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Homozygous deletions of cadherin genes in chondrosarcoma—an array comparative genomic hybridization study

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Chondrosarcoma is a malignant bone tumor that is often resistant to chemotherapy and radiotherapy. We applied high resolution oligonucleotide array comparative genomic hybridization to 46 tumor specimens from 44 patients with chondrosarcoma and identified several genes with potential importance for the development of chondrosarcoma. Several homozygous deletions were detected. The tumor suppressor genes *CDKN2A* and *MTAP* were each homozygously deleted in four of the cases, and the *RB1* gene was homozygously deleted in one. Two homozygous deletions of *MTAP* did not affect *CDKN2A*. Deletions were also found to affect genes of the cadherin family, including *CDH4* and *CDH7*, each of which had a targeted homozygous loss in one case, and *CDH19*, which had a targeted homozygous loss in two cases. Loss of the *EXT1* and *EXT2* genes was uncommon; *EXT1* was homozygously deleted in none and *EXT2* in two of the cases, and large heterozygous losses including *EXT1* and/or *EXT2* were seen in three cases. Targeted gains and amplifications affected the *MYC*, *E2F3, CDK6*, *PDGFRA*, *KIT,* and *PDGFD* genes in one case each. The data indicate that chondrosarcomas develop through a combination of genomic imbalances that often affect the RB1 signaling pathway. The inactivation of cadherin genes may also be critical in the pathogenesis of the tumor.

Keywords Chondrosarcoma, array comparative genomic hybridization, cadherin gene, MTAP, RB1 signaling pathway

Chondrosarcoma is a heterogeneous group of malignant tumors characterized by the production of cartilage. The most common subtype is known as *conventional chondrosarcoma,* which accounts for about 85% of all chondrosarcoma cases, and is the third most common primary malignancy of bone, after myeloma and osteosarcoma. Conventional chondrosarcoma is further classified into central and peripheral chondrosarcoma based on the tumor's location in the bone. Central chondrosarcoma arises from the medullary cavity of the bone, whereas peripheral chondrosarcoma originates from the surface of the bone. The central subtype is more common, accounting for about 75% of all chondrosarcomas (peripheral subtype: ~10%) (1–3).

Central chondrosarcomas are usually primary tumors, but some cases arise secondarily from a pre-existing benign enchondroma. Enchondromas are most often solitary lesions but can also occur as multiple lesions in a nonhereditary condition known as enchondromatosis. Peripheral chondrosarcomas, instead, are thought to almost always develop through malignant transformation of a pre-existing benign osteochondroma. Osteochondromas may occur as multiple

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lesions, as seen in the multiple osteochondroma syndrome, which is a hereditary disorder caused by mutations in the exostosin 1 (*EXT1*) or exostosin 2 (*EXT2*) tumor suppressor genes. (1,2,4). However, 85% of osteochondromas present as solitary, sporadic lesions caused by the somatic inactivation of *EXT1* (5,6).

In addition to conventional chondrosarcomas, several rare subtypes of chondrosarcomas are discerned. The most common of these is dedifferentiated chondrosarcoma, which is a high grade non-cartilaginous sarcoma juxtaposed with a malignant cartilage-forming tumor, typically a low grade conventional chondrosarcoma. Both components seem to share identical genetic alterations, with additional genetic changes in the dedifferentiated component (7).

The treatment for chondrosarcoma is surgery, as the tumors are largely resistant to currently available chemotherapy and radiotherapy. Prognosis is primarily based on histologic grade; high grade conventional chondrosarcomas often metastasize, resulting in adverse outcome. The grading of chondrosarcoma suffers, however, from interobserver variability. Therefore, leads for new targeted treatment strategies, as well as for prognostic markers, are urgently needed (1,2).

Studies using cytogenetics and comparative genomic hybridization (CGH) have revealed a wide spectrum of karyotypes in conventional chondrosarcoma, ranging from cases with a single numerical or structural aberration to cases with a very complex karyotype. Nevertheless, the pattern of changes tends to be nonrandom. As the most common subtype, genetic studies have mostly been focused on central chondrosarcoma. Most central chondrosarcomas, especially the high grade tumors, present complex karyotypes, dominated by low level gains and losses of whole or large parts of chromosomes (1,8–11).

Alterations of the RB1 signaling pathway are seen in 96% of high grade central chondrosarcomas (12). Moreover, several genes and pathways (e.g., hedgehog, insulin growth factor) are already under preclinical study as potential therapeutic targets in central chondrosarcoma (2). Three genome-wide array CGH studies on chondrosarcoma have been published to date, two using bacterial artificial chromosome (BAC) arrays and one using 44K oligonucleotide arrays (10,11,13). In this study, we applied 244K oligonucleotide CGH arrays on 44 conventional chondrosarcoma cases.

Materials and methods

Patients and materials

The study was performed on 46 fresh-frozen tumor specimens from 44 patients with conventional chondrosarcoma. Before the analysis, the proportion of neoplastic cells was ensured from frozen sections to be at least 80% in all the specimens. Of the 44 patients, 29 were treated at Helsinki University Central Hospital, Finland, and 15 at Lund University Hospital, Sweden. The clinical data of the patients are shown in Supplementary Table S1. Twenty-four of the tumors were classified as central and six as peripheral conventional chondrosarcomas. Five of the tumors were dedifferentiated chondrosarcomas, and the remaining nine were unclassified. The dedifferentiated non–cartilage-forming component and the cartilage-forming component were not distinguished for the analysis of the dedifferentiated chondrosarcomas. Six of the cases (marked in Supplementary Table S1) were included in a previous study using BAC array CGH (10). In addition, 12 of the cases were previously analyzed by conventional cytogenetic methods (8,10). The karyotypes are shown in Supplementary Table S2. This study was approved by the local ethics committees.

DNA isolation

Genomic DNA was extracted using the standard phenol–chloroform method. Two reference DNAs, one female and one male, were extracted from pools of blood samples (four individuals per pool). The concentration and the quality of DNA were measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and gel electrophoresis.

Array CGH hybridization

Digestion, labeling, and hybridization were performed by following Agilent's protocol version 4.0 for Agilent Human Genome CGH 244A oligonucleotide microarrays, which contain approximately 236,000 probes with a median spacing of 8.9 kb (7.4 kb in RefSeq sequences) (Agilent Technologies, Santa Clara, CA).

DNA digestions were performed on 1.5 μg samples of patient DNA and gender-matched reference DNA. The digested DNA samples were labeled by random priming with Cy3-dUTP (reference DNA) and Cy5-dUTP (patient DNA) using an Agilent Labeling Kit. The labeled DNA samples were purified, combined, and hybridized to the Agilent Human Genome CGH 244A microarrays at 65°C for 40 hours. The hybridized arrays were washed and then scanned using an Agilent scanner.

Data analysis

The array images were analyzed and dye normalization of the data was performed using the Agilent Feature Extraction Software (version 9.5.3.1). The normalized data were analyzed using the ADM2 algorithm in the Agilent DNA Analytics Software (version 4.0) with a user-defined threshold of 6.0. To exclude large aberrations with a low absolute mean ratio, the "fuzzy zero" algorithm was applied. An additional custom-made filter was used to exclude all alterations involving fewer than five consecutive probes, as well as losses and gains with a $\log_2$ ratio between -0.25 and 0.25. Minimal overlapping regions of the most frequent gains and losses were obtained from the Probe Penetrance Summary Report. Homozygous deletions ($\log_2$ ratio < -0.90) and high level gains or amplifications ($\log_2$ ratio > 1.5) were retrieved from the Interval-based Aberration Summary Report. The genomic profiles were compared to known copy number variable regions (Database of Genomic Variants, http://projects.tcag.ca/variation) by visual inspection to exclude the regions that most probably presented copy number variants instead of acquired alterations.