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Case report

Giant-cell tumor of bone, stage II, displaying translocation t(12;19)(q13;q13)

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Summary. A new case of giant-cell tumour (GCT) of bone with benign histological features, clinical stage II, has been reviewed with immunohistochemistry and electron microscopy. After short-term tissue culture the karyotype, using G-banding techniques, presented a consistent translocation t(12;19)(q13;q13). Nude mice xenografts of the tumour were unsuccessful after 6 months of follow-up. Presence of such chromosomal rearrangement may be related to locally aggressive, histologically benign giant-cell tumors of bone.

Key words: Giant-cell tumor of bone – Chromosome aberrations – Translocation (12;19)

Introduction

Giant-cell tumours (GCT) of bone are not an homogeneous type of tumour; they exhibit diverse biological behavior and vary from completely benign to malignant. Grading of this neoplasm, from the histological and cytological points of view, has been described by several authors (Jaffe et al. 1940; Murphy et al. 1956; Senerkin 1980). In practice, however, this grading has been fallible because the morphological criteria used are to a great extent subjective. Several pathologists (Goldenberg et al. 1970; McGrath 1972) found the system ineffective in distinguishing between these more aggressive GCT and those with a benign course. Therefore other approaches such as clinical and radiographic studies continue to be essential for classifying this entity (Campanacci et al. 1975; Hudson et al. 1984; Present et al. 1986).

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No reports of systematic cytogenetic analysis of GCT of bone have appeared hitherto in the literature. In the pre-banding era, one case of human GCT with a chromosome mode at 46 and superficially normal diploid karyotype was described (Ishii et al. 1978).

Here we present a new case of GCT of bone in stage II showing benign histology but cortical invasion and breakthrough of the bone cortex in the distal femur of a 36 year-old female. We were able to karyotype, using trypsin-Giemsa banding, in what we believe is the first bona fide case of aggressive human GCT of bone.

Case report

The patient was a 36 year-old female, who for three months experienced weakness and pain in the right knee with progressive swelling, following a fall. Roentgenograms showed an expanding and varying radiolucent area situated eccentrically at the distal epiphysis of the right femur. A well-marginated thin shell of reactive bone covered the cortex but some lytic foci were detected (Fig. 1a). The CT scan showed a lytic lesion with mixed density and apparent focal soft tissue extension. The adjacent subcondral plate was preserved. A systematic search for distal metastases with CT scan and bone scintigrams proved negative. A block resection of the lower end of the right femur was performed and a modular prothesis (type Kötz) was applied. Twelve months after surgery the recovery is complete and no signs of local recurrence or distant metastases have been detected.

Material and methods

Small representative tumour fragments were paraffin-embedded (formalin fixed). Several histological stains were made: hematoxylin-erithrosine-eosine, PAS (periodic acid Schiff) with and without treatment with diastase, Masson's trichrome and Gomori's silver impregnation. Several immunoperoxidase techniques were carried out following the peroxidase-antiperoxidase (PAP) or avidine-biotine complex (ABC) methods. We used polyclonal rabbit antisera for vimentin (1/30), desmine (1/200), neuronal specific enolase (1/200), alpha-1-antitrypsin (1/500),

alpha-1-antichimotrypsin (1/500), myoglobin (1/200) and fibronectin (1/200).

Tissue for TEM was fixed in glutaraldehyde, postfixed in osmium tetroxide and examined at 60–80 kv in a JEOL 100B electron microscope.

The tumour was also processed for in vitro culture studies. Tissue was minced and enzymatically disaggregated for 1 h at 37° C with 2 mg/ml of collagenase II (200 U/mg Cooper Biomedical). Cultures were initiated in Flaskettes (25 cm^2 culture flasks, Nunk) in RPMI 1640 medium to which was added 20% fetal bovine serum, 1% glutamine and 1% penicillin/streptomycin and incubated at 37° C in a humidified atmosphere with 5% CO_2 in air.

For cytogenetic analysis the cultures were harvested from 7 to 27 days. One hour before harvesting, 0.01 mg/ml colcemid was added to the culture medium, and hypotonic-trypsin-versene was used for 10 minutes to detach the cells, followed by a 10 minute exposure to 0.075 KCl and fixation in methanol/glacial acetic acid (3/1). Air-dried slide preparations were made by standard techniques. Karyotype analysis was performed using GTG-technique. Each well-banded metaphasis was karyotyped according to the ISCN (1985).

Results

Macrosections of the resected tumour were evaluated. A thin reactive fibrous capsule was present. The tumour eroded the cortex and foci of soft-tissue extension were observed. No articular cartilage infiltration was found but there was extension into the medullary canal. The muscles of the thigh were not involved in the neoplastic growth.

Several specimens of the neoplastic tissue were reviewed for histology. In the well-preserved areas the typical histological pattern was characterized by a large number of multinucleated giant-cells, homogeneously distributed (Fig. 1 b). All displayed the well-known osteoclastic cell type. No atypia was seen in such cells. The second cell type was represented by stromal cells which possessed an oval or spindle shaped contour. Isolated mitotic figures were observed among these cells but in low number (3 by 10 HPF); we could not observe stromal cell atypism but occasionally they possessed two nuclei. Other cells intermingled with these possessing an histiocytic appearance (Fig. 2a).

Neovascularisation was extensive and extravasation of erythrocytes and haemosiderin pigment was common. We found no vascular permeation by the tumour cells. Sarcomatous areas of well-differentiated fibrosarcoma with herring-bone cellular arrangement was lacking, as were patterns of malignant fibrohistiocytoma with pinwheel-storiform configurations. Reactive osteogenesis was detected focally in peripheral areas of the tumour but no malignant osteoblastic fields were seen.

Immunohistochemistry of the neoplasm showed activity of both stroma and giant multinucleated cells to vimentin. Stromal reactive histiocytes were positive for alpha-1-antitrypsin and alpha-1-antichimotrypsin, while the mononucleated stroma cells remained negative. Some positive

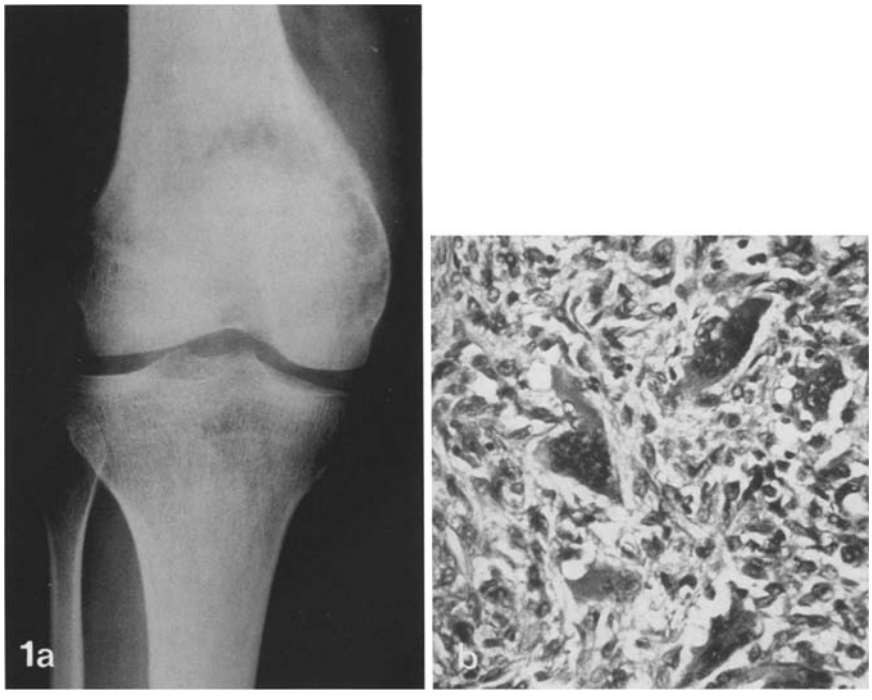

Fig. 1. a Roentgenogram of the knee showing a lytic lesion involving most of the condyle and thinning the cortex. Presence of a marginated shell of reactive bone. **b** Histological features of the neoplasm. Presence of giant-cells intermingled with fibroblast-like stromal cells (HE ×400)

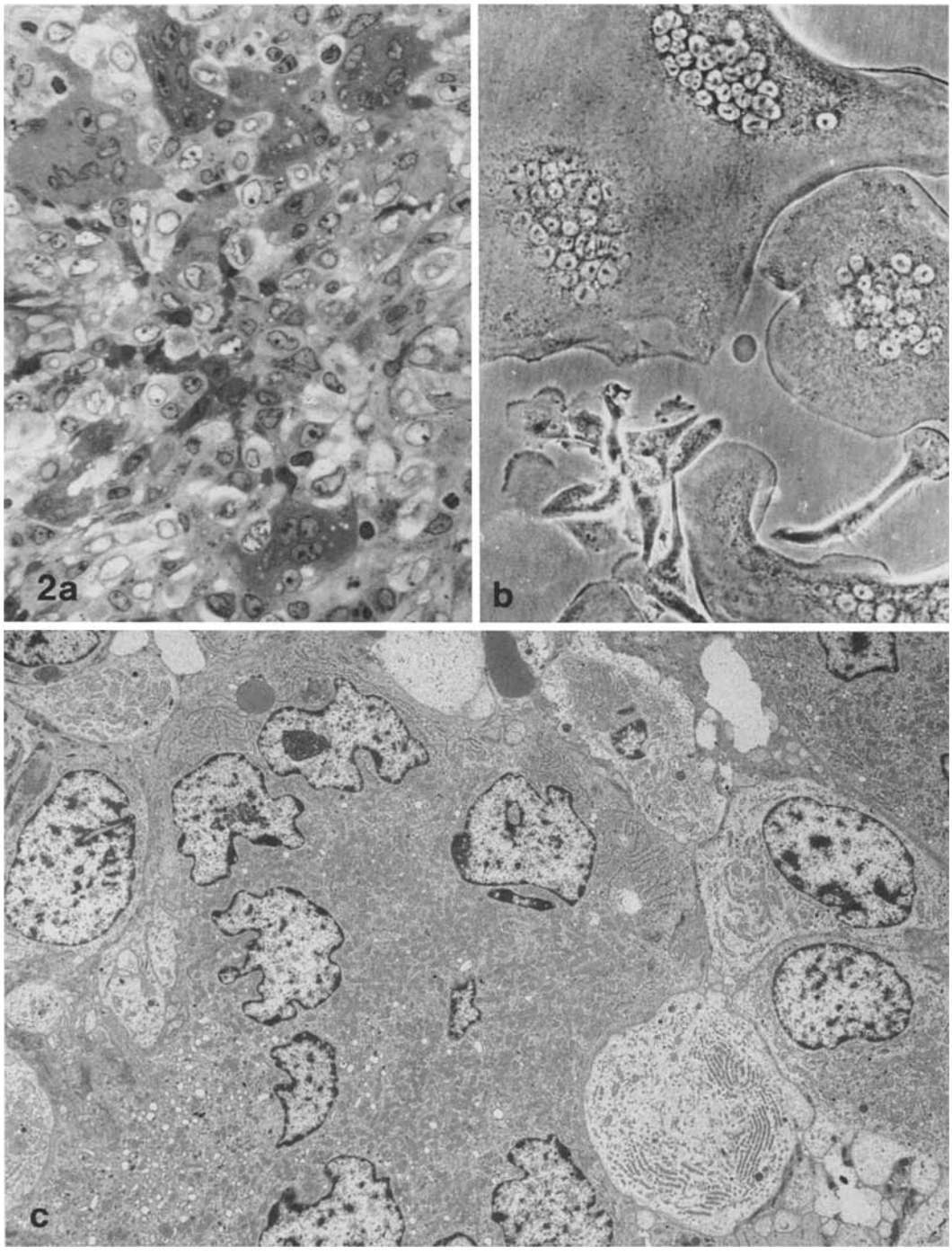

Fig. 2. **a** Tissue section of the giant-cell tumor of bone showing typical multinucleated giant cells associated with stromal cells (Toluidine blue, ×750). **b** Primary tissue cultures after 2 day-old culture with the presence of both types of giant-cells intermingled with the mononuclear cells of fibroblastic-like appearance. (Phase contrast, ×750). **c** Low power electron micrograph of the neoplasm. The GC cytoplasm contains a great number of mitochondria and sparse rough endoplasmic reticulum, the latter usually located at the periphery; several histiocyte-like and mesenchymal looking-cells are also present. (×4000)

staining for alpha-1-antichimotrypsin was occasionally found in the large multinucleated giant cells. Other reactivities were negative.

The ultrastructural studies of the neoplasm confirm previous analyses (Aparisi et al. 1977). Three types of cells could be differentiated: multinucleated giant cells similar in appearance to osteoclasts; mononuclear stromal cells with a fibroblast-like structure being spindle-shaped and provided with a very irregular outline; and macro-

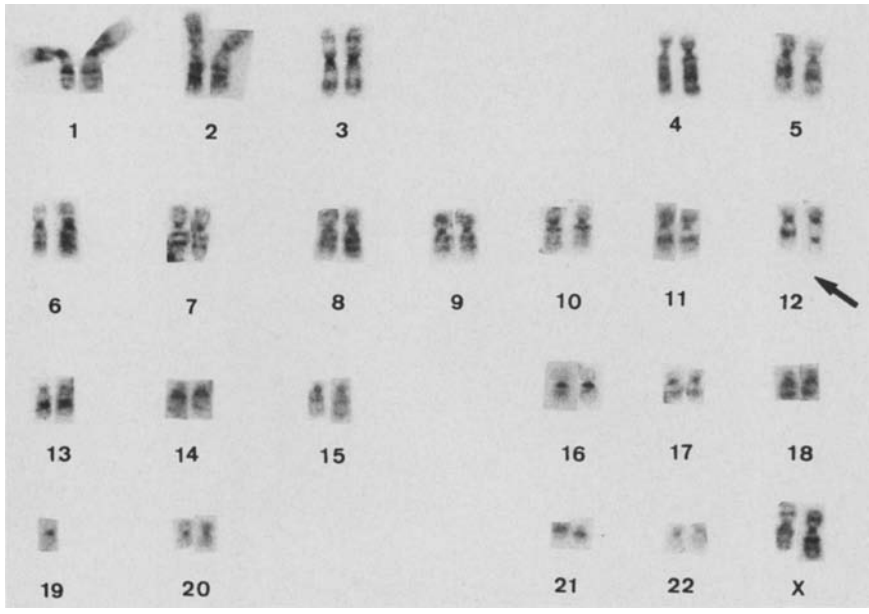

Fig. 3. G-banding of short-term cultured tumoral cells. Representative karyotype 45,XX, −12, −19, +t(12;19)(q13;q13). *Arrow* indicates the t(12;19)

phages of connective tissue type of histiocytic-like character. In addition to the stromal cells there were other cells exhibiting features of lymphocytes. High endothelial capillaries were seen irregularly distributed within the tumour, lacking any close relationship with the tumour cells (Fig. 2c).

In short-term tissue cultures the cells grew quickly and attached as monolayers on the first day after retrieval. The cultures survived for 48 days following 4 subcultures. Three major cell types were identified based on their morphological features: the main population was made up of mononuclear cells of fibroblastic-like appearance but some epithelioid histiocytes were also seen. A large number of giant-cells spread in between. Two types of multinucleated giant cells were observed: those with clear large cytoplasm with several well-delimited small nuclei and contracted ones with clumped conglomerated nuclei centrally or laterally located (Fig. 2b). Both types of multinucleated giant-cells appeared within 8 to 12 h and survived up to 21 days in primary tissue culture only. Several mitoses were seen in different stages of progression. Transitional variations from mononuclear to multinucleated cells could be observed.

For karyotyping a total of 34 cells from 7, 8 and 27 day-old cultures were analysed based on G-banding. The cells showed the following karyotypes: 45,XX, −12, −19, +t(12;19)(q13;q13) in 5 metaphases (Fig. 3). 44,XX, −19, −21, −22, + t(12;19)(q13;q13) in 10 metaphases; the other 19 metaphases were all normal.

A xenograft into three 6 week-old nude mice (nu-nu/bal b-c) of the original neoplasm was made six h after surgery. In a follow-up of six months, the subcutaneously implanted tumor was unsuccessful.

Discussion

The prediction of the biological behavior of a neoplasm is mainly based upon histological and cytological criteria.Nevertheless some tumors, as is the case for GCT of bone, may not express sufficient morphological features to allow histological grading as proposed by Jaffe et al. (1940), because the exact delimitation between the benign (grade I) and the locally malignant (grade II) remains artificial, no clear-cut delimitation can be fixed. While the number of primary malignant (grade III) sarcomatous GCT is quite low (Hutter et al. 1962; McGrath 1972; Nascimento et al. 1979; Senerkin 1980; Present et al. 1986) there are several communications in which an indistinct conventional-looking GCT has produced local recurrences and metastases (Murphy and Ackerman 1956; Jewell and Bush 1964; Pan et al. 1964; Goldenberg et al. 1970; Spjut 1971; Trifaud and Chaix 1975; Joly et al. 1984). Diverse morphological techniques, such as enzyme-histochemistry and immunohistochemistry as well as electron microscopy have clarified the histogenesis of this tumour in part, but have afforded little information regarding its grading (Joshida et al. 1982).

The case here discussed belongs to the category of benign-looking GCT (grade I). No signs of histological malignancy were found: the number of mitoses remained low and no atypia was ob-

served in the stromal cells. A point of reference for an aggressive behavior was clinically detected by radiographic and CT scanning because of the demonstration of an infiltrated periostal cortex with focal breakthrough of the cortical bone and initial soft-tissue extension. This fact was confirmed histologically. Following the criteria of Present et al. (1986) we classified this neoplasm clinically as stage II.

Numerical and structural chromosomal changes have been established in the last few years as consistent abnormalities in malignant cells of sarcomas in soft tissue and bone (Sandberg and Turc-Carel 1987). Although the number of cases is still very low, specific translocations have been found in several neoplasms: t(11;22)(q24;q12) in Ewing's sarcoma (Aurias et al. 1983; Turc-Carel et al. 1988), t(X;18)(p11;q11) in synovial sarcoma (Turc-Carel 1986a; Noguera et al. 1988) and t(12;16)(q13;p11) in myxoid liposarcoma (Turc-Carel et al. 1986; Mertens et al. 1987). In this case we observed a chromosomal rearrangement, t(12;19)(q13,p13) which has not been reported to date. One of the break points of this translocation, however, is preferentially involved in other types of neoplasms. Rearrangements of the 12 q13–15 have been communicated both in benign and malignant tumours: salivary mixed benign tumours show such rearrangements (Mark et al. 1982; Mark and Dahlenfors 1986), as do uterine benign leiomyomas (Heim et al. 1988; Gibas et al. 1988). Furthermore structural changes involving 12q13–14 have been reported repeatedly in benign lipomas (Turc-Carel et al. 1986b; Mandahl et al. 1987). In the malignant lipogenic tumour (myxoid liposarcoma) the involvement seems to be restricted to t(12;16)(q13;p11) (Turc-Carel et al. 1986; Mertens et al. 1987).

Therefore we agree with Heim et al. (1988) that the finding of an 12q-involvement in several types of neoplasms, including the GCT of bone, is compatible with the suspicion that 12q13–15 contains a rather non-specific neoplasia-associated gene.

Additional cases of GCT of bone have to be studied by cytogenetic methods in order to ascertain the frequency of the present translocation and its relationship to aggressivity (clinical stage and histological grading). Previous observations in our laboratory on two additional benign GCT (stage I) provided a normal chromosomal pattern of 46 and no rearrangements. Moreover an assay in order to ascertain the malignancy of the present case using nude-mice xenotransplants of the original neoplasm failed after 6 months of observation, as also occurred with the two other GCT possessing a normal chromosomal composition. This fact excludes a sarcomatous degeneration of the tumor (Giovanella et al. 1974).

From the present observations we hypothesize the existence of an intermediate grade of GCT with local aggressive behavior, lacking in morphological (optical and EM) specific peculiarities but with a consistent chromosomal rearrangement. Tissue culture and cytogenetic analysis are important complementary tools for the biological diagnosis and for prognosis of such tumours, and their use should be encouraged in pathological laboratories.

References

Jaffe HL, Lichtenstein L, Portis RB (1940) Giant cell tumor of bone: Its pathologic appearance, grading, supposed variants and treatment. Arch Pathol 30:993–1031

Murphy WR, Ackerman LV (1956) Benign and malignant giant-cell tumors of bone. A clinical-pathological evaluation of thirty-one cases. Cancer 9:317–339

Hutter RVP, Worcester JN Jr, Francis KC, Foote FW Jr, Stewart FW (1962) Benign and malignant giant cell tumors of bone: A clinicopathologic analysis of the natural history of the disease. Cancer 15:653–690

Jewell JH, Bush LF (1964) "Benign" giant-cell tumor of bone with a solitary pulmonary metastasis: A case report. J Bone Joint Surg 46-A:848–852

Pan P, Dahlin DC, Lipscomb PR, Bernatz PE (1964) "Benign" giant-cell tumor of the radius with pulmonary metastasis. Proc Staff Meet Mayo Clin 39:344–349

Goldenberg RR, Campbell C, Bonfiglio M (1970) Giant-cell tumor of bone: An analysis of two hundred and eighteen cases. J Bone Joint Surg 52A:619–664

Spjut HJ, Dorfman HD, Fechner RF, Ackerman LV (1971) Giant cell tumor. In tumors of bone and cartilage. Atlas of tumor pathology, second series, Fascicle 5. Washington, DC, Armed Forces Institute of Pathology, pp 293–315

McGrath PJ (1972) Giant cell tumor of bone: An analysis of 52 cases. J Bone Joint Surg 54-B:216–229

Giovanella BC, Stehlin JS, Williams LJ (1974) Heterotransplantation of human malignant tumors induced by cell cultures derived from human solid tumors. JNCI 52:921–930

Campanacci M, Giunti A, Olmi R (1975) Giant cell tumors of bone: A study of 209 cases with long-term follow-up in 130. Ital J Orthop Traumatol 1:249–277

Trifaud A, Chaix C (1975) Particularites des metastases pulmonares des tumeurs a cellules geantes. Rev Chir Orthop 61:391–413

Aparisi T, Arborgh B, Ericsson JLE (1977) Giant cell tumor of bone: Detailed fine structural analysis of different cell components. Virchows Arch [A] 376:273–298

Ishii S, Yamawaki S, Sasaki T, Usui M, Ubayama Y, Minami A, Yagi T (1978) Tissue culture studies on human giant cell tumour and osteosarcoma. Int Orthop (SICOT) 2:77–86

Nascimento AG, Huvos AG, Marcove RC (1979) Primary malignant giant cell tumor of bone: A study of eight cases and review of the literature. Cancer 44:1393–1402

Sanerkin NG (1980) Malignancy, aggressiveness and recurrence in giant cell tumor of bone. Cancer 46:1641–1649

Joshida H, Akeho M, Yumoto T (1982) Giant cell tumor of bone: Enzyme histochemical, biochemical and tissue culture studies. Virch Arch [A] 395:319–330

Mark J, Dahlenfors R, Ekedahl C, Stenman G (1982) Chromosomal patterns in a benign human neoplasm, the mixed salivary gland tumour. Hereditas 96:141–148

Aurias A, Rimbaut C, Buffe D, Dubousset J, Mazabraud A (1983) Chromosomal translocation in Ewing's sarcoma. N Engl J Med 309:496–497

Hudson TM, Schiebler M, Springfield DS, Enneking WF, Hawkins IF Jr, Spanier SS (1984) Radiology of giant cell tumors of bone: Computed tomography, arthrotomography and scintilography. Skel Radiol 11:85–95

Joly MA, Vazquez JJ, Martinez A, Guillen FJ (1984) Bloodborne spread of a benign giant cell tumor from the radius to the soft tissue of the hand. Cancer 54:2564–2567

ISCN (1985) An International System for Human Cytogenetic Nomenclature, Harnden DB and Klinger HP (eds); published in Collaboration with Cytogenet Cell Genet (Karger, Basel, 1985); also in Birth Defects: original Article Series, Vol. 21, No. 1 (March of Dimes Birth Defects Foundation, New York, 1985)

Mark J, Dahlenfors R (1986) Cytogenetical observations in 100 human benign pleomorphic adenomas: specificity of the chromosomal aberrations and their relationship to sites of localized oncogenes. Anticancer Res 6:299–308

Present D, Bertoni F, Hudson T, Enneking WF (1986) The correlation between the radiologic staging studies and histopathologic findings in aggressive stage 3 giant cell tumor of bone. Cancer 57:237–244

Tur-Carel C, Dal Clin P, Limon J, Li F, Sandberg A (1986a) Translocation X;18 in synovial sarcoma. Cancer Genet Cytogenet 23:93

Turc-Carel C, Dal Cin P, Rao U, Karakousis C, Sandberg AA (1986b) Cytogenetic studies of adipose tissue tumors. I. A benign lipoma with reciprocal translocation t(3;12)(q28;q14). Cancer Genet Cytogenet 23:283–289

Turc-Carel C, Limon J, Dal Cin P, Rao U, Karakousis C, Sandberg AA (1986) Cytogenetic studies of adipose tissue tumors. II. Recurrent reciprocal translocation t(12;16)(q13;p11) in myxoid liposarcomas. Cancer Genet Cytogenet 23:291–299

Mandahl N, Heim S, Johansson B, Bennet K, Mertens F, Olsson G, Rooser B, Rydholm A, Willen H, Mitelman F (1987) Lipomas have characteristic structural chromosomal rearrangements of 12q13–q14. Intl J Cancer 39:685–688

Mertens F, Johansson B, Mandahl N, Heim S, Bennet K, Rydholm A, Willen H, Mitelman F (1987) Clonal chromosome abnormalities in two liposarcomas. Cancer Genet Cytogenet 28:137–144

Sandberg AA, Turc-Carel C (1987) The cytogenetics of solid tumors. Relation to diagnosis, classification and pathology. Cancer 59:387–395

Gibas Z, Griffin CA, Emamel BS (1988) Clonal chromosome rearrangement in a uterine myoma. Cancer Genet Cytogenet 32:19–24

Heim S, Nilbert M, Vanni R, Floderus U-M, Mandahl N, Liedgren S, Lecca V, Mitelman F (1988) A specific translocation, t(12;14)(q14–15;q23–24), characterizes a subgroup of uterine leiomyomas. Cancer Genet Cytogenet 32:13–17

Noguera R, Lopez-Gines C, Gil R, Carda C, Pellin A, Llombart-Bosch A (1988) Translocation (X:18) in a synovial sarcoma: A new case. Cancer Genet Cytogenet 33-2:311

Turc-Carel C, Aurias A, Mugneret F, Lizard S, Sidaner I, Volk C, Thiery JP, Olschwang S, Philip I, Berger MP, Philip T, Lenoir GM, Mazabraud A (1988) Chromosomes in Ewing's sarcoma. I. An evaluation of 85 cases and remarkable consistency of t(11;22)(q24;q12). Cancer Genet Citogenet 32:229–238

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