HHS Public Access

Author manuscript

Clin Cancer Res. Author manuscript; available in PMC 2020 July 15.

Published in final edited form as:

Clin Cancer Res. 2020 January 15; 26(2): 419–427. doi:10.1158/1078-0432.CCR-18-4212.

Genomic profiling identifies association of *IDH1/IDH2* mutation with longer relapse free and metastasis free survival in high-grade chondrosarcoma

Guo Gord Zhu[1,5], **Khedoudja Nafa**[1], **Narasimhan P. Agaram**[1], **Ahmet Zehir**[1], **Ryma Benayed**[1], **Justyna Sadowska**[1], **Laetitia Borsu**[1], **Ciara Kelly**[2], **William D. Tap**[2], **Nicola Fabbri**[3], **Edward Athanasian**[3], **Patrick J. Boland**[3], **John H. Healey**[3], **Michael F. Berger**[1,4], **Marc Ladanyi**[1], **Meera R. Hameed**[1]

[1]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[2]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[3]Orthopaedic Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[4]Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[5]Cooper University Hospital, Cooper Medical School of Rowan University, Camden, NJ

Abstract

Purpose—Chondrosarcomas are the second most common primary malignant bone tumors. While histologic grade is the most important factor predicting the clinical outcome of chondrosarcoma, it is subject to interobserver variability. *IDH1* and *IDH2* hotspot mutations were recently found to be frequently mutated in central chondrosarcomas. However, a few published articles have been controversial regarding the association between *IDH1/IDH2* mutation status and clinical outcomes in chondrosarcomas.

Experimental Design—We performed hotspot sequencing of *IDH1* and *IDH2* genes in 89 central chondrosarcomas and targeted next-generation sequencing in 54 of them, then correlated the *IDH1/IDH2* mutation status with the patient's clinical outcome.

Results—Although no association was discovered between *IDH* mutation status and the patient's overall survival, *IDH1*/*IDH2* mutation was found to be associated with longer relapse free and metastasis free survival in high-grade chondrosarcomas. Genomic profiling reveals *TERT* gene amplification and *ATRX* mutation, for the first time, in addition to *TERT* promoter mutation in a subset (6/30, 20%) of high-grade and dedifferentiated chondrosarcomas. These abnormalities in telomere genes are concurrent with *IDH1*/*IDH2* mutation, and with *CDKN2A/2B* deletion or *TP53* mutation, suggesting a possible association and synergy among these genes in chondrosarcoma progression. We found twenty-one percent of chondrosarcoma patients also had

The corresponding author: Meera Hameed, mailing address: Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY 10065, hameedm@mskcc.org.

The authors declare no potential conflicts of interest.

histories of second malignancies unrelated to cartilaginous tumors, suggesting possible unknown genetic susceptibility to chondrosarcoma.

Conclusions—*IDH1/IDH2* mutations are associated with longer relapse free and metastasis free survival in high-grade chondrosarcomas and they tend to co-occur with *TERT* mutations, and with *CDKN2A/2B* and *TP53* alterations in a subset of high-grade chondrosarcomas.

Introduction

Chondrosarcomas are the second most common primary malignant bone tumors that usually affect adults with peak incidence in the fifth to seventh decade of life [1]. Most common skeletal locations are pelvis, femur, humerus and rib. Histologic grade is the single most important factor predicting the clinical outcome of chondrosarcoma in terms of local relapse and distant metastasis [2]. Low-grade (grade I) chondrosarcomas behave in a locally aggressive manner and rarely metastasize. They are usually treated with extended curettage and local adjuvants when intra-compartmental (intraosseous) and with wide excision when extra-compartmental [3]. Higher grade chondrosarcomas (grade II and grade III) have a worse prognosis with five-year survival of 53% compared to 83% in low grade tumors[3]. High-grade chondrosarcomas are usually treated surgically with en-bloc resection. Some low-grade chondrosarcomas can progress to high-grade chondrosarcomas at relapse, which is estimated to occur in 13% percent of cases [2]. Dedifferentiated chondrosarcomas are characterized by biphasic histomorphology where a low-grade chondrosarcoma component sharply transitions to a high-grade non-cartilaginous mesenchymal component. The prognosis of dedifferentiated chondrosarcoma is dismal with rapid development of widespread metastases within 2 years [4]. They are treated with wide surgical resection. The role of radiotherapy and chemotherapy are not fully established as standard of care in the treatment of high grade chondrosarcomas and dedifferentiated chondrosarcoma [3]. Various clinical trials are underway for chondrosarcoma treatment [5, 6].

Isocitrate dehydrogenase (IDH) is an enzyme of the Krebs's cycle which catalyzes isocitrate to α-ketoglutarate [7]. Somatic mutations in *IDH* genes (*IDH1* and *IDH2*) were first discovered in human glioblastomas through whole exome sequencing [8] and are associated with better overall survival (OS) in glioblastoma patients [8, 9]. About 50% of enchondromas and central chondrosarcomas possess *IDH1* or *IDH2* mutations. The incidence was found to be higher, up to 80% in patients with Ollier disease and Maffucci syndrome [10–13]. Unlike glioblastomas, published literature has been controversial regarding the association between *IDH1/IDH2* mutation and overall survival in chondrosarcoma patients. While no association was found between *IDH1*/*IDH2* mutation and OS in cartilaginous neoplasms in earlier studies [12, 14], a recent study has proposed chondrosarcoma patients with *IDH1*/*IDH2* mutation have significantly shorter OS than the patients without mutation [5].

Additionally, comprehensive genomic studies of chondrosarcomas have also identified genetic alterations involving *COL2A1* (37%), *TP53* (20%), RB1 pathway (33%) and Hedgehog pathways (18%) [13, 15]. *TERT* promoter mutation was found to be associated with higher grade chondrosarcoma in one recent study [6]. The molecular mechanisms and

genetic alterations underlying chondrosarcoma progression from lower grade to higher grade remain largely unknown. Since histologic grading is subject to interobserver variability [16], yet remains the gold standard for predicting biological behavior, it is desiring to identify biological/molecular markers that can aid in predicting clinical outcome and inform clinical decision making.

In the current study, we performed hotspot sequencing of *IDH1* and *IDH2* genes in 89 central chondrosarcomas and comprehensive genomic profiling of 54 central chondrosarcomas by targeted next-generation DNA sequencing by MSK-IMPACT (Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets) platform [17]. We then correlated *IDH1/IDH2* mutation status with the patient's clinical outcome. Although no association was discovered between *IDH* mutation status and patient's overall survival, *IDH1*/*IDH2* mutations were found to be associated with longer relapse free survival (RFS) and metastasis free survival (MFS) in high-grade chondrosarcomas. We also identified, for the first time, *TERT* gene amplifications and *ATRX* mutations in addition to *TERT* promoter mutation in a subset (6/30, 20%) of high-grade and dedifferentiated chondrosarcomas. These genetic abnormalities, involved in the telomere dysfunction, tend to be concurrent with *IDH1*/*IDH2* mutations, and with *CDKN2A/2B* deletion or *TP53* mutation.

Materials and Methods

Case selection

This study was approved by Institutional review board 15–273 and 12–245, and was conducted in accordance with the U.S. Common Rule. Patients with diagnosis of central chondrosarcoma and adequate tumor material were selected from September 1984 to July 2017. The follow-up data were available up to about 260 months (more than 21 years). The H&E slide subjected to molecular testing, molecular results and electronic medical records were reviewed manually. For 32 cases, tumor FFPE unstained slides and matched normal peripheral blood (stored in EDTA) were available. These cases were sequenced on MSK-IMPACT platform containing 342, 410 or 468 genes including TERT promoter region. For another 22 cases, only frozen tumor samples stored in liquid nitrogen were available. H&E sections of corresponding FFPE sections were examined for tumor confirmation. These cases were sequenced on MSK-IMPACT platform comprising of 279 genes and *TERT* promoter was not included. Complete gene lists of MSK-IMPACT platforms are included in the file "Supplementary Data #1".

Patient samples with adequate tumor material were selected after histological review. At least 50% tumor purity were available on H&E in all cases, which is more than the minimal requirement of tumor purity of 20% for the MSK-IMPACT platform [17]. Detailed Sequenom-based DNA genotyping can be found in our previous publication [18]. Besides, DNA QC metrics after extraction were also used to decide whether the sample is sufficient for sequencing [17]. Generally, 10 unstained slides for resection and 20 unstained slides for biopsy were required. The macro-dissection was determined and guided by the reviewing pathologist on a case-by-case basis depending on the tumor size, purity and the relationship of the tumor cells to the stromal cells etc.

All the clinical data are included in the file “Supplementary Data #2”.

Sequencing methods

Among 89 cases, hotspot mutation regions in exon 4 of *IDH1* (codon 132) and *IDH2* (codon 140 and 172) were genotyped using Sequenom-based mass spectrometry in 53 cases initially, then the mutation-positive cases were also confirmed using Sanger sequencing. Twelve cases were sequenced using a clinically validated Sanger sequencing assay only. Fifty-four cases were tested on MSK-IMPACT next generation sequencing platform.

MSK-IMPACT Next-Generation Sequencing

Mutation profile analyses of the 54 tumors were performed on MSK-IMPACT platform [17, 19], a validated custom hybridization capture-based assay, which is capable of detecting somatic mutations, small insertions and deletions, copy number alterations and selected structural rearrangements. Four different panels were used in this study containing 279 (22 cases), 341 (3 cases), 410 (19 cases) or 468 (10 cases) genes. All protein-coding exons of target genes, the promoter of *TERT* (32 cases, see figure 3) and selected introns of 17 recurrently rearranged genes were captured by custom designed synthetic DNA probes. Both matched tumor and normal specimens were sequenced in 32 cases. Unmatched tumors were sequenced in 22 cases and data were manually curated to exclude the germline SNP using the following criteria: allele frequency from 0.4–0.6 or 0.8–1.0 unless the tumor purity is greater than 80%, or reported in the gnomAD database for more than 10 times. The sequence read alignment processing, non-synonymous mutations, copy number alterations and rearrangements were determined as previously described [19]. All the curated mutations and CNAs are included in the file “Supplementary Data #3”.

Statistical analyses and graph plotting

Survival analyses based on *IDH1* or *IDH2* mutations were performed using Graphpad Prism 7.01. Kaplan–Meier curves were used to compare survival among different stratified groups. Gehan-Breslow-Wilcoxon tests or chi-square test were used to calculate P values. Maftools is used to generate the genomic profile diagram [20]. (https://bioconductor.org/packages/release/bioc/html/maftools.html)

Results

Clinicopathologic findings

Eighty-nine central chondrosarcomas were selected comprising 23 low-grade (grade I), 51 high-grade (43 grade II, 8 grade III), and 15 dedifferentiated chondrosarcomas. Tumor grade of the primary tumors was assessed using standard criteria [1]. The patients include 41 females and 48 males with ages ranging from 10 to 75 years (median: 51 years) at primary diagnosis. The locations of primary tumors included femur (22), tibia (6), fibula (2), humerus (12), phalanx (1), pelvis (17), spine(7), scapula (4), chest wall (total:15, clavicle:1, sternum:4, rib:7, or not specified:3) and skull base (3) (Table 1). Among the 84 patients for whom follow-up data was available, distant metastases were found in 50% (42/84) of cases and the most common metastatic sites are lung, pleura and bone (88%, 37/42). Other uncommon metastatic sites include liver, lymph node, brain and soft tissue.

All of the low-grade chondrosarcomas were treated with curetting or resection. High-grade chondrosarcomas (Grades II and III) were resected en bloc except three cases of grade II where the primary tumors were curetted. The dedifferentiated chondrosarcomas were treated with wide resection with or without adjuvant chemotherapy.

Notably, 19 of 89 (21%) of the patients also had histories of a second malignancy which were unrelated to cartilaginous tumors and developed metachronously or synchronously. These include colon adenocarcinoma, lung adenocarcinoma, high-grade uterine serous carcinoma, cholangiocarcinoma, hepatocellular carcinoma, glioblastoma, well-differentiated liposarcoma, renal cell carcinoma, breast carcinoma, thyroid carcinoma, melanoma, lymphoma, squamous cell carcinoma of skin and intraductal papillary mucinous neoplasm of pancreas. Most patients had no known cancer predisposition syndromes except, one case of Von Hippel–Lindau syndrome and another patient with Ollier disease.

IDH1 and *IDH2* mutations

IDH1 or *IDH2* hot-spot mutations (*IDH1* R132 and *IDH2* R172) were identified in 46% (41/89) of chondrosarcomas (Table 1). Of these, 73% (30/41) were *IDH1* (R132C: **8**, R132G: **10**, R132H: **5**, R132I: **1**, R132L: **4**, R132S: **2**) and 27% (11/41) were *IDH2* (R172G: **1**, R172M: **3**, R172S: **7**). No hotspot mutations at *IDH2* R140 residue were identified. The highest incidence was seen in chondrosarcomas of femur (17/22, 77%). Except for one skull base chondrosarcoma in a patient with Ollier disease, no *IDH1* or *IDH2* mutations were identified in chondrosarcomas arising from skull base, spine or sternum. No statistically significant associations were found between the types of IDH1/IDH2 amino acid change and tumor grade, size, anatomic location or patient age (data not shown).

No association between *IDH1/IDH2* mutation and overall survival

Chondrosarcomas arising from the skull base (n=2) or associated with Ollier disease (n=1) were excluded from the Kaplan-Meier survival analyses since the skull base chondrosarcomas were treated with radiation only and Ollier disease is a unique non-hereditary developmental disorder. Totally, 79 patients had informative follow-up data, who were followed up with imaging studies (radiograph, CT, and/or MRI) to assess local recurrence and distant metastasis. The OS times were significantly different among low-grade (grade I, median: >263 months, cannot be further defined since no patients died of disease), high-grade (grade II-III, median: 160 months) and dedifferentiated (median: 50 months) chondrosarcomas ($p < 0.0001$, Figure 1A). However, there was no statistically significant difference in the OS between *IDH1/IDH2* mutant and wild-type chondrosarcomas independent of grade ($p = 0.845$, median: *IDH1/IDH2* mutant: > 263 months, cannot be further defined since less than 50% of patients died of disease, wild-type: 226 months, Figure 1B, Table 2). No statistically significant difference in the OS was found between *IDH1/IDH2* mutant and wild-type chondrosarcomas in each of the grade groups either (low grade, high grade and dedifferentiated, Figure 1C)

IDH1/IDH2 mutation is associated with longer RFS and MFS in high-grade chondrosarcoma

Next, we analyzed if *IDH1/IDH2* mutation has an impact on the RFS time (measured by time to relapse (TTR): the time from surgical resection to local relapse or, the time from the

initial diagnosis to the appearance of distant metastasis, whichever comes first) and the metastasis free survival (MFS) (measured by time to metastasis, TTM, the time from the initial diagnosis to the appearance of distant metastasis).

The Kaplan-Meier RFS curves showed appreciable difference between *IDH1/IDH2* mutant and wild-type groups altogether independent of grade (overall TTR medians: mutant: 120 months, wild-type: 25 months) (Figure 2A, Table 2) although the p value is borderline (p=0.063). For high-grade chondrosarcoma, however, TTR is significantly longer for *IDH1/IDH2* mutant than wild-type group (TTR medians for high-grade: 45 vs 13 months, HR=3.5, p=0.003). No significant difference in RFS between *IDH1/IDH2* mutant and wild-type groups was found in either low-grade (p=0.258, median: undefined) or dedifferentiated chondrosarcomas (p=0.775, median: 4 vs 5.5 months).

As far as MFS analysis, TTM is significantly longer for *IDH1/IDH2* mutant than wild-type group in high-grade chondrosarcoma (TTM medians for high-grade: 50 months vs 19 months, HR=2.6, p=0.013). However, no statistically significant difference in TTM between *IDH1/IDH2* mutant and wild-type groups was found in either low-grade (p=0.433) or dedifferentiated chondrosarcomas (p=0.751, Figure 2 and Table 2). The overall percentages of metastasis in high-grade chondrosarcomas are 50% and 72% for *IDH1/IDH2* mutant and wild-type groups, respectively. Only one low-grade chondrosarcoma (*IDH* wild-type) developed distant metastasis. All dedifferentiated chondrosarcomas except one case (12/13) developed distant metastasis (interval: 0–49 months, median: 4 months). *IDH1/IDH2* mutations were present in 54% (7/13) of dedifferentiated chondrosarcomas (Table 2).

Only one low-grade chondrosarcoma, which was *IDH* wild-type, developed both local recurrence and metastasis. The primary tumor was a 19 cm pelvic chondrosarcoma from a 20-year-old girl which was surgically resected. The tumor recurred locally in the abdomen multiple times at 11 months, 22 months and 7 years after initial resection. Distant metastases also developed in the soft tissue of thigh and liver at 30 months after initial resection. The liver metastasis was treated with radiofrequency ablation and other relapsed tumors and metastases were surgically resected. All the resected tumors were low-grade chondrosarcomas. The patient is now free of relapse 11 years after surgical resection of her last relapse.

Genomic profiling of the chondrosarcomas

We subjected 54 cases of central chondrosarcoma from the cases studied above, including 12 low-grade (grade I), 33 high-grade (29 grade II, 4 grade III) and 9 dedifferentiated chondrosarcomas to targeted next-generation sequencing by MSK-IMPACT platform (see details in materials and methods). Genomic alterations that occur at least twice and in two cases are summarized in Figure 3 together with clinicopathologic information. Majority of cases (49/54), except 3 grade I and 2 grade II, harbor at least one gene-level genomic alteration (including single nucleotide variant (SNV), copy number alterations (CNA) and structural variants (SV)), or large-scale CNA at chromosomal and/or subchromosomal levels. The median number of non-synonymous SNV per sample is two. The most dominant nucleotide substitution is “C>T” followed by “C>A” and “C>G”. Low-grade (grade I) chondrosarcomas harbor 0–2 non-synonymous SNV with very few CNA (either focal or

large scale). High-grade and dedifferentiated chondrosarcomas tend to have higher numbers of genomic alterations than low-grade chondrosarcomas (Figure 3).

The most common recurrent genomic alterations (greater than 20%) are *IDH1/IDH2* hotspot mutations (24/54, 44%), *CDKN2A* and/or *CDKN2B* homozygous or heterozygous deletions (18/54, 30%), and *TP53* mutations or deletions (11/54, 20%) (Figure 3). Other gene-level genomic alterations, including SNV, CNA and SV, occur at lower frequencies. The genetic findings that distinguish grade I chondrosarcoma from higher grade chondrosarcomas are as follows, 1) lower mutation load, 0–2 events/case; 2) rare or no CNA; 3) not associated with *CDKN2A/2B* deletion; 4) not associated with *TP53* mutation. Only 3 of 12 low grade chondrosarcomas were informative for *TERT* and *ATRX* alterations, thus precluding further assessment of these alterations in these tumors.

Common signaling pathways harboring genomic alterations

Recurrent genomic alterations (events occur at least twice and in at least two cases) are plotted in groups based on the functional pathways of the involved genes (Figure 3). In the epigenetic regulation and chromatin remodeling pathways, *IDH1/IDH2* hotspot mutations are the most common genomic alterations. Other genomic alterations in this pathway include missense mutations, deletions or rearrangement in *DNMT1*, *EED* and *KMT2C* as well as those events that only occurred once (such as alterations in *ARID1A, ARID1B, CARM1, CENPA, EP300, EZH2, H3F3B, KDM5A, KDM2D, PBRM1, SMARCA4, SMARCB1, TET2* and *WHSC1L1*).

Regarding genes involved in cell cycle control, *CDKN2A/2B* deletion is the most common genomic alteration. Other less frequently mutated genes include *RB1* and other genes which were found to be mutated in a single case (*CCND1, CCND2, CCND3, CDK4* and *CDK6*). *TP53* is the most commonly mutated gene regulating apoptosis and DNA damage response. Other infrequent events include *CARD11, BCOR, MCL1, MDM2, BBC3, BCL2L1, BCL6* and *FAS* in apoptosis pathways, and *ATM, BRCA1, ERCC2, ERCC3, FANCA, MSH2, PALB2, RAD52* and *TOP1* in DNA damage repair pathways.

Genes encoding cell membrane receptors and their intracytoplasmic signaling proteins are also the common targets of the genomic alterations. These include *EPHA7, FGFR3, IGF1R, KIT, NOTCH3, KRAS, PTEN, PTPRT* and *PRKC1*, among others.

Concurrence of *TERT* or *ATRX* mutation with *IDH1/IDH2* mutation, and with *CDKN2A/2B* deletion or *TP53* mutation in a subset of high-grade chondrosarcomas

Six cases (6/32, 19%) harbored genomic alterations in *TERT* or *ATRX*, which are involved in telomere regulation (Figure 4). These include three cases of *TERT* 5' promoter hotspot mutation (g.1295228C>T, g.1295250C>T), one case of focal high-amplitude *TERT* amplification, one case of *ATRX* frame-shift deletion and one case of *ATRX* missense point mutation. While this information was only available in three low grade chondrosarcomas, all three lacked *TERT* or *ATRX* alterations. Interestingly, *TERT* promoter mutation was seen in a grade II chondrosarcoma of the finger, an uncommon site for chondrosarcoma. Four cases of *TERT* alterations and one case of *ATRX* frame-shift mutation co-occur with *IDH1/IDH2* mutation, and with *CDKN2A/2B* deletion or *TP53* mutation (Figure 3, 4). The single case of

ATRX missense mutation is wild-type for *IDH1/IDH2*. The co-occurrence of *IDH1/IDH2* mutation with *TERT/ATRX* alterations is statistically significant (p=0.02, note: this p value is not statistically significant after correction for indiscriminating multiple testing by Bonferonni method), which suggests a possible cooperation of telomere dysfunction with *IDH1*/*IDH2* mutation, and with *CDKN2A/2B* deletion or *TP53* mutation in chondrosarcoma progression.

Discussion

IDH1/IDH2 mutations and chondrosarcomas

IDH1 and *IDH2* mutations are prevalent in enchondroma and central chondrosarcoma as well as in Ollier disease and Maffucci syndrome [10–13]. In the current study, *IDH1* or *IDH2* mutations were identified in 46.2% of 89 chondrosarcomas, which is consistent with prior studies. Amino acid substitutions were only found at residues R132 in *IDH1* and R172 in *IDH2*, but not at R140 in *IDH2*. The ratio of *IDH1* to *IDH2* mutation is 30:11, which is slightly higher than previously reported [10]. As far as tumor location, femur has the highest *IDH1/IDH2* mutation percentage (77.3%) in our cohort. No *IDH1* or *IDH2* mutation was found in chondrosarcomas located at vertebrae or sternum. This is consistent with a previous study where no *IDH* mutations were found in four chondrosarcomas from vertebrae [10], indicating mutation prevalence in certain anatomic sites. *IDH1* or *IDH2* mutations were equally distributed among low-grade, high-grade and dedifferentiated chondrosarcomas suggesting *IDH1* and *IDH2* mutations occur as an early event during tumorigenesis similar to glioblastoma and acute myelogenous leukemia [10, 21].

IDH1/IDH2 mutation as a predictive marker for disease prognosis

Although *IDH1* and *IDH2* mutation has been associated with better OS in glioma patients [9], only one out of three studies have shown an association in chondrosarcoma patients [5, 12, 14]. In the current study, we found that *IDH1*/*IDH2* mutation is not associated with the OS either with or without tumor grade stratification, which is consistent with two previously published studies [12, 14]. In contrast a recent study by Lugowska et al [5], proposed that *IDH1*/*IDH2* mutations are predictors of shorter survival in chondrosarcomas. However, the tumors listed in this study were not pure conventional central chondrosarcomas and included other types (15 cases), for example, mesenchymal chondrosarcoma (a translocation associated tumor defined by *HEY1-NCOA2* rearrangement). Additionally, this study did not show data on PFS and MFS, which is the focus of our paper as further discussed below.

Since most chondrosarcomas are relatively slow-growing until they transform (dedifferentiation), they are often not the primary causes of death. Therefore, the OS is often confounded by other causes such as a second malignancy or non-neoplastic diseases. We further evaluated RFS and MFS (measured by TTR and TTM) which are more disease specific and may better reflect the differences in the biological behaviors of *IDH* mutant and wild-type chondrosarcomas.

In our study, we found that *IDH1*/*IDH2* mutation is strongly associated with longer RFS and MFS in high-grade chondrosarcomas. There were no statistically significant differences in

RFS and MFS between *IDH1/IDH2* mutant and wild-type low-grade or dedifferentiated chondrosarcomas. As most low-grade chondrosarcomas did not recur or metastasize in our cohort (only one case developed local recurrence and metastasis) and dedifferentiated chondrosarcomas progressed rapidly, a statistical difference could not be identified in these two cohorts between *IDH1/IDH2* mutant and wild-type chondrosarcomas.

Biochemically, IDH1 and IDH2 active site mutations result in neomorphic enzyme activity, which causes the accumulation of 2-hydroxyglutarate (2-HG) at supraphysiological levels within cells [22, 23]. Elevated 2-HG level competitively inhibits histone lysine demethylases and TET family of 5-methylcytosine hydroxylase, which results in increased levels of histone and DNA methylation [24–26]. The increased histone methylation and DNA hypermethylation inhibits normal cellular differentiation, promotes pathological self-renewal and allows subsequent malignant transformation processes [27, 28]. Although the oncogenic effect of *IDH1/IDH2* mutation is well illustrated, the mechanism underlying the association of *IDH1/IDH2* mutation with better OS in glioblastoma is largely unclear, except for that overexpression of IDH1 has been shown to cause decreased proliferation in established glioma cell lines [29, 30]. In the current study, we demonstrate, for the first time, the striking parallel phenomenon in human chondrosarcoma compared to glioblastoma by assessing RFS and MFS, which are more disease specific measurements of clinical outcomes in slow progressing tumors. Additional functional studies are needed to understand the biological role of *IDH1/IDH2* mutations in prolonging RFS and MFS in grade II and III chondrosarcomas.

Genomic profiling of chondrosarcomas

Consistent with sarcomas with complex genetic alterations [31], chondrosarcomas are also characterized predominantly by CNA with low mutation loads. Only a few genes are highly recurrently mutated, including *IDH1* and *IDH2* (46% combined, hotspot mutation), *CDKN2A* and *CDKN2B* (30% combined, often homozygous co-deletion), and *TP53* (20%). Other genomic alterations only occur infrequently. As low-grade chondrosarcomas showed lower mutation load, very few or no CNA, absence of *CDKN2A/2B* deletion and *TP53* mutation, these genomic findings could serve as adjunct markers to histological grading in morphologically challenging cases. The underlying genomic complexity in high-grade and dedifferentiated chondrosarcomas is reflected morphologically by increased mitoses and nuclear atypia as shown in other types of sarcomas [31]. Prior studies have also demonstrated that *CDKN2A* deletion and *TP53* mutation are associated with high-grade and dedifferentiated chondrosarcomas but not with low-grade chondrosarcomas [13, 32, 33]. Similar finding in astrocytoma has shown that *CDKN2A* deletion was strongly associated with poorer overall survival after adjustment for *IDH* mutation, sex, and age [34]. These findings suggest a role for dysregulation in cell cycle and DNA damage repair pathways as a result of inactivation of *CDKN2A/2B* and *TP53* in chondrosarcoma progression.

Twenty percent (20%, 6/30) of high-grade and dedifferentiated chondrosarcomas harbor *TERT* or *ATRX* alteration. None of the low-grade chondrosarcomas tested, albeit a small number (3 cases), showed these alterations, which is consistent with the recent study that suggests *TERT* promoter mutations may play a role in chondrosarcoma progression since its

association with increased histological grade [6]. Concurrence of *TERT* or *ATRX* mutation with *IDH1/IDH2* mutation, and with *CDKN2A/2B* deletion or *TP53* mutation in a subset of high-grade chondrosarcomas suggests a possible synergy among these pathways in the progression of chondrosarcoma. In line with our study, *TERT* promoter and *IDH1/IDH2* mutations co-occur in 79% of oligodendrogliomas [35]. Mechanistic study performed on glioma cells show that mutant IDH1 can indirectly reactivate TERT and contribute to astrocytic immortalization and transformation [36]. Mutant IDH1 can also initiate telomeric dysfunction and alter DNA repair pathway preferences at telomeres, cooperating with ATRX loss to drive the alternative lengthening of telomere phenotype for gliomagenesis [37]. *TERT* promotor and *IDH1/IDH2* mutation status can be used to guide glioma classification and diagnosis, categorize glioma patients into distinct survival subgroups, and direct individualized treatments for the distinct molecular subtypes [38, 39].

Telomere dysfunction has been found in a variety of human cancers and is associated with more aggressive behavior [40, 41]. This is the first study to our knowledge that identified *TERT* amplification and *ATRX* mutations in chondrosarcomas in addition to *TERT* promoter mutations. No significant difference in OS, RFS and MFS was identified between *TERT/ATRX* mutant and wild-type chondrosarcomas (data not shown). Additional studies may further elucidate the molecular mechanism underlying the telomere dysfunction associated progression of chondrosarcomas and the synergistic effect among the pathways.

Other genomic alterations in this study involved cell surface receptors which include *EPHA7*, *FGFR3*, *IGF1R*, *KIT* and *NOTCH3* etc. Many of these genes have been found to be critical regulators in the cartilage development. For instance, FGFR3 functions as an important physiological negative regulator of bone growth. *FGFR3* activation mutations are associated with human hypochondroplasia, achondroplasia due to growth attenuation of the cartilage while inactivating mutations are associated with skeletal overgrowth in human CATSHL syndrome [42]. We found *FGFR3* deletions in two cases suggesting that FGFR3 might function as a tumor suppressor in chondrosarcoma development. This is consistent with its role as physiological negative regulator of cartilage development. Eph, insulin-like growth factor and NOTCH signaling pathways have also been shown to be involved in the chondrocyte development [43–45]. We also found amplification of the receptor tyrosine kinases such as *IGF1R* and *KIT* which might potentially be targetable by tyrosine kinase inhibitors. Intracytoplasmic signaling factors downstream of these cell membrane receptors such as *KRAS* and *PTEN* are also found to be occasionally mutated, which include *KRAS G12A* mutation and *PTEN* missense mutation in one case each. In summary, it appears that some genes and pathways which are involved in normal cartilage development are disrupted in the chondrosarcoma development.

Second malignancies in chondrosarcoma patients

Twenty one percent of chondrosarcoma patients also had histories of second malignancies that are unrelated to cartilaginous tumors. Most patients did not have a known cancer predisposition syndrome, suggesting there might be unknown genetic susceptibility underlying the chondrosarcoma development in these patients. Investigation of the family

history and germline genetic association study might help elucidate the potential genetic susceptibility in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research is supported by the NCI of the NIH (P30 CA008748)

Abbreviations list

IDH	Isocitrate dehydrogenase
MSK-IMPACT	Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets
OS	overall survival
RFS	relapse free survival
MFS	metastasis free survival
TTR	time to relapse
TTM	time to metastasis

References

1. Fletcher CDM, World Health Organization., and International Agency for Research on Cancer., WHO classification of tumours of soft tissue and bone. 4th ed. World Health Organization classification of tumours. 2013, Lyon: IARC Press 468 p.
2. Bjornsson J, et al., Primary chondrosarcoma of long bones and limb girdles. Cancer, 1998 83(10): p. 2105–19. [PubMed: 9827715]
3. Gelderblom H, et al., The clinical approach towards chondrosarcoma. Oncologist, 2008 13(3): p. 320–9. [PubMed: 18378543]
4. Czerniak B and Dorfman HD, Dorfman and Czerniak's bone tumors. Second edition. ed. 2016, Philadelphia, PA: Elsevier xiii, 1506 pages.
5. Lugowska I, et al., IDH1/2 Mutations Predict Shorter Survival in Chondrosarcoma. J Cancer, 2018 9(6): p. 998–1005. [PubMed: 29581779]
6. Lin Y, et al., hTERT promoter mutations in chondrosarcomas associate with progression and disease-related mortality. Mod Pathol, 2018 31(12): p. 1834–1841. [PubMed: 30065261]
7. Lehninger AL, Nelson DL, and Cox MM, Lehninger principles of biochemistry. 6th ed. 2013, New York: W.H. Freeman.
8. Parsons DW, et al., An integrated genomic analysis of human glioblastoma multiforme. Science, 2008 321(5897): p. 1807–12. [PubMed: 18772396]
9. Yan H, et al., IDH1 and IDH2 mutations in gliomas. N Engl J Med, 2009 360(8): p. 765–73. [PubMed: 19228619]
10. Amary MF, et al., IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. J Pathol, 2011 224(3): p. 334–43. [PubMed: 21598255]

11. Amary MF, et al., Ollier disease and Maffucci syndrome are caused by somatic mosaic mutations of IDH1 and IDH2. Nat Genet, 2011 43(12): p. 1262–5. [PubMed: 22057236]
12. Pansuriya TC, et al., Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. Nat Genet, 2011 43(12): p. 1256–61. [PubMed: 22057234]
13. Tarpey PS, et al., Frequent mutation of the major cartilage collagen gene COL2A1 in chondrosarcoma. Nat Genet, 2013 45(8): p. 923–6. [PubMed: 23770606]
14. Cleven AHG, et al., IDH1 or −2 mutations do not predict outcome and do not cause loss of 5-hydroxymethylcytosine or altered histone modifications in central chondrosarcomas. Clin Sarcoma Res, 2017 7: p. 8. [PubMed: 28484589]
15. Gao L, et al., Targeted next-generation sequencing of dedifferentiated chondrosarcoma in the skull base reveals combined TP53 and PTEN mutations with increased proliferation index, an implication for pathogenesis. Oncotarget, 2016 7(28): p. 43557–43569. [PubMed: 27248819]
16. Skeletal Lesions Interobserver Correlation among Expert Diagnosticians Study, G., Reliability of histopathologic and radiologic grading of cartilaginous neoplasms in long bones. J Bone Joint Surg Am, 2007 89(10): p. 2113–23. [PubMed: 17908885]
17. Zehir A, et al., Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med, 2017 23(6): p. 703–713. [PubMed: 28481359]
18. Shukla N, et al., Oncogene mutation profiling of pediatric solid tumors reveals significant subsets of embryonal rhabdomyosarcoma and neuroblastoma with mutated genes in growth signaling pathways. Clin Cancer Res, 2012 18(3): p. 748–57. [PubMed: 22142829]
19. Cheng DT, et al., Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. J Mol Diagn, 2015 17(3): p. 251–64. [PubMed: 25801821]
20. Mayakonda AK, Maftools HP, Efficient analysis, visualization and summarization of MAF files from large-scale cohort based cancer studies. bioRxiv, 2016: p. doi: 10.1101/052662.
21. Dang L, Jin S, and Su SM, IDH mutations in glioma and acute myeloid leukemia. Trends Mol Med, 2010 16(9): p. 387–97. [PubMed: 20692206]
22. Dang L, et al., Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature, 2009 462(7274): p. 739–44. [PubMed: 19935646]
23. Ward PS, et al., The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. Cancer Cell, 2010 17(3): p. 225–34. [PubMed: 20171147]
24. Chowdhury R, et al., The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. EMBO Rep, 2011 12(5): p. 463–9. [PubMed: 21460794]
25. Koh KP, et al., Tet1 and Tet2 regulate 5-hydroxymethylcytosine production and cell lineage specification in mouse embryonic stem cells. Cell Stem Cell, 2011 8(2): p. 200–13. [PubMed: 21295276]
26. Xu W, et al., Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer Cell, 2011 19(1): p. 17–30. [PubMed: 21251613]
27. Figueroa ME, et al., Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell, 2010 18(6): p. 553–67. [PubMed: 21130701]
28. Lu C, et al., IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature, 2012 483(7390): p. 474–8. [PubMed: 22343901]
29. Bralten LB, et al., IDH1 R132H decreases proliferation of glioma cell lines in vitro and in vivo. Ann Neurol, 2011 69(3): p. 455–63. [PubMed: 21446021]
30. Nie QM, et al., IDH1R(1)(3)(2)H decreases the proliferation of U87 glioma cells through upregulation of microRNA-128a. Mol Med Rep, 2015 12(5): p. 6695–701. [PubMed: 26324126]
31. Cancer Genome Atlas Research Network. Electronic address, e.d.s.c. and N. Cancer Genome Atlas Research, Comprehensive and Integrated Genomic Characterization of Adult Soft Tissue Sarcomas. Cell, 2017 171(4): p. 950–965 e28. [PubMed: 29100075]

32. Rozeman LB, Hogendoorn PC, and Bovee JV, Diagnosis and prognosis of chondrosarcoma of bone. Expert Rev Mol Diagn, 2002 2(5): p. 461–72. [PubMed: 12271817]
33. van Beerendonk HM, et al., Molecular analysis of the INK4A/INK4A-ARF gene locus in conventional (central) chondrosarcomas and enchondromas: indication of an important gene for tumour progression. J Pathol, 2004 202(3): p. 359–66. [PubMed: 14991902]
34. Reis GF, et al., CDKN2A loss is associated with shortened overall survival in lower-grade (World Health Organization Grades II-III) astrocytomas. J Neuropathol Exp Neurol, 2015 74(5): p. 442–52. [PubMed: 25853694]
35. Killela PJ, et al., Mutations in IDH1, IDH2, and in the TERT promoter define clinically distinct subgroups of adult malignant gliomas. Oncotarget, 2014 5(6): p. 1515–25. [PubMed: 24722048]
36. Ohba S, et al., Mutant IDH1 Expression Drives TERT Promoter Reactivation as Part of the Cellular Transformation Process. Cancer Res, 2016 76(22): p. 6680–6689. [PubMed: 27758882]
37. Mukherjee J, et al., Mutant IDH1 Cooperates with ATRX Loss to Drive the Alternative Lengthening of Telomere Phenotype in Glioma. Cancer Res, 2018 78(11): p. 2966–2977. [PubMed: 29545335]
38. Karsy M, et al., New Molecular Considerations for Glioma: IDH, ATRX, BRAF, TERT, H3 K27M. Curr Neurol Neurosci Rep, 2017 17(2): p. 19. [PubMed: 28271343]
39. Vuong HG, et al., TERT promoter mutation and its interaction with IDH mutations in glioma: Combined TERT promoter and IDH mutations stratifies lower-grade glioma into distinct survival subgroups-A meta-analysis of aggregate data. Crit Rev Oncol Hematol, 2017 120: p. 1–9. [PubMed: 29198322]
40. Maciejowski J and de Lange T, Telomeres in cancer: tumour suppression and genome instability. Nat Rev Mol Cell Biol, 2017 18(3): p. 175–186. [PubMed: 28096526]
41. Zhang C, et al., The Association between Telomere Length and Cancer Prognosis: Evidence from a Meta-Analysis. PLoS One, 2015 10(7): p. e0133174. [PubMed: 26177192]
42. Foldynova-Trantirkova S, Wilcox WR, and Krejci P, Sixteen years and counting: the current understanding of fibroblast growth factor receptor 3 (FGFR3) signaling in skeletal dysplasias. Hum Mutat, 2012 33(1): p. 29–41. [PubMed: 22045636]
43. Guntur AR and Rosen CJ, IGF-1 regulation of key signaling pathways in bone. Bonekey Rep, 2013 2: p. 437. [PubMed: 24422135]
44. Hosaka Y, et al., Notch signaling in chondrocytes modulates endochondral ossification and osteoarthritis development. Proc Natl Acad Sci U S A, 2013 110(5): p. 1875–80. [PubMed: 23319657]
45. Matsuo K and Otaki N, Bone cell interactions through Eph/ephrin: bone modeling, remodeling and associated diseases. Cell Adh Migr, 2012 6(2): p. 148–56. [PubMed: 22660185]

Translational Relevance

High grade Chondrosarcomas (Grade II and III) have a 53% 5-year survival and the prognosis of dedifferentiated chondrosarcomas is dismal with less than 2-year survival. The role of chemotherapy in recurrent/metastatic chondrosarcomas is not well-established and is generally poor. As 50% of chondrosarcomas possess IDH1/2 mutations, we sought to correlate IDH1/2 mutations to clinical outcome in 89 patients. We found IDH1/2 mutations in 46% of the patients and while overall survival was not affected in this cohort, we found IDH mutations were associated with longer relapse free survival (RFS) and metastases free survival (MFS) in high grade chondrosarcomas. We also found TERT gene amplification and ATRX mutation, in addition to TERT promoter mutation in a subset of high-grade chondrosarcomas. As IDH1 inhibitors are being used in clinical trials for solid tumors, this data provides an opportunity to further explore the above markers in chondrosarcoma biology.

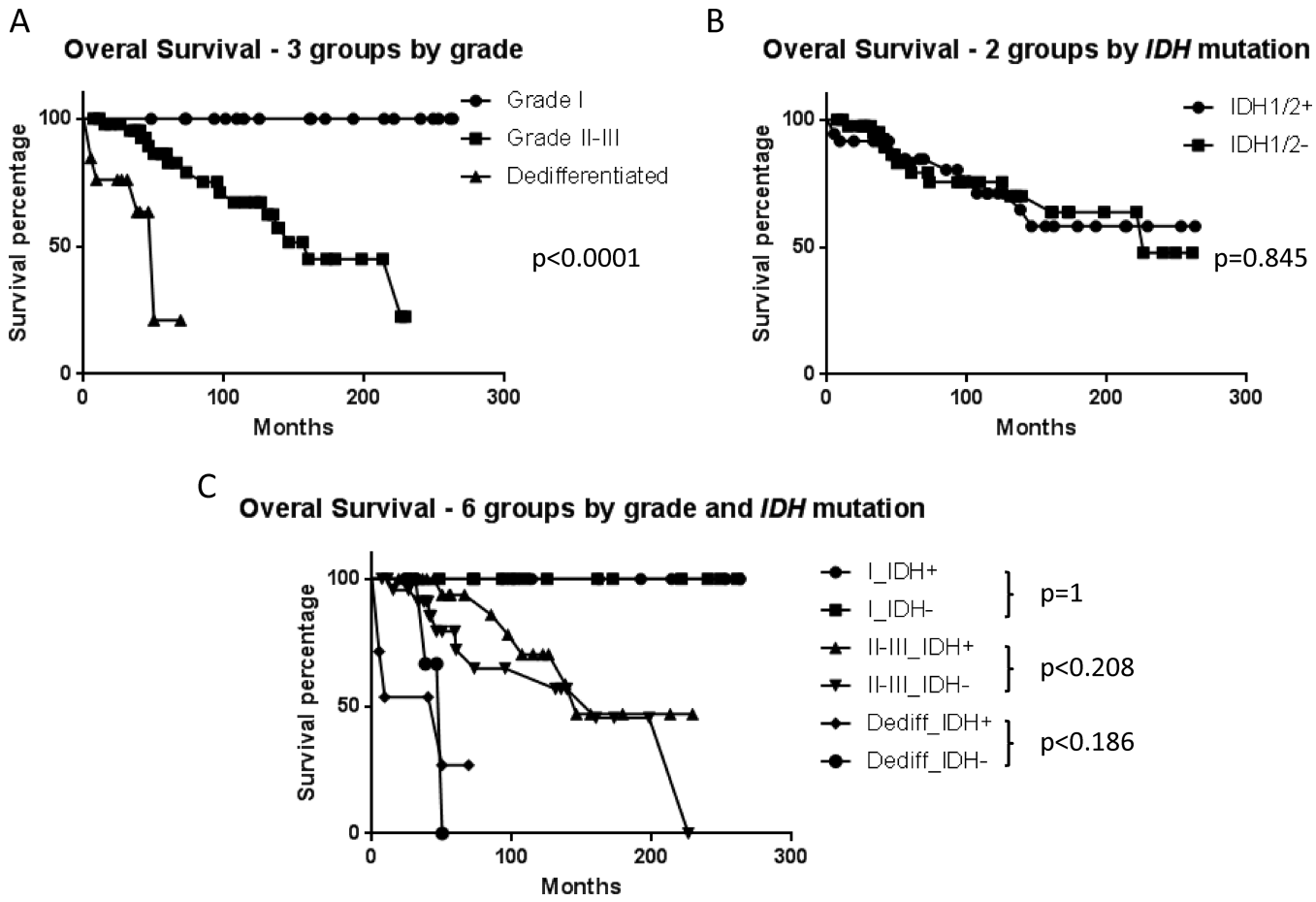


Figure 1.

Kaplan-Meier survival analysis of the overall survival (OS) of 79 chondrosarcoma patients. A. OS curves stratified by tumor grade only; B. OS curves stratified by *IDH1/2* mutation status only; C. OS curves stratified by tumor grade and *IDH1/IDH2* mutation status.

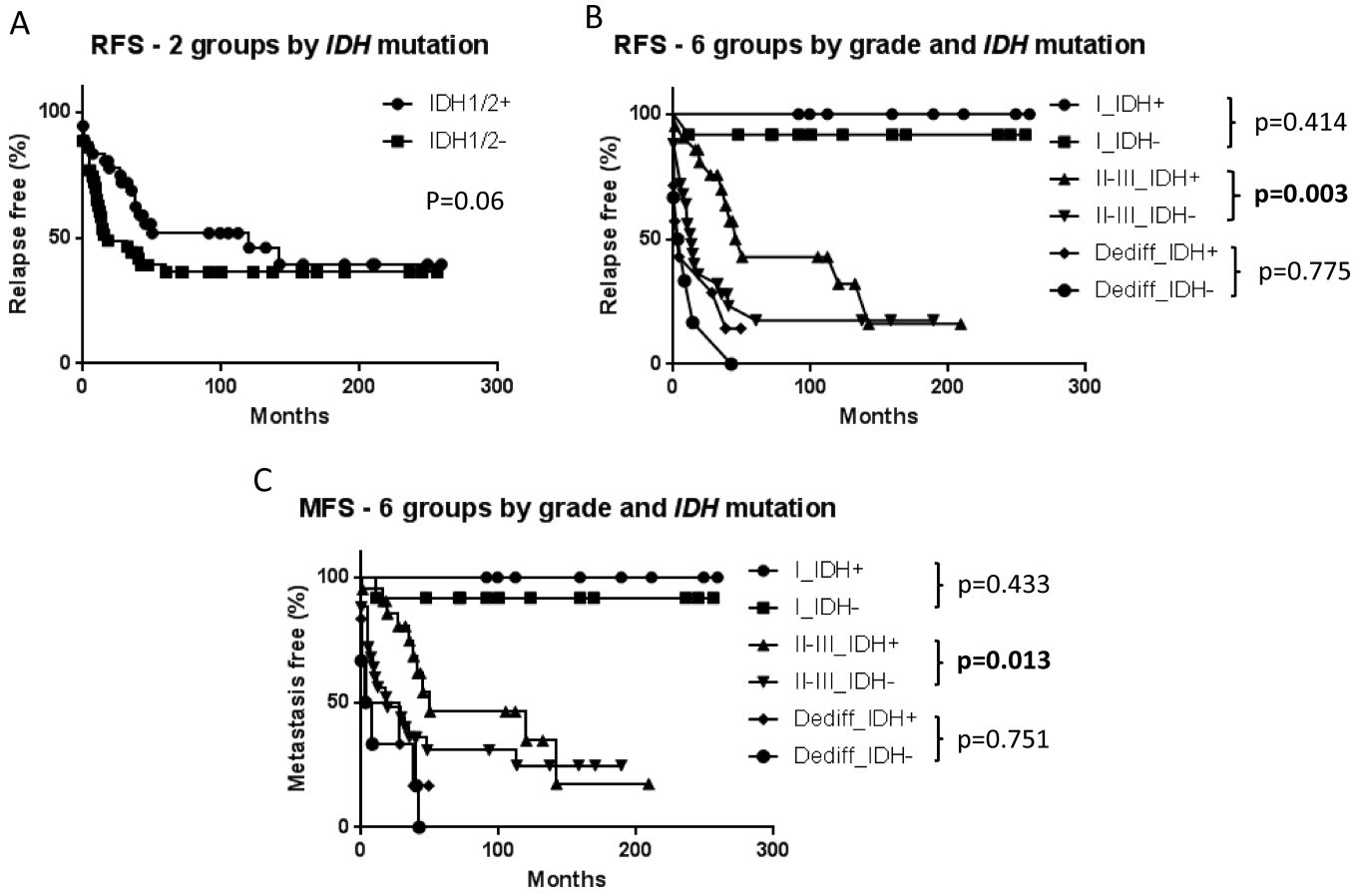


Figure 2.

Kaplan-Meier survival analysis of relapse free survival (RFS) of 79 chondrosarcoma patients. A. RFS curves by time to relapse (TTR) stratified by *IDH1*/*IDH2* mutation status only; B. RFS curves by TTR stratified by IDH1/2 mutation status and tumor grade; C. Metastasis free survival (MFS) curves by time to metastasis (TTM) stratified by *IDH1/IDH2* mutation status and tumor grade.

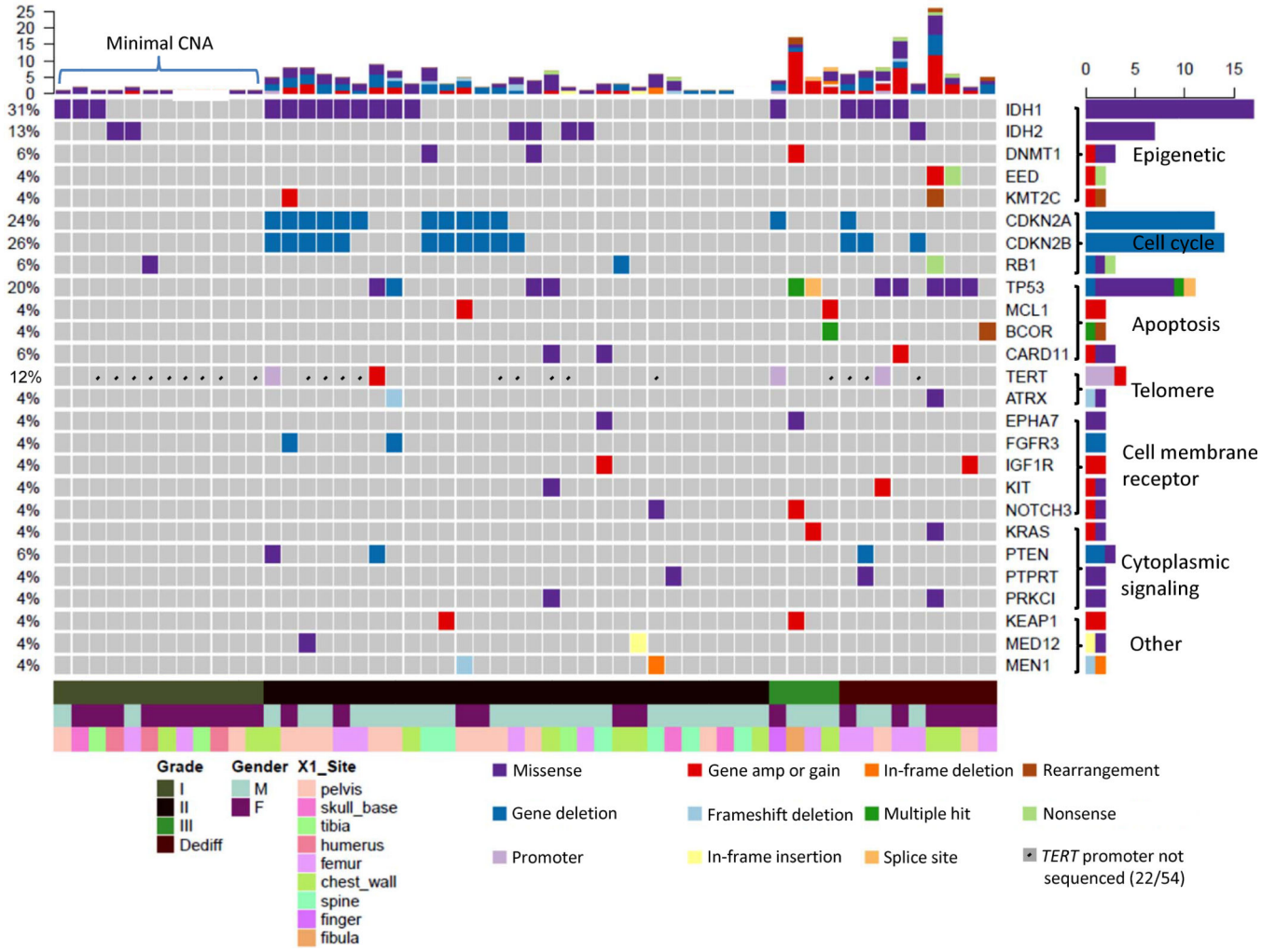
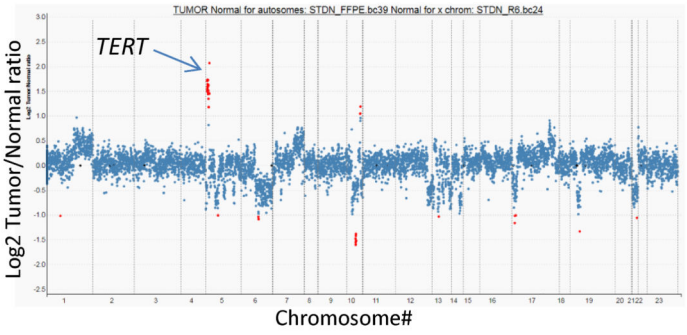


Figure 3.

Genomic alteration profiles of 54 chondrosarcomas together with clinicopathologic information. Only events >= 2 are included in the plot. Each row represents a gene and each column represents a patient. The patients are sorted by tumor grades horizontally.

A

#	Gene	Mutation type	gDNA	Amino Acid
1	TERT	5′Flank missense	g.1295228 C>T	
2	TERT	5′Flank missense	g.1295250 C>T	
3	ATRX	Frame-shift deletion	c.2656_2659delGAGA	p.E886Lfs*18
4	ATRX	Missense	c.5773 G>A	p.D1925N
5	TERT	Amplification		



B

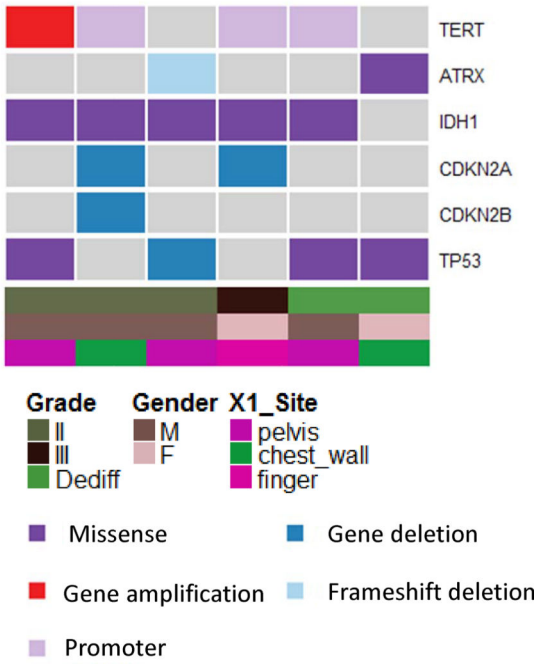


Figure 4.

A) Six chondrosarcomas demonstrate *TERT* (NM_198253) and *ATRX* (NM_000489) mutations. B) Five cases are concurrent with *IDH1* mutation while all cases are concurrent with *CDKN2A/2B* deletion or *TP53* mutation. One case of *ATRX* missense mutation (p.D1925N) is wild-type for *IDH1/IDH2*. The co-occurrence of *IDH1/IDH2* mutation with *TERT/ATRX* alterations is statistically significant (p=0.02).

Table 1.

IDH1/IDH2 mutations summary

Location (n)	Grading (n)	*IDH1/2* Mutations (n)	Mutation (percentage)
Femur (22)	I (4) II-III (10) De-diff (8)	R132C (3), R132G (3), R132H (3), R132L (1), R172M (3), R172S (4)	77% (17/22)
Tibia (6)	I (3) II-III (3)	R132C (1), R132G (1), R172S (1)	50% (3/6)
Fibula (2)	I (1) II-III (1)	R132G (1)	50% (1/2)
Humerus (12)	I (8) II-III (4)	R132H (1), R132S (1), R172G (1), R172S (1),	33% (4/12)
Phalanx (1)	II-III (1)	R132G (1)	100% (1/1)
Pelvis (17)	I (3) II-III (10) De-diff (4)	R132C (2), R132G (2), R132H (1), R132I (1), R132L (1), R132S (1), R172S (1)	53% (9/17)
Spine (7)	II-III (7)		0%
Scapula (4)	I (1) II-III (2) De-diff (1)	R132G (1) R132L (1)	50% (2/4)
Chest wall (15)	I (2) II-III (11) De-diff (2)	R132C (1) R132G (1) R132L (1)	20% (3/15)
Skull base (3)	I (1) II-III (2)	R132C (1, Ollier)	33.3% (1/3)
Total (89)	I (23) II-III (51) De-diff (15)	R132C (8), R132G (10), R132H (5), R132I (1), R132L (4), R132S (2), R172G (1), R172M (3), R172S (7)	46% (41/89)

Table 2.

Survival analysis summary

	IDH1/2 mutant (n=36)	IDH1/2 wild-type (n=43)
Female | Male	14 | 22	23 | 20
Median age at 1° diagnosis	53.5 (10–73)	46 (18–75)
Grade		
Low-grade (I)	7 (19.4%)	12 (27.9%)
High-grade (II-III)	22 (61.2%)	25 (58.1%)
Dedifferentiated	7 (19.4%)	6 (14.0%)
Relapse Free Survival (months)		
Median TTR, overall	120	18 (p=0.060)
Median TTR, low-grade	> 259 *	> 256 * (p=0.414)
Median TTR, high-grade	45	13 (HR=4.5, p=0.003)
Median TTR, dedifferentiated	4	5.5 (p=0.775)
Metastasis Free Survival (months)		
Median TTM, overall	120	40 (p=0.083)
Median TTM, low-grade	> 259 *	> 256 * (p=0.414)
Median TTM, high-grade	50	19 (HR=2.6, p=0.013)
Median TTM, dedifferentiated	16	5.5 (p=0.751)
Percent metastasis		
Low-grade	0% (0/7)	8.3% (1/12)
High-grade	50% (11/22)	72% (18/25)
Dedifferentiated	85.7% (6/7)	100% (6/6)
Overall survival (months)		
Median OS	> 263 *	226 (p=0.845)

TTR: time to relapse; TTM: time to metastasis, OS: overall survival

* cannot be further defined due to less than 50% of patients developed relapse or metastasis, or died.