Unique mutation portraits and frequent COL2A1 gene alteration in chondrosarcoma

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Chondrosarcoma is the second most frequent malignant bone tumor. However, the etiological background of chondrosarcomagenesis remains largely unknown, along with details on molecular alterations and potential therapeutic targets. Massively parallel paired-end sequencing of whole genomes of 10 primary chondrosarcomas revealed that the process of accumulation of somatic mutations is homogeneous irrespective of the pathological subtype or the presence of IDH1 mutations, is unique among a range of cancer types, and shares significant commonalities with that of prostate cancer. Clusters of structural alterations localized within a single chromosome were observed in four cases. Combined with targeted resequencing of additional cartilaginous tumor cohorts, we identified somatic alterations of the COL2A1 gene, which encodes an essential extracellular matrix protein in chondro-osseous development, in 19.3% of chondrosarcoma and 31.7% of enchondroma cases. Epigenetic regulators (IDH1 and YEATS2) and an activin/BMP signal component (ACVR2A) were recurrently altered. Furthermore, a novel FNI-ACVR2A fusion transcript was observed in both chondrosarcoma and osteochondromatosis cases. With the characteristic accumulative process of somatic changes as a background, molecular defects in chondrogenesis and aberrant epigenetic control are primarily causative of both benign and malignant cartilaginous tumors.

[Supplemental material is available for this article.]
Table 1. Summary of clinical data and somatic alterations of 10 chondrosarcoma cases analyzed by whole-genome sequencing

|             | CS01  | CS02  | CS03  | CS04  | CS05  | CS06  | CS07  | CS08  | CS09  | CS10  |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Age         | 14    | 32    | 58    | 43    | 62    | 86    | 50    | 58    | 54    | 33    |
| Gender      | F     | M     | M     | M     | F     | F     | F     | F     | M     |       |
| Tumor site  | Rib   | Pelvis| Pelvis| Pelvis| Humerus| Knee | Rib   | Pelvis| Scapula| Tibia |
| Subtype     | Peripheral | Central | Peripheral | Peripheral | Central | Synovial | Central | Central | Central |
| Tumor grade | Multiple osteochondromatosis | Multiple osteochondromatosis | Multiple osteochondromatosis |       |       |       |       |       |       |       |
| Clinical background |       |       |       |       |       |       |       |       |       |       |
| Average sequence depth (tumor) | 42.6  | 34.2  | 27.6  | 30.8  | 33.4  | 37.7  | 37.2  | 37.2  | 29    | 24.7  |
| Average sequence depth (normal) | 32.7  | 31.6  | 25.4  | 32.9  | 32.8  | 32.7  | 27.6  | 31.6  | 35    | 31.6  |
| Number of somatic substitutions | 3521  | 5005  | 6128  | 2215  | 5043  | 3705  | 4100  | 5326  | 2288  | 7014  |
| Number of nonsynonymous substitutions | 10    | 26    | 37    | 7     | 26    | 11    | 29    | 26    | 12    | 27    |
| Rearrangement break points | 66    | 60    | 45    | 44    | 24    | 23    | 1     | 40    | 10    | 37    |
Results

Whole-genome sequencing (WGS) of chondrosarcoma

Massively parallel paired-end sequencing of the whole genomes of 10 pairs of primary chondrosarcoma and matched normal muscle tissues was performed. The cases included five central, four peripheral, and one synovial—a rare subtype (Table 1). The median sequence coverage was 33.4x for tumor tissue and 31.4x for normal tissue (Table 1). All peripheral cases were associated with osteochondromatosis and harbored germline EXT1 or EXT2 mutations (Supplemental Table S1). No IDH1/2 or other enchondromatosis-associated gene mutations (PTH1R, PTPN11, or ACP5) (Hopyan et al. 2002; Bowen et al. 2011; Briggs et al. 2011) were observed in the germline genomes. In total, 44,345 somatic single-nucleotide variations (SNVs; ranging from 2215–7014 per genome, 1.55/Mb on average) and 4096 small insertions/deletions (indels, ranging from 269–544 per genome) were identified (Fig. 1A; Supplemental Table S2). The somatic point mutations included 211 nonsynonymous mutations (21.1 per genome on average) and 14 indels (1.4 per genome on average) in the coding regions (Supplemental Table S3). More than 91% (78/85) of somatic substitutions and 63% (17/27) of somatic indels were validated by Sanger sequencing. These mutations were significantly enriched in membranous proteins, especially those with transmembrane receptor activity (Supplemental Table S4).

Unique somatic substitution signatures in the chondrosarcoma genomes

Analysis of genome-wide somatic mutation signatures revealed that C:G>T:A transitions are dominant, followed by T:A>C:G, T:A>A:T, and C:G>A:T substitutions in all chondrosarcoma cases (Fig. 1A). These somatic substitution signatures at CpG sites were not associated with subtype classification or the presence of IDH1 mutations (Supplemental Figs. S1, S2). Principal component analysis of trans-cancer genome data showed that the substitution pattern in these chondrosarcomas was most similar to those of prostate (Berger et al. 2011) and liver (Fujimoto et al. 2012) cancers and chronic lymphocytic leukemia (permutation test; P = 0.0010) (Fig. 1B; Puente et al. 2011). A significant reduction in C:G>A:T transversions on the
transcribed strand was observed in both central (CS-2T, 7T, 8T, and 9T) and peripheral (CS-1T, 3T, and 10T) cases (Fig. 1C; Supplemental Fig. S3), which correlated with gene expression level (Fig. 1D). To explore any sequence context–dependent substitutions in the chondrosarcoma genomes, we measured the frequencies of immediate 5' and 3' nucleotides for all substitutions. This analysis revealed significant increases in C>T transitions at TpCpT, C>A transversions at ApCpA, and T>A transversions at ApTpA in all cases except CS5T (Fig. 2A; Supplemental Fig. S4). No context-specific T>C transitions were observed. This triplet landscape differs from those of liver cancer and CLL and those caused by known etiological factors such as C>T in UV-associated melanoma (Pleasance et al. 2010a) or C>A in smoking-associated lung cancer (Pleasance et al. 2010b), but shares significant commonalities with that of prostate cancer (permutation test; P = 0.0017) (Fig. 2B; Supplemental Figs. S5, S6). A further context survey of the 10 nucleotides stretching in the 5' and 3' directions from each somatic substitution identified a predominance of A/T around the sites of the C>A substitutions (particularly on the 3' side), and found that T was dominant at either side of the C>T substitution (Fig. 3). This pattern was also observed in prostate cancer but not in melanoma and smoking-associated lung cancer (Fig. 3; Supplemental Fig. S7).

Structural alterations in chondrosarcoma

We determined copy number and structural alterations by analyzing sequence depth and paired sequence reads (Supplemental Tables S5, S6). We predicted 350 rearrangements in 10 chondrosarcomas. Validation analysis by genomic PCR and Sanger sequencing of randomly selected breakpoints verified >98% of predictions (67/68) as somatic. No recurrent rearrangements were detected. A remarkably complex rearrangement—a cluster of structural alterations localized within a single chromosome—was observed in four cases (Supplemental Figs. S8, S9A–C). Massive rearrangements were involved in CS2T with amplification in the short arm of chromosome 5, including the TERT gene (Fig. 4A, B). Interstitial deletion of exons 2 and 3 and a resulting premature stop codon in the WNK2 tumor suppressor gene (Hong et al. 2007), which encodes a negative regulator of the MEK/ERK pathway (Moniz et al. 2007), was detected in CS6T (Fig. 4C).

Novel driver genes in chondrosarcoma

To explore driving alterations in chondrosarcomagenesis, we calculated the expected number of somatic nonsynonymous and splice site substitutions, coding indels, and rearrangements with an adjustment for background mutation rate and gene length in each gene, and we identified five recurrently altered genes (IDH1, TP53, ACVR2A, COL2A1, and YEATS2) with a false-discovery rate of <1% (Supplemental Table S7). Among these, recurrent mutations of the IDH1 and TP53 genes have been previously reported in chondrosarcoma (Wadayama et al. 1993; Amary et al. 2011a). To validate mutation frequency in a larger number of cases, we performed target exon resequencing of three potential new driver genes (COL2A1, YEATS2, and ACVR2A) and IDH1/2 in an additional 47 chondrosarcoma, with 19 corresponding adjacent nontumor tissues, and in 41 enchondroma samples (Fig. 5; Supplemental Tables S8, S9). COL2A1 encodes the alpha 1 chain of type II collagen which is enriched in cartilage (Cheah et al. 1985) and was mutated in 11 chondrosarcoma (19.3%) and 13 enchondroma cases (31.7%). Structural alterations (intragenic tandem duplication and deletion) of the COL2A1 gene were also detected. YEATS2 alterations, in-

Figure 2. Somatic mutation portraits in the chondrosarcoma genome. (A) Frequencies of 96 mutation portraits (combination of immediate 5' and 3' bases with six substitutions) in peripheral (CS1T) and central (CS2T and CS5T) cases. Sixteen triplet sequence patterns (mutations with immediate 5' and 3' nucleotides) for each substitution are indicated by different color columns. (B) Frequencies of 96 mutation portraits in chondrosarcoma (average), prostate cancer (average), UV-associated melanoma, and smoking-associated lung cancer genomes.
Including one nonsense mutation and an intrachromosomal inversion, were detected in seven chondrosarcoma (12.3%) cases and one enchondroma case (2.4%). YEATS2 is a scaffolding subunit of the nuclear acetyltransferase complex which targets histone H3 and represses transcription of target genes (Wang et al. 2008), and a nonsense mutation (p.W1229*) that disrupts the regulatory histone fold module (Fig. 5B). They are significantly more frequent in higher-grade (grades 2 and 3) cases than grade 1 chondrosarcoma/enchondroma cases ($P = 0.023$) and tend to be mutually exclusive to COL2A1 mutation ($P = 0.07$).

ACVR2A mutations were observed in four chondrosarcoma cases (7%) and three enchondroma cases (7.3%). IDH1 mutations at codon 132 were observed in 12 chondrosarcoma (21.1%) and five enchondroma cases (12.2%) and were not associated with any other mutations. The presence of a COL2A1 or IDH1 mutation was not associated with patients’ prognoses (Supplemental Fig. S10).

### Novel fusion gene in chondrosarcoma

Four in-frame fusion transcripts were predicted by structural rearrangements (Table 2). Among these, two (ACVR2A-FN1 and FN1-ACVR2A) were expressed and validated in the synovial CS6T case (Fig. 6A–C; Supplemental Fig. S11). Paired-end whole-transcriptome sequencing demonstrated that expression of the ACVR2A gene transcript increased sharply—more than 10-fold—in the exons fused to the FN1 gene (Fig. 6D), without any overt change in FN1 gene expression at the fusion junction (Supplemental Fig. S12). This suggests that promoter activity of the FN1 gene conferred increased FN1-ACVR2A transcript in the tumor. RNA sequencing further revealed that expression of the ACVR2A gene increased more than 25-fold in the FN1-ACVR2A fusion-positive case (CS6T) compared with that of other samples (CS1T and CS7T) (Supplemental Table S10). Since this fusion gene was detected in a chondrosarcoma with features suggestive of preexisting synovial osteochondromatosis (Fig. 6A), we further surveyed a range of cartilaginous tumors, including extramedullary ones (17 chondrosarcomas, seven synovial chondromatoses, and 13 osteochondromas) using RT-PCR, and detected expression of the FN1-ACVR2A—but not the ACVR2A-FN1—fusion transcript in a case of osteochondromatosis (Fig. 6E; Supplemental Fig. S13).

### Discussion

Using the whole-genome sequencing (WGS) approach, the present study revealed comprehensive landscapes of genetic alterations in the chondrosarcoma genome that include global mutational signatures, structural alterations including copy number changes and rearrangements, and new driver genes.

Somatic mutation signatures are affected by both environmental carcinogen exposures and defects in DNA repair systems (Stratton et al. 2009; Nik-Zainal et al. 2012). Previous WGS analyses of small-cell lung cancer and melanoma cell lines showed an intimate association between specific carcinogens (smoking or UV exposure) and the patterns of base substitutions (Pleasance et al. 2010a,b). Furthermore, WGS of breast cancers have reported that BRCA1-mutated breast cancers showed a unique mutational signature (Nik-Zainal et al. 2012). Our analysis showed that C>G>T:A transitions with significant transcription-coupled repair are broadly observed in chondrosarcoma, which is also characteristic of smoking-associated lung cancer (Pleasance et al. 2010b; Govindan et al. 2012).
Nonetheless, further mutation trait analysis demonstrated that the mutagenesis process in chondrosarcoma seems to be distinct from mutagenesis induced by known carcinogens, including tobacco smoking. The presence of an \textit{IDH1} mutation is associated with epigenetic alterations such as CpG hypermethylation in tumors, including enchondroma (Pansuriya et al. 2011; Turcan et al. 2012). It could be possible that frequent CpG methylation induces the chance of somatic C-to-T transition by spontaneous deamination of 5-methylcytosine. However, the genome sequencing described here revealed that the \textit{IDH1} mutation exerts no significant effect on the somatic mutation frequency at CpG sites during chondrosarcomagenesis.

Our analysis uncovered the unexpected similarity of the mutation portraits (C$\rightarrow$T transitions atTpCpT, C$\rightarrow$A transversions at ApCpA, and T$\rightarrow$A transversions at ApTpA) of chondrosarcoma and prostate cancer, both of which frequently occur in older and male patients. Nine out of 10 cases showed similar mutational signatures, and these nine cases harbored a distinct set of somatic mutations, suggesting that this signature could be caused by common etiological factors but not affected by somatic deficiency in the DNA repair system. The etiological risk factors, including genetic susceptibility for chondrosarcoma, remain unknown partly because of the small number of patients; however, our observation implies that aging, hormonal status, or dietary factors, all of which are suggestive etiological factors of prostate cancer (Hsing and Chokkalingam 2006), or genetic risk factors, may be associated with this sarcoma.

WGS of 10 cases followed by further validation of a larger cohort identified a few common alterations (\textit{COL2A1} and \textit{IDH1}) and other rarer events, the latter of which include progression-associated changes such as \textit{YEATS2}, in chondrosarcomagenesis. \textit{COL2A1}, which is rarely mutated in other tumor types, particularly emerged as a new frequently altered gene in chondrosarcoma. The pattern of \textit{COL2A1} mutation in this study is different from that reported in the previous study (Tarpey et al. 2013). A preponderance of missense mutation has been observed in the present study whereas truncating mutations were predominant in the previous report. This discrepancy could be due to the difference in ethnicity (Japanese and Caucasian) or unknown carcinogenesis background of each cohort, and a larger collection of samples by an international collaboration will be required to explore this. Germline \textit{COL2A1} mutations are associated with a number of chondroskeletal malformation syndromes, so-called type II collagenopathies (Spranger et al. 1994;
Nishimura et al. (2005), including spondyloepiphyseal dysplasia congenita, achondrogenesis type II, and osteoarthritis associated with chondrodysplasia. Col2a1 mutant mice demonstrated growth plate disorganization with reduced elaborate collagen fibrils (Esapa et al. 2012). However, no clinical association between these congenital diseases and cartilaginous tumors has been reported so far. The presence of frequent COL2A1 as well as IDH1/2 mutations in both chondrosarcoma and enchondroma demonstrated by the present and previous studies (Amary et al. 2011a) supports a model of progression from enchondroma to chondrosarcoma. Our analysis also identified that aberrations in the epigenetic regulators play important roles in cartilaginous tumors. The presence of an IDH1 mutation is associated with CpG hypermethylation in tumors including enchondroma (Pansuriya et al. 2011; Turcan et al. 2012). YEATS2 is a scaffolding subunit of the nuclear acetyltransferase complex and harbors a histone-like module that interacts with TATA-binding protein and negatively regulates gene transcription (Wang et al. 2008). Somatic mutations in the YEATS2 gene have also been reported in lung (3.8%), colorectal (1.8%), and endometrial (1.8%) cancers in the COSMIC database (Forbes et al. 2011).

Table 2. Inframe fusion genes detected in chondrosarcoma

| Fusion gene     | Sample | Rearrangment type | Chromosome 1 | Position 1 | Chromosome 2 | Position 2 |
|-----------------|--------|-------------------|--------------|------------|--------------|------------|
| PDE1C–MACC1     | CS01T  | Deletion          | chr7         | 20,201,367 | chr7         | 32,263,702 |
| LIP1–FAM176C    | CS03T  | Inversion         | chr21        | 15,528,857 | chr21        | 33,858,432 |
| ACVR2A–FN1      | CS06T  | Inversion         | chr2         | 148,646,674| chr2         | 216,289,052|
| FN1–ACVR2A      | CS06T  | Inversion         | chr2         | 148,646,754| chr2         | 216,289,134|
identified recurrent FN1-ACVR2A fusion transcript, which further suggests a molecular link between chondrosarcoma and osteochondromatosis. ACVR2A encodes a membrane serine/threonine protein kinase which functions as a receptor for the activin and bone morphogenetic proteins 4 and 6 (Donaldson et al. 1992) and is involved in skeletal development (Matzuk et al. 1995). The FN1-ALK fusion transcript has been identified as a potential therapeutic target in ovarian cancer (Ren et al. 2012).

Collectively, our analysis demonstrated that molecular defects in chondrocyte differentiation and epigenetic regulators are synergistically causative of both benign and malignant cartilaginous tumors (Supplemental Fig. S14). A recent study reported the potential efficacy of an IDH1 inhibitor for treating IDH1-mutated tumors (Roehle et al. 2013). Aberrant FN1-ACVR2A gene fusion and recurrent ACVR2A mutations also warrant small-molecular inhibitors targeting activin receptor kinases (Harrison et al. 2005) as potential therapeutic modalities against a subset of chondrosarcoma.

Methods

Clinical samples

The clinical and pathological features of 10 patients and their tumors are shown in Supplemental Table S1. Tissue samples were provided by the National Cancer Center Biobank, Japan. High-molecular-weight genomic DNA and RNA were extracted from fresh frozen tumor specimens and noncancerous muscle tissue. The study protocol was in agreement with the Ethical Committee of the National Cancer Center, Tokyo, Japan.

Whole-genome and transcriptome sequencing

We prepared 300- to 500-bp insert libraries from 3 μg of genomic DNA and 150- to 200-bp insert libraries from 2 μg of total RNA using the TruSeq DNA sample preparation kit and mRNA-Seq sample preparation kit (Illumina, respectively). The libraries were
subjected to paired-end sequencing of 100 bp on the HiSeq 2000 (Illumina) according to the manufacturer's instructions.

Detection of somatic point mutations and short indels
Paired-end reads were aligned to the human reference genome (GRCh37) using the Burrows-Wheeler aligner (BWA) (Li and Durbin 2009). Probable PCR duplications, in which paired-end reads aligned to the same genomic positions, were removed using SAMtools (Li et al. 2009) and a program developed in-house. To find somatic point mutations and short indels, SAMtools was applied with stringent confidence filtering conditions we developed (Totoki et al. 2011). The details of our filtering conditions are described in the Supplemental Information.

Validation of candidate driver genes in an additional cohort
To validate the mutation frequencies of COL2A1, YEATS2, and ACVR2A with recurrent mutations in benign and malignant cartilaginous tumors, we amplified all protein-coding exons of those genes using formalin-fixed, paraffin-embedded (FFPE) DNA from 47 chondrosarcoma, 19 corresponding adjacent nontumor tissues, and 41 enchondroma samples. Six sequencing libraries were prepared from 115 amplicon mixtures of pooled DNA from three chondrosarcomas, pooled normal DNA, and pooled DNA from two enchondromas. The 115 amplicons covered a total of 20 kb of coding regions of the three genes. The six libraries were subjected to paired-end sequencing of 100 bp using an Illumina GA IIx sequencer. Paired-end reads were aligned to the human reference genome (GRCh37) using BWA, and somatic mutations were called using SAMTools (Li et al. 2009) and programs developed in-house. All candidate 137 SNVs and 13 short indels for ACVR2A, COL2A1, and YEATS2 and the mutational hot spots for ID1H and IDH2 were further verified in individual cases by the MassARRAY system (Sequenom). The primer sets, which include a pair of amplicon primers and an extension primer for each SNP, were designed using the MassARRAY Designer software (Sequenom) (Supplemental Table S11). The details of our filtering conditions of the mutation call and the verification by the MassARRAY system are described in the Supplemental Information.

Fusion gene validation by RT-PCR and sequencing
Total RNA was reverse-transcribed to cDNA using SuperScript III (Invitrogen). cDNA was subjected to PCR amplification using Ex Taq (Takara Bio). The PCR products were directly sequenced in both directions by Sanger sequencing using the BigDye Terminator kit (Applied Biosystems).

Data access
Sequence and mutation/indel data have been submitted to the European Genome-phenome Archive (EGA; https://www.ebi.ac.uk/ega/) under accession number EGAS00001000505.

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References

Amary MF, Bacci K, Maggiani F, Damato S, Halai D, Berisha F, Pollock R, O'Donnell P, Grigoriadis A, Duss T, et al. 2011a. IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and peristeal chondromas but not in other mesenchymal tumours. J Pathol 224: 334–343.

Amary MF, Damato S, Halai D, Eskandarpour M, Berisha F, Bonar F, McCarthy S, Fantin VR, Straley KC, Lobo S, et al. 2011b. Ollier disease and Maffucci syndrome are caused by somatic mosaic mutations of IDH1 and IDH2. Nat Genet 43: 1262–1265.

Asp J, Inerot S, Block JA, Lindahl A. 2001. Alterations in the regulatory pathway involving p16, p1b, and cdk4 in human chondrosarcoma. J Orthop Res 19: 149–154.

Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AV, Shomer A, Esgueva R, Pflueger D, Sougnez C, et al. 2011. The genomic complexity of primary human prostate cancer. Nature 470: 214–220.

Bovée JV, Hogendoorn PC, Wunder JS, Alman BA. 2010. Cartilage tumours and bone development: molecular pathology and possible therapeutic targets. Nat Rev Cancer 10: 481–488.

Bowen ME, Boydén ED, Campos Xavier B, Bonafé L, Superti-Furga A, Ikegawa S, Comerrier-Daïe V, Bovée JV, Pansyrui-Ty, et al. 2011. Loss-of-function mutations in PITPN1 cause metachondromatosis, but not Ollier disease or Maffucci syndrome. PLoS Genet 7: e1002050.

Briggs TA, Rice GI, Daly S, Urquhart J, Gornall H, Bader-Meunier B, Baskar K, Baskar S, Baudouin V, Beresford MW, et al. 2011. Tartrate-resistant acid phosphatase deficiency causes a bone dysplasia with autoimmune and a type I interferon expression signature. Nat Genet 43: 127–131.

Cheah KS, Stoker NG, Griffin JR, Grosveld FG, Solomon E. 1985. Identification and characterization of the human type II collagen gene (COL2A1). Proc Natl Acad Sci USA 82: 2553–2559.

Donaldson CJ, Mathews LS, Vale WW. 1992. Molecular cloning and binding properties of the human type II activin receptor. Biochem Biophys Res Commun 184: 310–316.

Esapa CT, Hough TA, Testori S, Head RA, Crane EA, Chan CP, Evans H, Bassett JH, Tylzanski P, McNally EG, et al. 2012. A mouse model for spondyloepiphyseal dysplasia congenita with secondary osteoarthritis due to a Col2a1 mutation. J Bone Miner Res 27: 413–428.

Fletcher CDM, Unni K, Mertens F. 2002. Pathology and genetics of tumours of soft tissues and bone. IARC Press, Lyon, France.

Forbes SA, Bindal N, Bambard S, Cole C, Kok CY, Bearde D, Jia M, Shepherd R, Leung K, Menzies A, et al. 2011. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res 39: D945–D950.

Fujimoto A, Totoki Y, Gornall H, Bader-Meunier B, Baskar K, Baskar S, Baudouin V, Beresford MW, et al. 2011. Tartrate-resistant acid phosphatase deficiency causes a bone dysplasia with autoimmune and a type I interferon expression signature. Nat Genet 43: 127–131.

Cheah KS, Stoker NG, Griffin JR, Grosveld FG, Solomon E. 1985. Identification and characterization of the human type II collagen gene (COL2A1). Proc Natl Acad Sci USA 82: 2553–2559.

Donaldson CJ, Mathews LS, Vale WW. 1992. Molecular cloning and binding properties of the human type II activin receptor. Biochem Biophys Res Commun 184: 310–316.

Esapa CT, Hough TA, Testori S, Head RA, Crane EA, Chan CP, Evans H, Bassett JH, Tylzanski P, McNally EG, et al. 2012. A mouse model for spondyloepiphyseal dysplasia congenita with secondary osteoarthritis due to a Col2a1 mutation. J Bone Miner Res 27: 413–428.

Fletcher CDM, Unni K, Mertens F. 2002. Pathology and genetics of tumours of soft tissues and bone. IARC Press, Lyon, France.

Forbes SA, Bindal N, Bambard S, Cole C, Kok CY, Bearde D, Jia M, Shepherd R, Leung K, Menzies A, et al. 2011. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res 39: D945–D950.

Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, Aoki M, Hosono N, Kubo M, Miya F, et al. 2012. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet 44: 760–764.

Govindan R, Ding L, Griffith M, Subramanian J, Deeds ND, Kanchi KL, Maher CA, Fulton R, Fulton L, Walls J, et al. 2012. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. Cell 150: 1121–1134.

Harrison CA, Gray PC, Vale WW, Robertson DM. 2005. Antagonists of activation signaling: mechanisms and potential biological applications. Trends Endocrinol Metab 16: 73–78.

Hecht JT, Hogue D, Wang Y, Blanton SH, Wagner M, Strong LC, Raskind W, Hansen MF, Wells D. 1997. Hereditary multiple exostoses (EXT): mutational studies of familial EXT1 cases and EXT-associated malignancies. Am J Hum Genet 60: 80–86.

Hong C, Moorfield KS, Jun P, Aldape KD, Kharbanda S, Phillips HS, Costello Jr. 2007. Epigenome scans and cancer genome sequencing converge on WNK2, a kinase-independent suppressor of cell growth. Proc Natl Acad Sci 104: 10974–10979.
Hopyan S, Golgzo N, Poon R, Gensure RC, Yu C, Cole WG, Bell RS, Jüppner H, Andrilis I, Wunder JS, et al. 2002. A mutant PTH/PTHrP type I receptor in enchondromatosis. Nat Genet 30: 306–310.

Hsing AW, Chokkalingam AP. 2006. Prostate cancer epidemiology. Front Biosci 11: 1388–1413.

Koziel J, Kunath M, Kelly OG, Vortkamp A. 2004. Ext1-dependent heparan sulfate regulates the range of Ihh signaling during endochondral ossification. Dev Cell 6: 801–813.

Krzywinski M, Schein J, Briel I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. 2009. Circos: an information aesthetic for comparative genomics. Genome Res 19: 1639–1645.

Larramendy ML, Tarkkanen M, Valle J, Kivioja AH, Ervasti H, Karaharju E, Salmivalli T, Elomaa I, Knuttila S. 1997. Gains, losses, and amplifications of DNA sequences evaluated by comparative genomic hybridization in chondrosarcomas. Am J Pathol 150: 685–691.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754–1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Matzuk MM, Kumar TR, Bradley A. 1995. Different phenotypes for mice deficient in either activins or activin receptor type II. Nature 374: 356–360.

Moniz S, Vennstrom S, Matos P, Brazão R, Silva E, Kotelevets O, Chastre E, Gespach C, Jordan P. 2007. Protein kinase WNK2 inhibits cell proliferation by negatively modulating the activation of MEK1/ERK1/2. Oncogene 26: 6071–6081.

Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, Jones D, Hinton J, Marshall J, Stebbings LA, et al. 2012. Mutational processes molding the genomes of 21 breast cancers. Cell 149: 979–993.

Nishimura G, Haga N, Kitoh H, Kurokawa H, Ando H, Nakashima E, Ohashi H, et al. 2005. The phenotypic spectrum of chondrosarcoma reveals SRC-pathway activity and dasatinib as an option for treatment. Cancer Res 69: 6216–6222.

Turcan S, Biaire-de Bruijn IH, de Miranda NJ, van Oosterwijk TJAM, Taminiau AH, van Wezel T, Hogendoorn PC. Bovée JV. 2009. Kinome profiling of chondrosarcoma reveals SRC-pathway activity and dasatinib as an option for treatment. Cancer Res 69: 6216–6222.

Sgroi DC, Fundele RJ. 1996. Cell cycle regulated transcription of the human type I collagen gene. Cancer Res 56: 2991–2997.

Sørum PH, Krokstad S, Mysterud A. 2010. Mortality from acute lymphoblastic leukemia. Nature 475: 101–105.

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