 2013; Zhu et al., 2014).

Inactivation of *SETD2* by copy number loss and mutations has been reported as a common event in sporadic clear cell renal cell carcinoma (Dalglish et al., 2010; Duns et al., 2010; Cancer Genome Atlas Research Network, 2013; Hakimi et al., 2013; Sato et al., 2013). Recurrent inactivating mutations in *SETD2* were also identified in acute leukemia (Zhu et al., 2014). Recently, functional studies have suggested a role for *SETD2* in regulating RNA processing/splicing (Simon et al., 2013; Grosso et al., 2015; Ho et al., 2015) and in coordination of DNA repair and maintaining genome integrity (Carvalho et al., 2014; Jha et al., 2014; Pfister et al., 2014; Kanu et al., 2015). Intriguingly, cancer cells deficient for *SETD2* and H3K36me3 demonstrate marked sensitivity towards inhibition of WEE1, a G2 checkpoint kinase (Pfister et al., 2015). Therefore, our findings suggest that small molecule inhibitor of WEE1, which is being evaluated in clinical trials, can be potentially explored for its therapeutic effects in chordomas with loss of *SETD2*. To explore this, it would be important to know whether hemizygous loss of *SETD2* also sensitizes cells to WEE1 inhibition, which was not tested in the previous report. The H3K36me3 immunohistochemical (IHC) staining used as a surrogate marker for *SETD2* loss-of-function in the present study is not a quantitative assay and lacks the sensitivity to detect the moderate reduction in H3K36me3 by heterozygous loss of *SETD2*. Some previous studies (Hu et al., 2010; Fontebasso et al., 2013) and our unpublished observations in other cell types have found that heterozygous mutations in *SETD2* led to a partial loss of H3K36me3 that could be detected with immuno-blotting or chromatin immuno-precipitation. To facilitate the proposed functional study, we are developing chordoma cell lines bearing hemizygous and homozygous loss of *SETD2*. We will quantify the levels of H3K36me3 in these cells and test the anti-proliferative effect of WEE1 inhibition.

In conclusion, our comprehensive genomic profiling of chordoma demonstrated that chordoma belongs to the C-class (copy number changes) tumors (Ciriello et al., 2013) whose oncogenic signature is nonrandom multiple copy number alterations across the genome, whereas somatic mutations in common cancer-associated genes were quite rare. Chromatin regulatory genes, notably *SETD2*, are frequently altered in chordomas, and co-occurrence of alterations in both chromatin regulatory genes and *CDKN2A/2B* are common and frequent events. Our data point to a role for chromatin regulators in chordoma pathogenesis. Functional studies focusing on chromatin regulators will be needed to further investigate this tumorigenic mechanism in chordoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Copy number changes and allelic imbalances detected in 22 chordoma tumor samples by OncoScan SNP-array analysis. (a) Genome-wide frequency plot of DNA copy number gains (blue) and losses (red) for all 22 chordoma samples with chromosomes organized in columns and indicated by labels on the top. (b) Copy number changes and copy neutral loss of heterozygosity (CN-LOH) across chromosome 3 are displayed for each individual chordoma case (rows). Two minimum deleted regions on 3p (3–4 Mb each) was shown. Red, losses; Yellow, LOH without copy loss. (c) SNP-array analysis results of chromosome

segment 9p21.3. Copy number changes are displayed for each individual chordoma case (rows). The minimum deleted region on 9p was mapped to *CDKN2A/2B* gene loci. Thick red bar, homozygous deletion; Thin red bar, hemizygous deletion. (d) A representative chromothripsis-like finding in this study. SNP-array result for 16q of case CD19. Red, losses; Blue, gains.

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Figure 2.

Integrated copy number changes and mutations in selected chromatin regulatory genes as well as *CDKN2A* and *CDKN2B* genes identified in 21 chordoma samples on which both SNP-array analysis and targeted-NGS were performed. Each column stands for one case. Note: The “double-green” symbols for *EP300* in the figure stands for two mutations identified in the gene in one sample.

Figure 3.

Immunohistochemistry staining of H3K36me3. (a) Sample CD16 showing positive nuclear staining of H3K36me3 antibody (×200). (b) Complete loss of H3K36me3 staining in CD19 in which both *SETD2* gene alleles were inactivated (×200). (c) Weak staining and focal loss of staining of H3K36me3 in CD5 (×200).

TABLE 1

Clinical Information of all Chordoma Cases and Mutations Identified in This Study

Case ID	Gender	Age	Tumor site	Metastasis	Follow-up (6 months to 12 years)	Mutation identified
CD1	M	55	Sacrum	n/a	A/NED	<i>SUFU</i> p.R146Q
CD2	M	68	Sacrum	n/a	DOD	<i>TRAF7</i> p.D381E
CD3	F	53	Sacrum	n/a	A/NED	none
CD4	M	36	Sacrum	n/a	A/NED	none
CD5	F	56	Pelvis soft tissue	yes	AWD	<i>SETD2</i> p.2517_2519del
CD6	F	69	Sacrum	yes	DOD	none
CD8	M	71	Sacrum	n/a	A/NED	none
CD9	F	63	Sacrum	n/a	A/NED	none
CD10	M	73	Sacrum	yes	DOD	<i>DNMT3A</i> p.S337L
CD11	F	30	Sacrum	yes	AWD	none
CD12	F	54	Sacrum	n/a	A/NED	<i>NF2</i> p.R57X <i>MTOR</i> p.A129S
CD13	M	24	Sacrum	n/a	A/NED	none
CD14	M	52	Sacrum	n/a	A/NED	none
CD15	M	41	Sacrum	n/a	A/NED	none
CD16	M	39	Sacrum	n/a	DOD	<i>PAK7</i> p.T547I
CD17 [#]	M	65	Sacrum	yes	DOD	<i>ARID1B</i> p.V602A <i>RUNX1</i> p.P333R & p.M267I <i>ASXL1</i> p.T822A <i>CREBBP</i> p.W1676C <i>PTPRD</i> p.M1253I
CD18 [#]	M	40	T1, T3	yes	DOD	<i>PBRM1</i> p.S1315F <i>ATR</i> p.D1280N <i>MDCI</i> p.S190fs*
CD19 [#]	F	74	Sacrum	n/a	DOD	<i>CDH1</i> p.G441D <i>SETD2</i> p.P2381fs*
CD20	M	52	Spine - Cervical	yes	DOD	n/a
CD21 [#]	F	70	Spine - Cervical	yes	AWD	<i>FUBP1</i> p.Y58C <i>FGFR2</i> p.R6H
CD22 [#]	M	61	Sacrum	n/a	A/NED	<i>ARID1B</i> p.315_315del <i>JAK2</i> p.V617F
CD23 [#]	F	58	Pelvis soft tissue	yes	DOD	<i>EP300</i> p.Q2343H & p.2266_2266del <i>HNFLA</i> p.T354M
CD24	M	70	Pelvic Soft tissue	n/a	A/NED	<i>SMARCB1</i> p.E95K
CD27 [#]	M	80	Sacrum	yes	DOD	<i>MDCI</i> p.S1542F

A/NED, alive and no evidence of disease; AWD, alive with disease; DOD, died of disease.

n/a, not available.

none, mutation was not detected.

[#] Matched normal was not available, instead, pooled normal was used.

TABLE 2

Frequency of Unbalanced Genomic Alterations in Chromosomes Commonly Changed in Chordomas (>30%) Revealed in This Study, Starting With the Most Common Alterations

Chromosome	Copy number loss only	Copy number loss + CN-LOH
3	72.7%	~77%
9p	~59%	~59%
1p	~55%	~59%
14	50%	50%
10	~45%	50%
13	~41%	~41%

TABLE 3

Chromothripsis-like Aberrations Detected in Four Chordoma Samples by SNP-Array Analysis in This Study

Case ID	Chromosomes affected by eth-like aberrations	Status at last follow up
CD12	22q	A/NED
CD16	11q, 16q	DOD
CD19	16q, X	DOD
CD21	12p	AWD