Low-grade Central Osteosarcoma of the Metatarsal Bone: A Clinicopathological, Immunohistochemical, Cytogenetic and Molecular Cytogenetic Analysis

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Abstract. *Low-grade central osteosarcoma (LGCOS) is a very rare low-grade malignant neoplasm that is often confused with a variety of benign fibro-osseous lesions. It rarely involves the small tubular bones of the feet. We present an unusual case of LGCOS arising in the third metatarsal bone of a 16-year-old boy. The radiographic appearance was suggestive of a benign lesion. An open biopsy was performed and the initial diagnosis was fibrous dysplasia. The patient underwent curettage of the lesion and packing of the bony defect with a synthetic bone substitute. Histologically, the curetted specimens consisted of spindle cells admixed with irregular bony trabeculae and osteoid. The spindle cells were fairly uniform with mild atypia, and cellularity varied from low to high. Immunohistochemistry showed that the tumor cells were focally-positive for cyclin-dependent kinase 4 and p53, but negative for murine double minute-2. The MIB-1 labeling index was 36.7% in the highest focus. Cytogenetic analysis exhibited the following clonal karyotypic abnormalities: 48,XY,del(6)(p11),add(8)(q24),add(12)(p11.2),+mar1,+mar-2. Spectral karyotyping demonstrated that marker chromosomes were composed mainly of chromosome 6. Metaphase-based comparative genomic hybridization analysis showed a high-level amplification of 6p12-p21 and gains of 8q21-q24, 10p15, 12q13-q15, and 16q23-q24. Based on these findings, the final diagnosis was revised to LGCOS and the patient was treated with an additional wide excision, followed by reconstruction with a free-vascularized osteocutaneous scapular flap. At 18 months of follow-up, the patient is well with no evidence of local recurrence or distant metastasis. Our case highlights the diagnostic difficulty of this tumor with limited tissue samples and the importance of immunohistochemical and molecular cytogenetic analyses in ambiguous cases.*

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Low-grade central osteosarcoma (LGCOS) is a rare but distinct variant of osteosarcoma, accounting for only 1-2% of all osteosarcomas (1). It tends to occur in the metaphysis of long bones, particularly the distal femur and the proximal tibia, and has a peak incidence in the second and third decades of life with no gender predilection. Patients present with intermittent pain, with or without swelling, usually of long duration. The treatment of choice for LGCOS is wide excision, and there is no role for chemotherapy. Unlike conventional osteosarcoma, LGCOS has a relatively good prognosis when treated appropriately.

Histologically, LGCOS consists of spindle cells arranged in an interlacing pattern. These cells exhibit subtle cytological atypia, and rarely carry mitotic figures. The matrix is produced as well-formed bony trabeculae. In some cases, the bone has a classic 'Chinese character' appearance of fibrous dysplasia. De-differentiation may be found either at the time of initial presentation (2-4) or at recurrence (5) and is associated with a poor prognosis (5). Immunohistochemically, the tumor cells are frequently positive for murine double minute-2 (MDM2) and/or cyclin-dependent kinase-4 (CDK4) (6, 7).

There have been very few cytogenetic studies of LGCOS (8, 9), and no recurrent chromosomal abnormalities have been detected. Metaphase and array-based comparative genomic hybridization (CGH) analyses have demonstrated gain or amplification of 12q13-q15 containing *MDM2* and *CDK4* (7, 10). Amplification of *tetraspanin-31* (formerly known as *SAS*), located on chromosome 12q13, has also been identified in 15% of LGCOS by quantitative polymerase chain reaction (11).

Only a very small number of LGCOS involving the metatarsal bone have been reported in the literature (12-14).

Herein we report an unusual example of LGCOS arising in the third metatarsal bone of an adolescent male with chromosome 6p alterations. We also review the cytogenetic and molecular cytogenetic characteristics of LGCOS, as well as its clinicopathological and immunohistochemical features.

Case Report

A previously good in general health, 16-year-old boy presented with an 18-month history of intermittent pain and swelling in the dorsum of the left foot. There was no history of antecedent trauma. Radiographs demonstrated an expansive lytic lesion in the third metatarsal bone without evidence of cortical destruction or periosteal new bone formation (Figure 1). No cortical destruction or soft tissue extension was seen on computed tomography nor on magnetic resonance imaging. The lesion exhibited intermediate signal intensity on T1-weighted sequences (Figure 2A) and moderately high signal intensity on T2-weighted sequences (Figure 2B). T1-weighted contrast-enhanced fat-suppressed sequences showed marked enhancement of the lesion (Figure 2C). A bone scintigraphy showed markedly increased uptake in the third metatarsal bone corresponding to the lytic lesion. Based on the clinical and radiological features, a benign condition, such as giant-cell reparative granuloma, was strongly suggested.

The patient underwent an open biopsy, and the initial pathological diagnosis was fibrous dysplasia (Figure 3). Intralesional curettage and packing of the bony defect with a synthetic bone substitute were performed. Grossly, the tumor was well-demarcated, firm, and grayish-white. Microscopically, the tumor consisted of spindle cells admixed with irregular bony trabeculae and osteoid (Figure 4A). Hypercellularity, mild cellular atypia, and scattered mitotic figures were observed (Figure 4B). Immunohistochemically, the tumor cells were focally positive for CDK4 (Figure 4C) and p53, but negative for MDM2. The MIB-1 labeling index was 36.7% in the highest focus (Figure 4D). The pathological diagnosis of the curetted specimens was revised to LGCOS.

Cytogenetic analysis revealed a complex karyotype-including marker chromosomes (Figure 5). The karyotype was as follows: 48,XY,del(6)(p11),add(8)(q24), add(12)(p11.2), +mar1,+mar2[3]/48,idem,add(15)(q24),add (20)(p11.2)[2]/46,XY[15]. Spectral karyotyping (SKY) analysis showed that marker chromosomes were mainly composed of chromosome 6 (Figure 6). Metaphase-based CGH analysis showed a high-level amplification of 6p12-p21 and gains of 8q21-q24, 10p15, 12q13-q15, and 16q23-q24 (Figure 7). No significant loss of DNA sequences was found.

Two months after curettage, the patient underwent an additional wide excision, followed by reconstruction with a free vascularized osteocutaneous scapular flap. No chemotherapy was administered. At 18 months of follow-up, the patient is well with no evidence of local recurrence or distant metastasis.

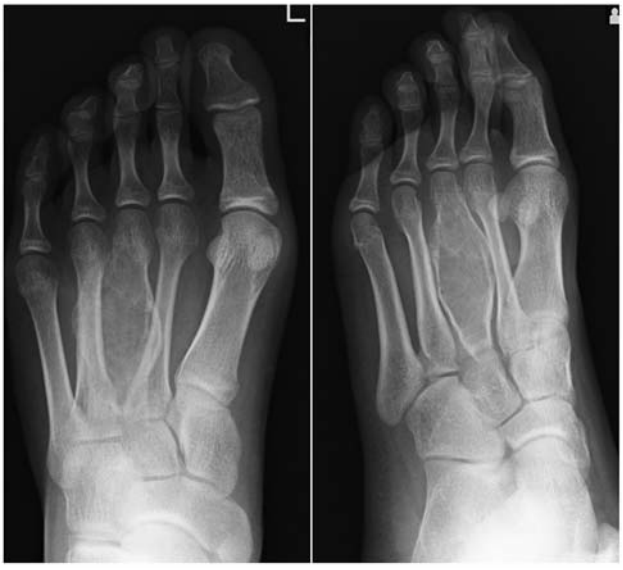

Figure 1. *Plain radiographs of the left foot showing an expansive lytic lesion in the third metatarsal bone, without evidence of cortical destruction.*

Discussion

LGCOS of the metatarsal bone is extremely rare; only four cases have been to date reported in literature (12-14). As in our case, a preoperative diagnosis of benign conditions, such as enchondroma and fibrous dysplasia, was made in two of those cases (12, 14). Moreover, even LGCOS involving the long bones was initially diagnosed as a benign condition in 40% of cases (15). These findings suggest that it may be difficult or impossible to clinico-radiologically distinguish LGCOS from benign fibro-osseous lesions without the presence of some degree of cortical destruction and/or soft tissue extension.

The differential diagnosis of LGCOS includes fibrous dysplasia and desmoplastic fibroma. The absence of cytological atypia and the presence of a permeative growth pattern may help to distinguish LGCOS from similar benign lesions histologically. In the present case, the open biopsy specimen showed a proliferation of plump spindle cells admixed with branching and anastomosing trabeculae of woven bone. There was neither cytological atypia nor mitotic activity. These features resulted in the initial mis-diagnosis of fibrous dysplasia. In fact, there are several reported cases of LGCOS mimicking fibrous dysplasia, where a limited tissue sample is used (15-17).

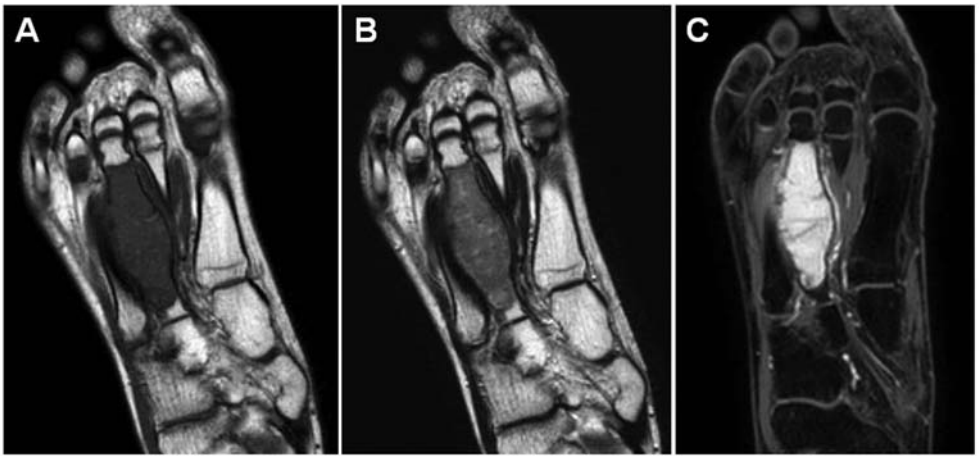

Figure 2. *Coronal magnetic resonance images of low-grade central osteosarcoma involving the third metatarsal bone. The lesion exhibits intermediate signal intensity on T1-weighted sequence (A) and moderately-high signal intensity on T2-weighted sequence (B). T1-weighted contrast-enhanced fat-suppressed sequence reveals marked enhancement of the lesion (C).*

Recent immunohistochemical studies demonstrated that MDM2 and/or CDK4 were expressed in LGCOS, but not in benign fibrous and fibro-osseous lesions (6, 7). The combination of these two markers can therefore serve as a valuable adjunct in the diagnosis of LGCOS. In the present case, we observed that the tumor cells were positive for CDK4, but negative for MDM2. Previous immunohistochemical studies also showed that LGCOS was more commonly positive for CDK4 than MDM2 (6, 11). These observations may be explained by the possibility that CDK4 staining is relatively resistant to de-calcification by acid-based products. On the other hand, Okada *et al*. (18) showed that proliferative cell activity evaluated by MIB-1 staining was significantly higher in LGCOS than in fibrous dysplasia, offering an advantage in differential diagnosis. Park *et al*. (19) also reported that the MIB-1 labeling index was lower in LGCOS than in conventional osteosarcoma. These results indicate that the overall mean MIB-1 labeling index is approximately 10% in LGCOS. In contrast, the present case had a high MIB-1 labeling index of 36.7%.

CGH analyses of LGCOS have revealed the gain or amplification of 12q13-q15 involving *MDM2*, *CDK4*, and *TSPAN31* genes (7, 10). It is interesting to note that the 12q13-q15 amplification is more common in low-grade osteosarcoma than in high-grade osteosarcoma (20, 21). Moreover, the gain of 12q13-q15 sequences has been shown to correlate with the presence of ring chromosomes in parosteal osteosarcoma (22). The amplification of *MDM2* and *CDK4* can lead to the deregulation of the cell cycle and may therefore play an important role in the progression of LGCOS.

Cytogenetic analysis of LGCOS revealed an inv(6)(p23q15) as the sole anomaly (9). In addition, a previous CGH study showed a gain of 6p21 in 33% of LGCOS (10). We also identified a rearrangement and an amplification involving chromosome 6p by SKY and CGH. These findings suggest that chromosome 6p alterations seem to recur in LGCOS. On the other hand, amplification of 6p12-p21 has frequently been observed in conventional osteosarcoma (23-27) and appears to be an early event in its pathogenesis. Interestingly, the gain and amplification of murine chromosome regions homologous to human chromosome 6p have also been reported in conditional mouse models of osteosarcoma (28). Several genes with

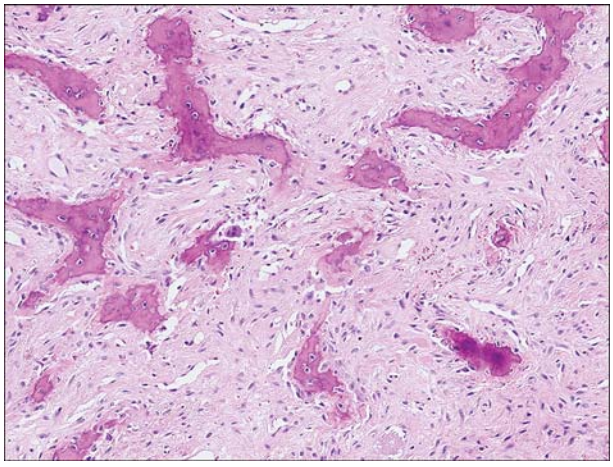

Figure 3. *The open biopsy specimen shows a proliferation of spindle cells admixed with bracing trabeculae of woven bone lacking osteoblastic rimming. There is no evidence of cytological atypia. Based on these features, we first made a diagnosis of fibrous dysplasia.*

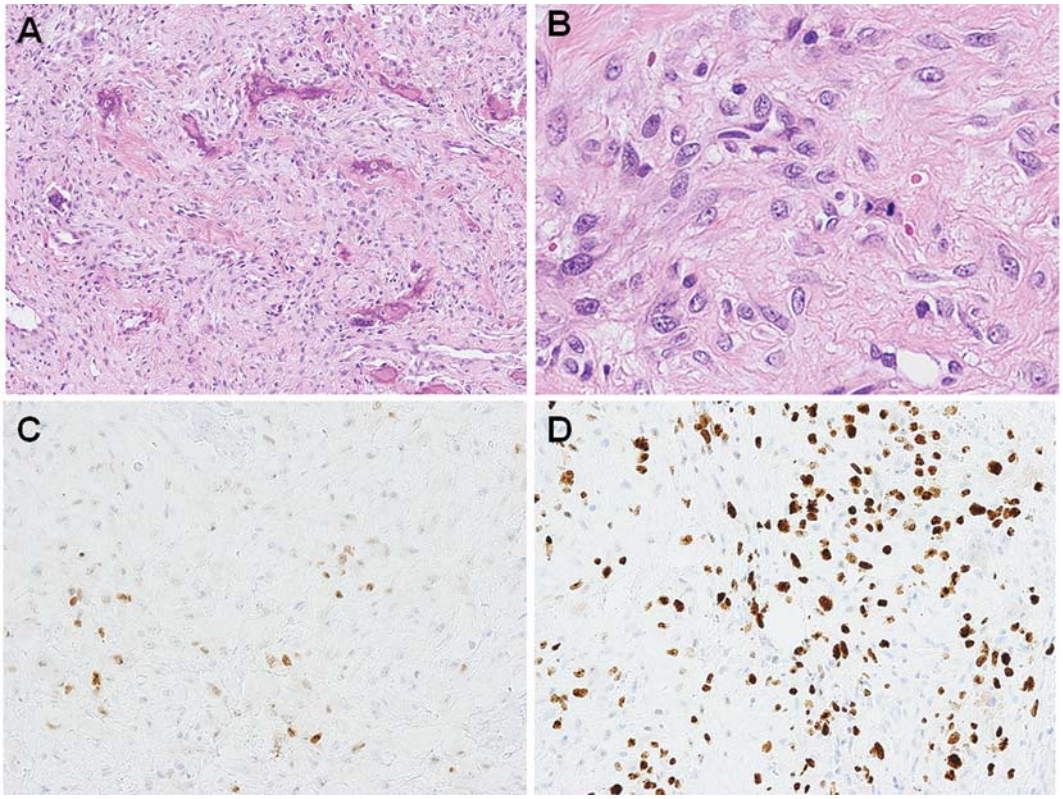

Figure 4. *Histological and immunohistochemical findings of low-grade central osteosarcoma. The tumor is composed of spindle cells admixed with irregular bony trabeculae and osteoid (A). Mild cellular atypia and mitotic activity are found (B). The tumor cells are focally positive for cyclin-dependent kinase-4 (C). The MIB-1 labeling index is 36.7% (D).*

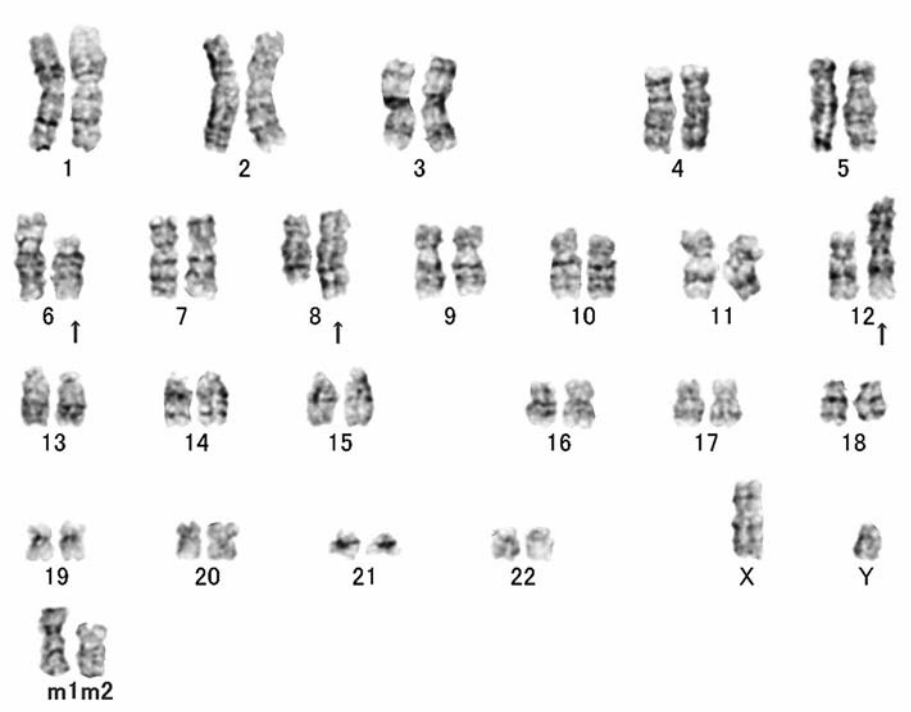

Figure 5. *A representative G-banded karyotype of low-grade central osteosarcoma, including two marker chromosomes. Arrows indicate the structural chromosomal aberrations.*

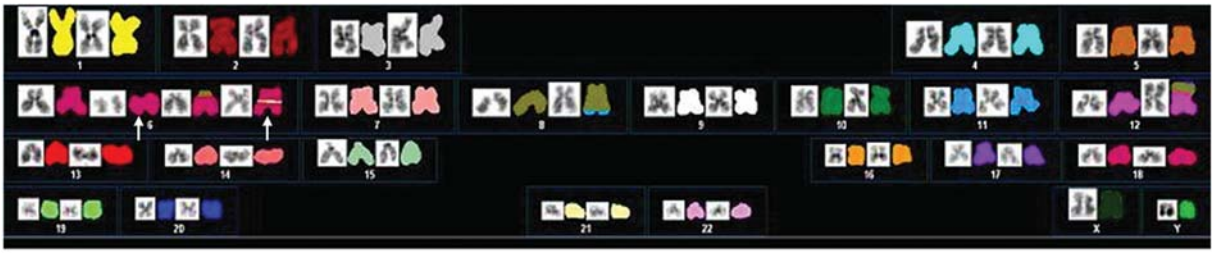

Figure 6. *Spectral karyotyping of low-grade central osteosarcoma illustrating the origins of two marker chromosomes (arrows). A classified image is displayed alongside the reverse 4',6-diamidino-2-phenylindole image.*

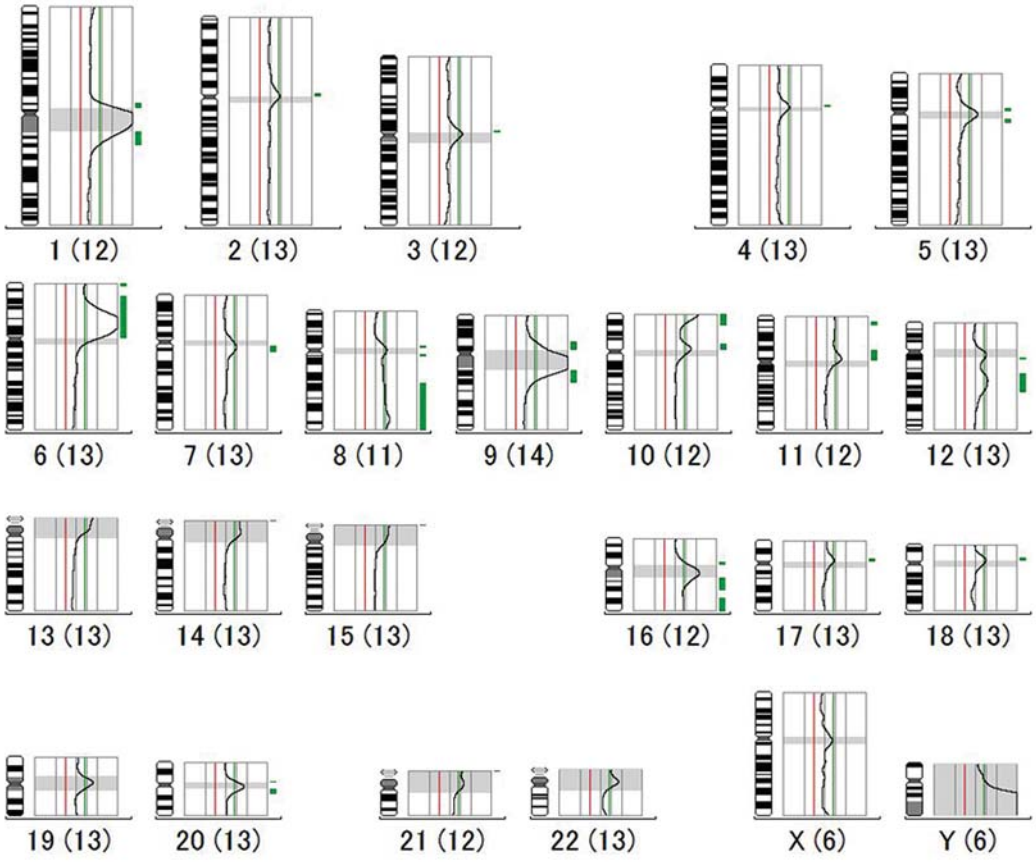

Figure 7. *Comparative genomic hybridization profile of low-grade central osteosarcoma showing a high-level amplification of 6p12-p21 and gains of 8q21-q24, 10p15, 12q13-q15, and 16q23-q24. The line in the middle (gray) is the baseline ratio (1.0); the left (red) and right (green) lines indicate ratio values of 0.8 and 1.2, respectively. Bars on the left (red) and right (green) of each frame indicate losses and gains, respectively. The terminology "1 (12)" represents 12 aberrations detected on chromosome 1; the same applies to other chromosomes shown in the profile.*

oncogenic potential lie within this chromosome region, such as *CDC5 cell division cycle 5-like* (*CDC5L*), *runt-related transcription factor 2* (*RUNX2*), *Cyclin D3* (*CCND3*), *vascular endothelial growth factor A* (*VEGFA*), and *pim-1 oncogene* (*PIM1*). *CDC5L* is a cell cycle regulator important for the G_2/M transition, and its overexpression may promote mitotic entry and shorten the G_2 phase (29). Lu *et al*. (26) suggested that overexpression of *CDC5L* through genomic amplification is likely to lead to aberrant cell-cycle control and may contribute to the malignant phenotype of osteosarcoma. *RUNX2* is a member of the RUNX family of transcription factors and encodes a nuclear protein with a Runt DNA-

binding domain. RUNX2 regulates osteoblast lineage determination and expansion, osteoblast maturation, and terminal differentiation *via* a complex variety of pathways (30). Recently, Sadikovic *et al*. (31) reported that RUNX2 overexpression was correlated with a poor response to chemotherapy in osteosarcoma. *CCND3* is a member of the cyclin-D family and encodes a protein involved in the regulation of the G_1/S transition (32). The amplification of *CCND3* has been reported in other types of cancer (33-35). *VEGFA* is a member of the platelet-derived growth factor/VEGF growth factor family and encodes a protein that is often found as a disulfide-linked homodimer. Its amplification and overexpression have been shown to be poor prognostic factors for survival in osteosarcoma (36, 37). *PIM1* is a proto-oncogene and encodes a serine/threonine protein kinase that is involved in cell proliferation, survival, differentiation, apoptosis, and tumourigenesis (38). PIM1 overexpression has been found in several cancer types (39). However, the precise roles of these amplifications in the pathogenesis and progression of LGCOS remain to be elucidated.

In summary, we have reported a rare case of LGCOS of the metatarsal bone with complex chromosomal rearrangements. Our case highlights the diagnostic difficulty of this tumor with limited tissue samples and the importance of immunohistochemical and molecular cytogenetic analyses in ambiguous cases. Moreover, our results indicate that chromosome 6p alterations may play a critical role in the pathogenesis of LGCOS.

Acknowledgements

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