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Telomeric Associations and Consistent Growth Factor Overexpression Detected in Giant Cell Tumor of Bone

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Abstract

Tum or specimens from 15 patients with giant cell tumor (GCT) of bone were cytogenetically analyzed. A subset of five individuals had tumor cells harvested and polyadenylated RNA isolated. Multiple Northern blots were performed utilizing radiolabeled probes for the growth factors TGFβ1, TGFβ2, TGFβ3, and TGFα (TGF, transforming growth factor). RNAs from other types of neoplasms and nonneoplastic cells were examined as controls. The most consistent cytogenetic abnormality detected involved multiple telomeric associations (TAs), most frequently involving the terminus of the long arm of chromosome 19 (19q). Northern blot analysis revealed a consistent expression of TGFβ1 and TGFβ2 with an inconsistent mRNA expression for the other TGFs. There was a relative overexpression of mRNA for TGFβ2. The gene location for TGFβ1 is near the 19q terminus and thus it is speculated that TGFβ may play a role in the neoplastic transformation of GCT.

INTRODUCTION

Giant cell tumor (GCT) is a benign, primary bone neoplasm that has an unpredictable pattern of biologic aggressiveness. This neoplasm has a proclivity to recur locally, and demonstrates the ability for benign pulmonary metastases on rare occasions. Surgical resection is the mainstay of therapeutic intervention. Recent large retrospective series reviewing GCT have demonstrated a reduction in the local recurrence rate from 40—60% in the past, to under 25% currently [1–6]. The histologically documented benign pulmonary metastasis rate remains unchanged at 2% [3, 7], These studies suggest that local recurrence is inversely proportional to the adequacy of the surgical margin. The further the plane of dissection from the tumor, the lower the recurrence rate. Unfortunately, GCT frequently occurs around major joints, such as the knee in young adults. W ide surgical resections performed in an attempt to lower the recurrence rate therefore often necessitate compromising musculoskeletal performance and function.

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Clearly, not all GCTs recur, even after intralesional procedures. Histology and radiology alone or in combination do not accurately predict which GCTs are biologically aggressive and thus dictate the margin of resection required for tumor eradication [2, 3, 5, 6,8–10], Other diagnostic modalities, such as flow cytometry, have similarly yielded poor prognostic results [11–16].

Initial cytogenetic investigations of GCT have supported generalized chromosomal instability manifested by multiple telomeric associations [17]. Specific assays for transforming growth factor beta (TGFβ) have indicated high levels of TGFβ in GCT [18]. The purpose of this study is to carry out the cytogenetic and growth factor analyses of a large series of GCTs in an effort to improve our understanding of the underlying neoplastic event in GCT, and possibly provide guidelines to suggest which tumors are clinically more aggressive.

MATERIALS AND METHODS

Specimens

Fifteen patients with histologically documented GCT of bone had 0.5–2.0 cubic cm of fresh tumor intraoperatively harvested and sterilely transported to the cell culture laboratory between January 1987 and October 1989. Clinical, radiographic, and surgical data were recorded on each individual. Sterile cellular suspensions were prepared and cultured as previously described [17].

Cytogenetics

Metaphases were stained for chromosomal analysis with Quinacrine mustard or Giemsa after trypsinization. Thirty consecutive metaphases were analyzed and photographed from each specimen. Slides that were cultured the fewest number of days were examined first. No cells examined were cultured longer than 3 weeks.

Nonrandom translocations involving the break and fusion of chromosomal telomeres were termed telomeric associations and were frequently observed. The precise relationship between the telomeres of two individual chromosomes at this “association” has not been clearly elucidated. An abnormal clone was defined as the occurrence in the same sample of two or more metaphases with the same chromosomal abnormality.

Competitive Binding Assay

Competitive binding assays for TGFβ were performed as described previously [19], AKR-2B (clone 84A) cells were utilized as indicator cells. Acid-activated, serum-free, conditioned media from cultured tumor cells of two patients were incubated with the indicator cells for 2 hours at room temperature with ^{125}I-TGFβ2. After binding, the cells were washed three times with PBS containing 0.1% BSA, followed by a 10-minute incubation with PBS containing 1% Triton X-100 to lyse the cells. The cell lysate was counted in a liquid scintillation counter. This assay will detect TGFβ1, TGFβ2, and TGFβ3 [20].

Northern Blot Analysis

Polyadenylated mRNA was isolated from GCT cells derived from long-term cultures. The cells were lysed and DNA mechanically sheared. The lysate was treated with 100 μg/ml proteinase K at 37°C for 30 minutes. Poly (A^+) RNA was collected following oligo-DT cellulose column chromatography. Two to five micrograms of polyadenylated mRNA was electrophoresed in a formaldehyde/agarose gel. The RNAs were transferred to nitrocellulose and Northern hybridizations were carried out under high stringency conditions [21] using ^{32}P-labeled murine TGFβ1 cDNA probes, ^{32}P-labeled riboprobes for murine TGFβ2, TGFβ3, and a human TGFα [22, 23].

RESULTS

The clinical data on the 15 patients whose tumor cell specimens were cytogenetically studied are listed in Table 1. The patients' ages, genders, and the locations and treatments of their GCTs are characteristic of this neoplasm. One patient presented with pulmonary metastases (number 4), and one patient presented with locally recurrent disease after multiple operative procedures (number 9). Adjunctive therapy consisting of external beam radiotherapy was utilized in only one patient (number 12). No patient received preoperative radiotherapy. The clinical follow-up ranges from 2 to 4 years. No locally recurrent GCT has been detected in any patient.

Cytogenetics

The cytogenetic analyses of the 15 patients studied are presented in Table 2. Clonal and nonclonal abnormalities are listed. No structural clonal changes were found to be identical in any more than two patients. Telomeric associations (TAs) were technically classified as nonclonal because no two metaphases in the same specimen had the same abnormality. However, the TAs seemed to be nonrandom because certain telomeres seemed to be predominantly involved.

Telomeric associations were detected in several metaphases obtained at direct harvest. One cell might contain multiple copies of a specific TA (e.g., a tetraploid cell) or a diploid cell might contain many TAs, each involving different termini. TAs were tabulated so that only two termini of any chromosome could be involved in TAs for any given cell, and for any given specimen. Termini that were involved in only one TA were discounted. In this manner, it was hoped that a conservative estimate of TA frequency would be reported and the possibilities of random associations minimized.

Eleven of fifteen patients demonstrated TAs by the above criteria. Examples of representative TAs are shown in Figure 1. Figure 2 is a histogram summarizing the occurrences of specific chromosomal termini involved in TAs following the above criteria. The terminus of the long arm (qter) of chromosome 19 was most frequently involved.

Competitive Binding

Mouse AKR-2B (clone 84A) cells have been shown to have high affinity and saturable binding sites for the TGFβs [19]. The binding of the TGFβs to surface receptors is

responsible for their biologic activity. The serum-free conditioned media of two individuals' (numbers 7 and 8) GCTs were both shown to displace radiolabeled TGFβ from its receptors on AKR-2B cells in a saturable manner (Figure 3). Because standard curves with pure TGFβ1 give 50% competition with 1 ng/ml, the GCTs are estimated to produce 3–5 ng/ml of TGFβ1, TGFβ2, and/or TGFβ3.

Northern Blot Analysis

Northern blots were prepared and hybridized with probes for TGFα, TGFβ1, TGFβ2, and TGFβ3. The gels were loaded with a variety of combinations of polyadenylated mRNA from five patients (numbers 7, 8, 11, 12, and 13) and several neoplastic and nonneoplastic control cell lines. Patient 11's muscle-derived fibroblasts were grown separately in culture, and proved to be cytogenetically normal. Using twice the amount of mRNA (4 μg) in the Northern blot analysis, the normal fibroblasts had no detectable signal with any of the four probes. Other control cell lines utilized for lane comparisons were: AKR-2B mouse embryo fibroblasts; HT1080, a human fibrosarcoma; BSC-1 green monkey kidney epithelial cells; human pancreatic carcinoma cells; and gastrointestinal epithelial cells.

The results of the Northern assays revealed that TGFβ1 and TGFβ2 expression was reproducibly detected in all the GCT cell lines. Figure 4 is a Northern blot probed with TGFβ1 demonstrating greater expression of TGFβ1 mRNA in the tumor cell lines as compared to rapidly growing control AKR-2B cells. TGFβ2 expression was compared using neoplastic and nonneoplastic cell lines (Fig. 5). This Northern blot demonstrates stronger TGFβ2 signal intensity in the GCT cell lines as compared to that seen in the normal gastrointestinal epithelium or in pancreatic tumor cell line mRNAs. In GCT, TGFβ2 mRNA expression was similar to that seen in BSC-1 cells, a cell line that markedly 6verexpresses this factor [24]. Figure 6 shows TGFβ2 expression in both GCT tumor cell lines of intensities comparable to that seen in a fibrosarcoma (HT1080). A comparison of TGFα, TGFβ1, TGFβ2, and TGFβ3 mRNA expression for the five GCTs and the normal fibroblast cell line derived from patient 11 is shown in Table 3. It is concluded that TGFβ2 was consistently overexpressed when compared to the other transforming growth factors studied in GCT cultured cells, and that TGFβ2 expression in GCT was usually elevated in comparison to different nonneoplastic and neoplastic cell lines. TGFβ1 mRNA was uniformly expressed in all GCTs, however, at a qualitatively lower level than TGFβ2. TGFβ3 and TGFα were not consistently expressed by these tumor cells.

DISCUSSION

This paper describes cytogenetic TGFα and TGFβ subtype analyses for individuals with GCT of bone. The most interesting and consistent cytogenetic finding was multiple telomere-telomere chromosome associations. This may suggest generalized chromosomal instability [25], In one instance, four chromosomes were attached to one another at only their telomeric termini (Fig. 1). In some tetraploid cells, duplicates of two chromosomes involved in a TA were observed. This suggests that the telomere-telomere association is stable in GCT chromosomes with TAs, and that it can survive and be duplicated during

mitosis. The terminus on the long arm of chromosome 19 (19qter) was the terminus most frequently involved in telomeric associations.

Interestingly, TGFβ1 is mapped to 19 q13.1–13.2. Although its location may be many kilobase pairs from the telomere, we postulated that a terminal translocation may affect TGFβ expression. It is for this reason that we have undertaken studies on TGFβ expression in GCT. Three lines of evidence support the hypotheses that there may be abnormalities in the TGFβ-receptor response pathway in GCT. First, the proximity of the TGFβ gene to a genomically unstable area may predispose to alteration of expression. Secondly, GCT, unlike many other solid tumors, grows with ease and rapidity in culture. This suggests an autocrine mechanism of growth stimulation by which TGFβ has been postulated to act [20], Finally, TGFβ is a known stimulator of proliferation for mesenchymal cells such as osteoblasts. This research did demonstrate that TGFβ was consistently expressed in each of the five patients tested when compared to neoplastic and nonneoplastic controls.

Giant Cell Tumor of Bone

Three major cell populations have been characterized with in vitro cell culture techniques [26]. A mononuclear cell line is the first, expressing monocyte-macro-phage markers that did not persist with prolonged culture time. A second mononuclear cell population did proliferate in culture and possessed the ability to synthesize collagens. This second cell type may be the neoplastic cellular element of GCT. The third cell population identified was a line of multinucleated giant cells bearing surface receptors in common with osteoclasts. This cell line had a poor proliferative capacity and was present during early cell culture. In vitro growth studies of GCT by time-lapse cinemicrography explains these findings [27–29], Multinucleated giant cells in culture continue to divide until only mononuclear cells remain. These end-stage mononuclear cells are morphologically indistinguishable from the original stromal cells. Additionally, Wood found that GCT giant cells were nonphagocytic, non-specific esterase negative and failed to exhibit IgG Fc and C3 receptors characteristic of macrophages [30], Therefore, it may be postulated that the stromal mononuclear cell and the giant cell in GCT are homologous and represent the neoplastic element in this tumor. The origin of this cell line remains uncertain, but recent studies demonstrate that the mononuclear cells express phenotypic relationships to cells of the monocyte-macrophage lineage [31].

A single, long-term (G20) in vitro cultured GCT has been studied [32], This cell line consisted of two cell populations initially, a stromal, fibroblastic element that had a high concentration of rough, endoplasmic reticulum on electron microscopy, and giant cells. The giant cell characteristics included multinucleation and a modified, ruffled border in the endoplasmic reticulum. The adherent stromal cells were similar to the giant cells. Midway through the culture time, a transformed cell line emerged that grew in clumps, was adherent to one another, and was not adherent to the flask. The authors concluded that a spontaneous transformation occurred. We have not observed such a morphologic change.

Cytogenetics

The cytogenetic characteristics of 15 patients with GCT of bone have been studied. We observed dissimilar clonal abnormalities in six patients, suggesting no consistent clonal abnormality. Nonrandom translocations involving the break and fusion of chromosomal telomeres or telomeric associations were observed in 11 of 15 individuals. This occurred in cells harvested from short-term cultures of less than 21 days. Chromosomal abnormalities identified in short-term cultures as well as at direct harvest are probably attributable to the neoplasm itself and not the senescence of deteriorating cultured cells.

Cocultivation studies with GCT cells and control fibroblasts showed that no TAs were found in the countersex fibroblasts. In addition, no abnormal metaphases were cytogenetically identified in normal fibroblasts harvested from a patient with GCT. Therefore, there is no evidence for a constitutional chromosomal abnormality or obvious chromosome breakage [33].

Generally, telomeric associations are random. However, in our study several telomeres seemed to predominate in their involvement. The 19qter was the most frequently involved chromosome in TAs. Using a conservative tabulation method of scoring telomeres when involved in at least two cells per patient, 19qter was observed in 31 TAs in five cases. The next most frequently involved termini were 11pter (10 TAs in three cases), 20qter (8 TAs in two cases), and 16pter (6 TAs in three cases).

Telomeric associations may represent chromosome instability and may be associated with preclonal stages in neoplastic disorders [25, 34, 35], Our findings suggest that telomeric associations are stable and may be copied and maintained during mitosis. TAs have been previously reported [25, 34, 35], They have occurred in hematopoietic malignancies, solid tumors, and senescent cells. Bridge et al. [35] has described TAs in GCT from 15 patients and 19qter was also frequently observed. It is not clear at this time whether the telomeric instability of GCT represents a cause or an effect of the neoplasm, or whether it is a finding common to other solid musculoskeletal tumors. The recent report of a GCT with a 12;19 chromosome translocation further supports the findings that chromosome 19 structural abnormalities represent a nonrandom event in this neoplasm [36]. The relationship of telomeric association frequency to clinical outcome or biologic aggressiveness cannot be determined. Thus far, no tumor has recurred, and only one patient had metastatic disease, which was evident on presentation. The fact that not every GCT cytogenetically examined exhibits TAs can be interpreted in several ways. Sampling error, biologic diversity, and incorrect light microscopic diagnosis of GCT are all possibilities.

TGFβ Gene Expression

The locus for TGFβ1 is at 19ql3.1–13.2. The TGFβ2 gene is located on chromosome 1q41, and TGFβ3 on chromosome 14q24 [37], TGFβ1, TGFβ2, and TGFβ3 share about 70% amino acid homology in the active region of the protein. They have similar receptor binding characteristics and biologic activities [20, 38], TGFβ2 has been shown to induce chondrogenesis and osteogenesis in the rat femur more effectively than TGFβ1 [39]. The in vivo biologic properties of TGFβ3 are similar to those of TGFβ1 and TGFβ2 [20]. The

radioreceptor assay demonstrated TGFβ activity present in the conditioned media from GCTs. This assay detects all three TGFβs. Northern blot analyses revealed rather consistent findings of messenger RNA for each of the five tumor specimens analyzed. Nonuniform trace gene expression was detected for TGFβ3, and low levels of TGFβ1 mRNA were uniformly identified in all GCT cell lines. TGFβ2 mRNA expression was always present and was elevated when compared to neoplastic and non-neoplastic controls, indicating that most of the TGFβ protein detected in the radioreceptor assay was probably TGFβ2.

An attempt was made to compare levels of gene expression present in the tumor with control tissue from the same patient. Both tumor and anatomically noncontiguous muscle fascia were harvested from patient 11. The subsequent fibroblasts had a normal phenotype and were cytogenetically normal. The fibroblasts did not contain detectable TGF mRNA.

The TGFβ family of polypeptide factors regulate cell growth and differentiation. In addition to TGFβ1, β2, and β3, this expanding family includes the more distantly related inhibins, activins, mullerian-inhibitor, and, most recently, bone morphogenic protein (BMP 2A and 3) [40]. The two BMP recombinant human proteins share between 34% and 38% amino acid residues with TGFβ1 and β2. BMP was originally described as causing the induction of bone formation at an extraskeletal ectopic site [41]. Bone is the most abundant human source for TGFβ2. The precise role of TGFβ and its isoforms in bone formation and remodeling is complex. This process is dependent upon many factors, and has not yet been completely elucidated [42].

The effect of TGFβ on bone cell replication in vitro is biphasic and depends upon both TGFβ concentration and cell density. Studies in fetal rat bone and adult human trabecular bone corroborate that TGFβ is a bi-functional osteoblast regulator [43, 44]. In both culture systems variable effects on DNA synthesis, type 1 collagen production, and alkaline phosphatase activity were detected dependent upon the cell density of the osteoblast and concentration of exogenous TGFβ. These studies suggest that TGFβ is an important regulator of osteoblastic differentiation at a local level in concert with other factors. TGFβ1 and β2 do exhibit coregulator dependency [45].

Osteoblasts both directly and indirectly mediate the coupling between bone formation and bone resorption. It is not surprising to find that a primary neoplasm of bone expresses elevated TGFβ message. However, from our results it remains unclear why TGFβ2 is expressed at elevated levels.

TGFα Gene Expression

TGFα mRNA was detected in three of five GCT cultures examined. TGFα has been implicated as a local factor in bonelysis, especially in the hypercalcemia of metastatic cancer to bone [46, 47]. Additionally, TGFα, once termed sarcoma growth factor, has been isolated in a number of human cancer cell lines [48]. It competes for the EGF receptor. GCT is osteolytic and its cell of origin is unknown. Thus, there were two reasons to investigate this probe in our study, although no increased TGFα mRNA was detected.

SUMMARY

This study demonstrates that specific, consistent, and nonrandom cytogenetic abnormalities are present in giant cell tumor of bone, particularly telomeric associations. The telomeric associations cluster around several telomeric termini, the most frequent of which is the 19q terminus. This unique phenomenon is detected in direct harvest and short-term cultures. One can speculate as to whether the TAs result from tumor progression or are an integral element of the initial neoplastic transforming event.

These data demonstrate high levels of TGFβ2 and mRNA in GCT that appear to be abnormal when compared to normal control fibroblasts and other neoplastic and nonneoplastic samples. This suggests that TGFβ2 may be a fundamental component in the neoplastic event in this tumor. More detailed studies are required to delineate the complete role of TGFβs in this tumor system and to rule out the possibility that TGFβ2 is secondarily elevated.

Longer clinical follow-up of the patients may be required to ascertain which giant cell tumors are more biologically aggressive, resulting in tumor recurrence. Prognostic correlation of a specific cytogenetic pattern or growth factor profile with a more biologically aggressive tumor (given similar anatomic sites of the neoplasm and similar surgical resection techniques) remains a goal.

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REFERENCES

1. Dahlin DC, Cupps RE, Johnson EW (1970): Giant cell tumor: A study of 195 cases. Cancer 25:1061–1070. [PubMed: 4910256]
2. Goldenberg RR, Campbell CJ, Bonfiglio M (1970): Giant cell tumor of bone. J Bone Joint Surg 52A:619–664.
3. Campanacci M, Guinti A, Olmi R (1975): Giant-cell tumours of bone. Italian J Orthop Traumat 1:249–277.
4. Larsson SE, Lorentzon R, Boquist L (1975): Giant cell tumor of bone. J Bone Joint Surg 57A: 167–173.
5. McDonald DJ, Sim FH, McLeod RA, Dahlin DC (1986): Giant cell tumor of bone. J Bone Joint Surg 68A:235–242.
6. Campanacci M, Baldini N, Boriani S, Sudanesa A (1987): Giant-cell tumor of bone. J Bone Joint Surg 69A: 106–114.
7. Rock MG, Pritchard DJ, Unni KK (1984): Metastases from histologically benign giant cell tumor of bone. J Bone Joint Surg 66A: 269–274.
8. Dahlin DC, Unni KK (1986): Bone Tumors CC Thomas, Springfield, Illinois.
9. 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.
10. Present D, Bertoni F, Hudson T, Enneking WF (1986): The correlation between the radiologic staging and histopathologic findings in aggressive stage 3 giant cell tumor of bone. Cancer 57:237–244. [PubMed: 3942958]

11. Helio H, Karaharju E, Nordling S (1985): Flow cytometric determination of DNA content in malignant and benign bone tumors. Cytometry 6:165–171. [PubMed: 3979218]
12. Kreicbergs A, Silvfersward C, Tribukait B (1984): Flow DNA analysis of primary bone tumors: Relationship between cellular DNA content and histopathologic classification. Cancer 53:129–136. [PubMed: 6580940]
13. Mankin HJ, Conner JF, Schiller AL, Perlmutter N, Alho A, McGuire M (1985): Grading of bone tumors by analysis of nuclear DNA content using flow cytometry. J Bone Joint Surg 67A:404–413.
14. Xiang JH, Spanier SS, Benson NA, Braylan RC (1987): Flow cytometric analysis of DNA in bone and soft-tissue tumors using nuclear suspensions. Cancer 59:1951–1958. [PubMed: 3032396]
15. Ladanyi M, Traganos F, Huvos AG (1989): Benign metastasizing giant cell tumors of bone: A DNA flow cytometric study. Cancer 64:1521–1526. [PubMed: 2776111]
16. Scott SM, Pritchard DJ, Unni KK, Rainwater LM, Lieber MM (1989): “Benign” metastasizing giant cell tumor: Evaluation of nuclear DNA patterns by flow cytometry. J Orthopaed Res 7:463–467.
17. Schwartz HS, Jenkins RB, Dahl RJ, DeWald GW (1989): Cytogenetic analyses in giant-cell tumors of bone. Clin Orthop Rel Res 240:250–260.
18. Schwartz HS, Jenkins RB, Moses HL (1988): The cytogenetic and growth factor analysis of giant cell tumor (Abstract) The Molecular Diagnostics of Human Cancer. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 82.
19. Tucker RF, Branum EL, Shipley GD, Ryan RJ, Moses HL (1984): Specific binding to culture cells of ^{125}I-labeled type β transforming growth factor from human platelets. Proc Natl Acad Sci USA 81:6757–6761. [PubMed: 6208555]
20. Graycar JL, Miller DA, Arrick BA, Lyons RM, Moses HL, Derynck R (1989): Human transforming growth factor-β3: recombinant expression, purification, and biological activities in comparison with transforming growth factors-β1 and -β2. Mol Endo 3:1977–1986.
21. Derynck R, Jarrett JA, Chen EY, Eaton DH, Bell JR, Assoian RK, Roberts AB, Sporn MB, Goeddel DV (1985): Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells. Nature 316:701–705. [PubMed: 3861940]
22. Feinberg AP, Vogelstein B (1983): A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Analyt Biochem 132:6–13. [PubMed: 6312838]
23. Miller DA, Lee A, Pelton RW, Chen EY, Moses HL, Derynck R (1989): Murine transforming growth factor-β2 cDNA sequence and expression in adult tissues and embryos. Molec Endocrinol 3:1108–1114. [PubMed: 2797004]
24. Derynck R, Lindquist PB, Lee A, Wen D, Tamm J, Graycar J, Rhee L, Mason A, Miller D, Coffey R, Moses H, Chen E (1988): A new type of transforming growth factor-β, TGF-β3. EMBO J 7:3737–3743. [PubMed: 3208746]
25. DeWald GW, Dahl RJ, Spurbeck JL, Carney JA, Gordon H (1987): Chromosomally abnormal clones and nonrandom telomeric transformations in cardiac myxomas. Mayo Clinic Proc 62:558–567.
26. Goldring SB, Schiller AL, Mankin HJ, Goldring SR, Schiller AL, Mankin HJ, Dayer JM, Krane SM (1986): Characterization of cells from human giant cell tumors of bone. Clin Orthop Rel Res 204:59–75.
27. Chuanhan F, Yuhvei C, Ziaqing Z, Hualong J, Xianzheng L (1981): In vitro tissue culture study on giant cell tumor of bone. Chinese Medical Journal 94(11):709–717. [PubMed: 6800711]
28. Jia-qing Z, Hua-long J, Chuan-han F, Yu-hui C (1982): Cinemicrographic observations of bone giant cell tumor cultured in vitro. Chinese Medical Journal 95(5):337–342. [PubMed: 6814847]
29. Fan Q, Lu Y (1989): The study of giant cell tumors in bone. Orthopedics 12(4):619–625. [PubMed: 2710721]
30. Wood GW, Neff JR, Gollahon KA, Gourley WK (1978): Macrophages in giant cell tumors of Bone. J Pathol 125(1):53–58. [PubMed: 722390]
31. Komiya S, Sasaguri Y, Inoue A, Nakashima M, Yamamotot S, Yanagida I, Morimatsu M (1990): Characterization of cells cultured from human giant-cell tumors of bone. Phenotypic relationship to the monocyte-macrophage and osteoclast. Clin Orthop Rel Res 258:304–309.

32. Marshall GJ, Kirchen ME, Schwinn CP, Stover S, Tsay A, Mendez L (1988): Spontaneous transformation of a giant cell tumor in vitro. Orthop Trans 12(2):500–501.
33. Butler MG, Allen A, Haynes JL, Scwartz HS: Cytogenetic abnormalities with telomeric associations in five giant cell tumors of bone (submitted).
34. Schwartz HS, Allen GA, Butler MG (1990): Telomeric associations. App Cytogenet 16:133–137.
35. Bridge JA, Sanger W, Neff JR (1990): Cytogenetic findings and biologic behavior of giant cell tumors of bone. Cancer 65:2697–2703. [PubMed: 2340469]
36. Noguera R, Llombart-Bosh A, Lopez-Gines C, Carda C, Fernandez CI (1989): Giant-cell tumor of bone, stage II, displaying translocation t(12;19)(ql3;ql3). Virchows Arch A Path Anat His 415:377.
37. Barton DE, Foellmer BE, Du J, Tamm J, Derynck R, Francke V (1988): Chromosomal mapping of genes for transforming growth factors Beta2 and Beta3 in man and mouse: Dispersion of TGF-Beta gene family. Oncogene Res 3:323–331. [PubMed: 3226728]
38. Cheifetz S, Weatherbea JA, Tsang MLS, Anderson JK, Mole JE, Lucas R, Massague J (1987): The TGFβ system: A complex pattern of cross-reactive ligands and receptors. Cell 48:409–415. [PubMed: 2879635]
39. Joyce ME, Roberts AB, Sporn MB, Bolander ME (1990): Transforming growth factor-β and the initiation of chondrogenesis and osteogenesis in the rat femur. J Cell Biol 110:2195–2207. [PubMed: 2351696]
40. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA (1988): Novel regulators of bone formation: Molecular clones and activities. Science 242:1528–1534. [PubMed: 3201241]
41. Urist MR, DeLange RJ, Finerman GAM (1983): Bone cell differentiation and growth factors. Science 220:680–686. [PubMed: 6403986]
42. Sporn MB, Roberts AB (1990): TGF-β: Problems and prospects. Cell Reg 1:875–882.
43. Centrella M, McCarthy TL, Canalis E (1987): TGFβ is a bifunctional regulator of replication and collagen synthesis in osteoblast-enriched cell cultures from fetal rat bone. J Biol Chem 262:2869–2874. [PubMed: 3469200]
44. Wergedal JE, Strong DD, Mohan S, Baylink DJ (1989): TGFβ is produced by and acts on human bone cells to stimulate protein synthesis and alkaline phosphatase activity by mechanism involving increased message levels (Abstract). Orthopaedic Research Society, Chicago, IL p. 85.
45. Bascom CC, Wolfshohl JR, Coffey RJ, Madigen L, Webb NR, Purchio AR, Derynck R, Moses HL (1989): Complex regulation of transforming growth factor β1, β2, and β3 mRNA expression in mouse fibroblasts and keratinocytes by transforming growth factors β1 and β2. Mole Cell Biol 9:5508–5515.
46. Stem PH, Krieger NS, Nissenson RA, et al. (1985): Human TGFβ stimulates bone resorption in vitro. J Clin Invest 76:2016–2019. [PubMed: 3877079]
47. Ibbotson KJ, Harrod J, Gowen M, D'Souza S, Smith DD, Winkler ME, Derynck R, Mundy GR (1986): Human recombinant TGFβ stimulates bone resorption and inhibits formation in vitro. Proc Natl Acad Sci USA 83:2228–2232. [PubMed: 3485799]
48. Todaro GJ, Fryling C, DeLarco JE (1980): TGF produced by certain human tumor cells: Polypeptides that interact with EGF receptors. Proc Natl Acad Sci USA 77:5258–5262. [PubMed: 6254071]

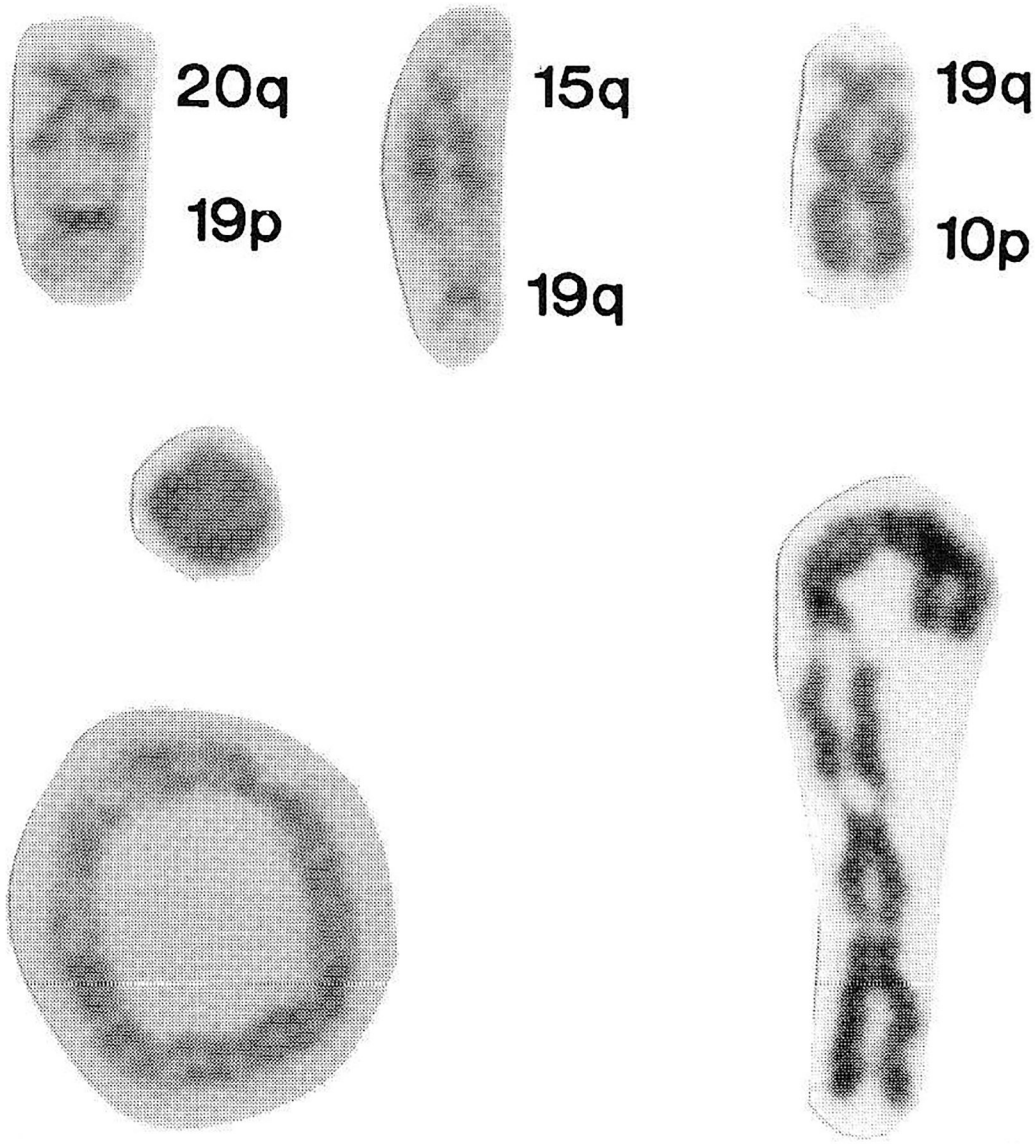

Figure 1.

Representative telomeric associations. Top row from left to right: tas(19;20) (pter;qter), tas(15;19)(qter;qter), and tas(10;19)(pter;qter). Bottom row from left to right: Two ring chromosomes and TAs of four chromosomes from groups D, C, G, and D.

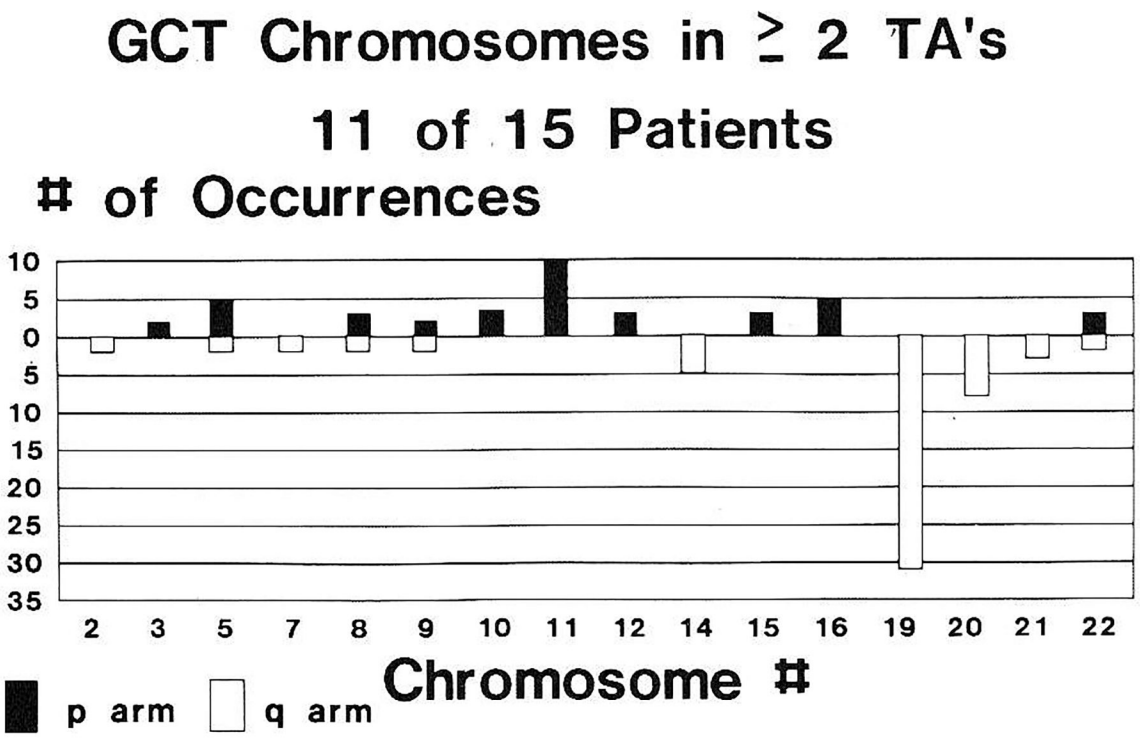

Figure 2.

Histogram of giant cell tumors of bone (GCT) involved in at least two telomeric associations (TAs). Eleven of 15 patients demonstrated TAs. The 19q terminus is most frequently involved.

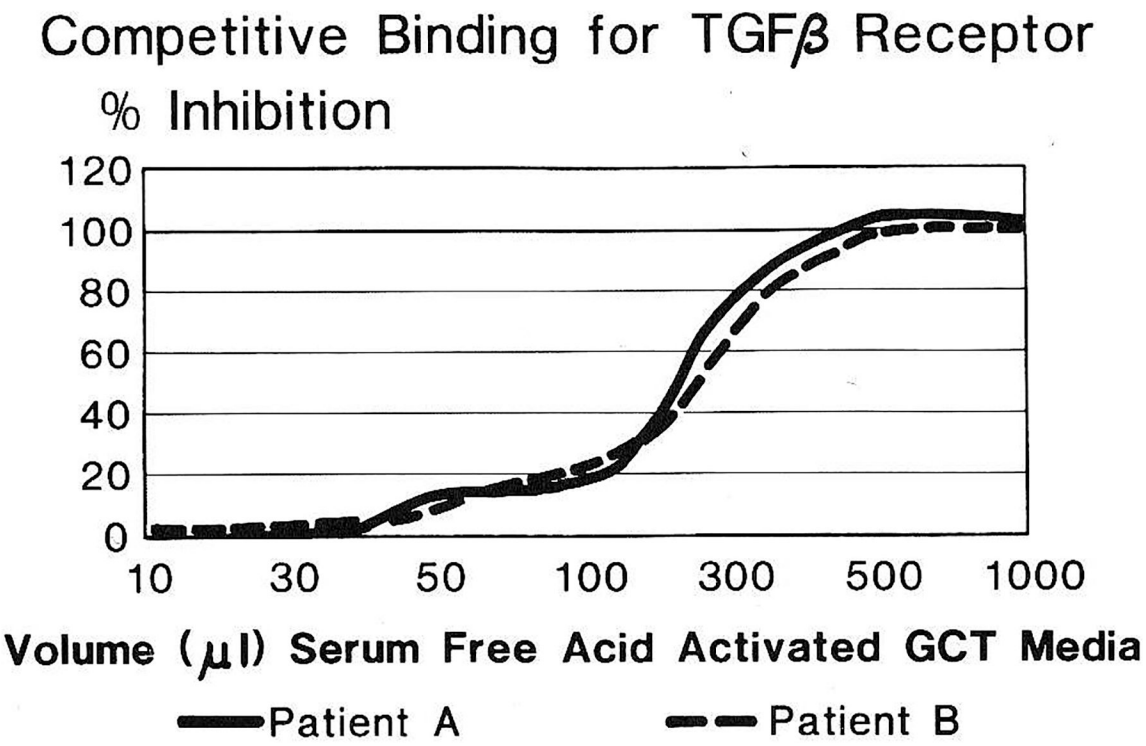

Figure 3.

Competitive binding for TGFβ receptor in giant tumor of bone. Increasing amounts of serum-free, acid-activated GCT media were added to ^{125}I-labeled TGFβ-saturated receptors on AKR-2B fibroblasts.

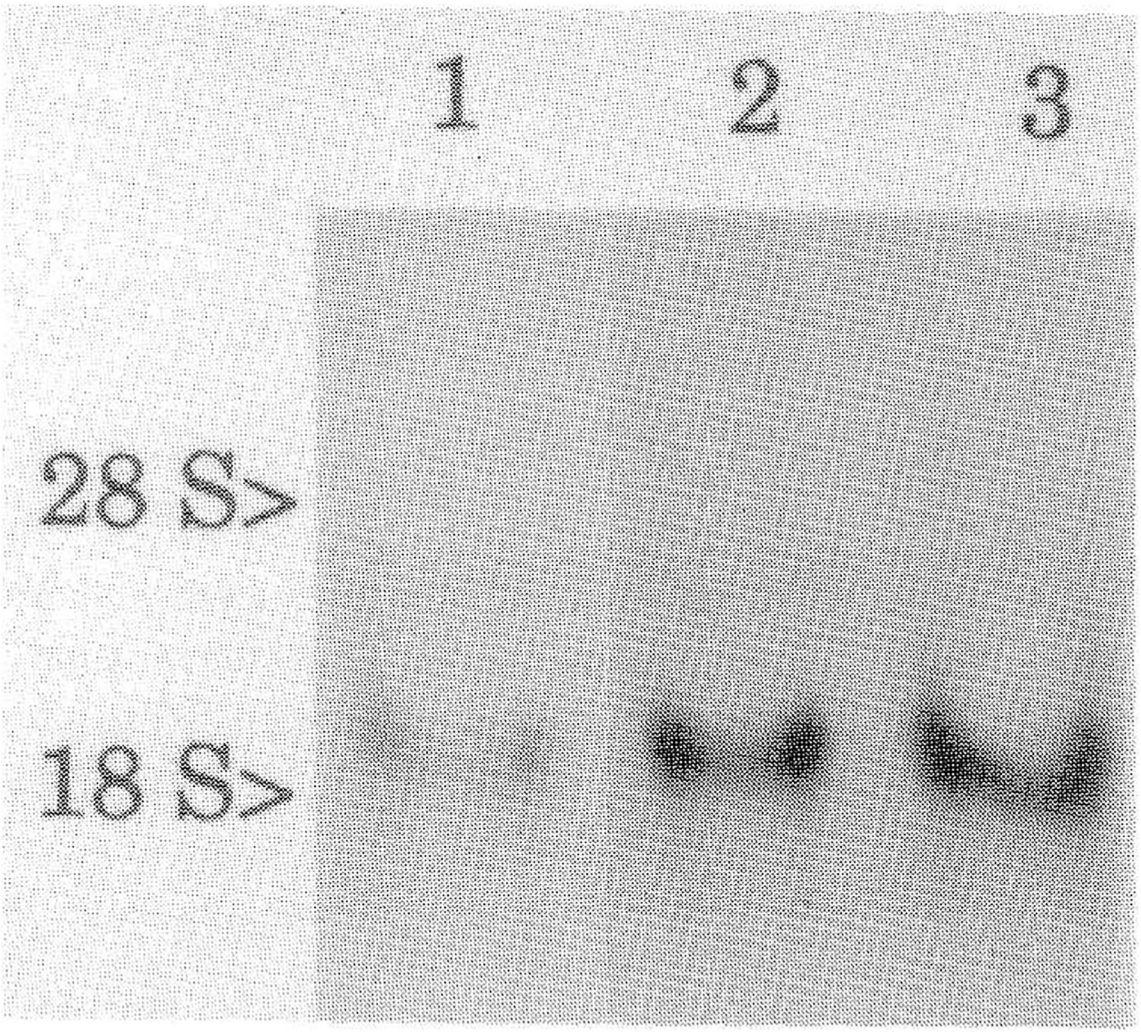

Figure 4.

Northern blot of AKR-2B fibroblasts and two individuals with giant cell tumors of bone. Two micrograms of polyadenylated RNA were used in all lanes. Lane 1 = rapidly growing AKR-2B fibroblasts. Lane 2 = GCT from patient 7. Lane 3 = GCT from patient 8. The radiolabeled TGFβ1 probe was prepared as previously described by Derynck et al. [24].

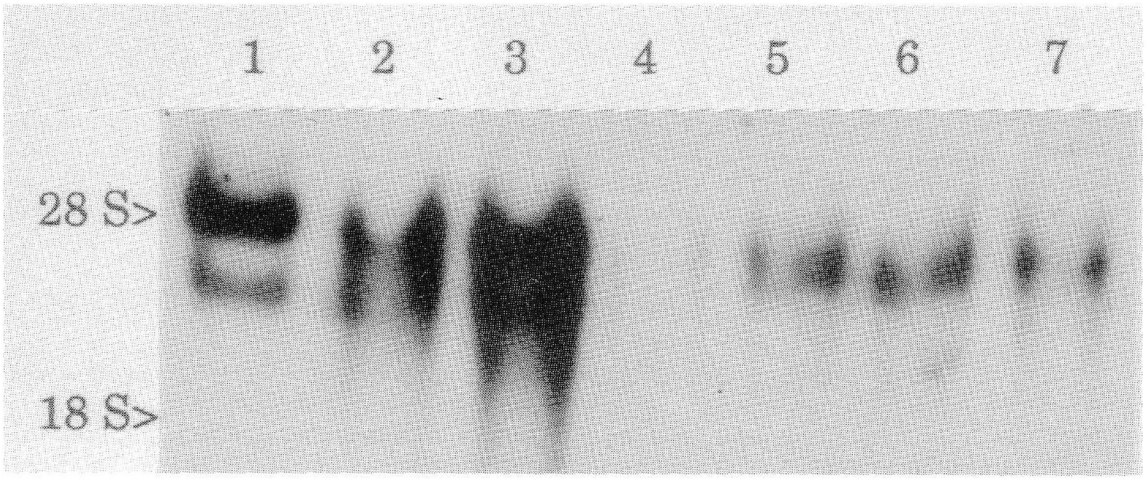

Figure 5.

Northern blot of giant cell tumor of bone compared to other neoplastic and nonneoplastic cell lines. Four micrograms of tumor and GI epithelium mRNA were used. One microgram of BSC-1 RNA was used. Lane 1 = BSC-1 kidney epithelium. Lane 2 = GCT from patient 7. Lane 3 = GCT from patient 8. Lane 4 = GI epithelium. Lanes 5, 6 = a Panc-1 human pancreatic carcinoma cell line. Lane 7 = Biopsied pancreatic carcinoma cell RNA. The radiolabeled TGFβ2 probe was prepared as previously described [24].

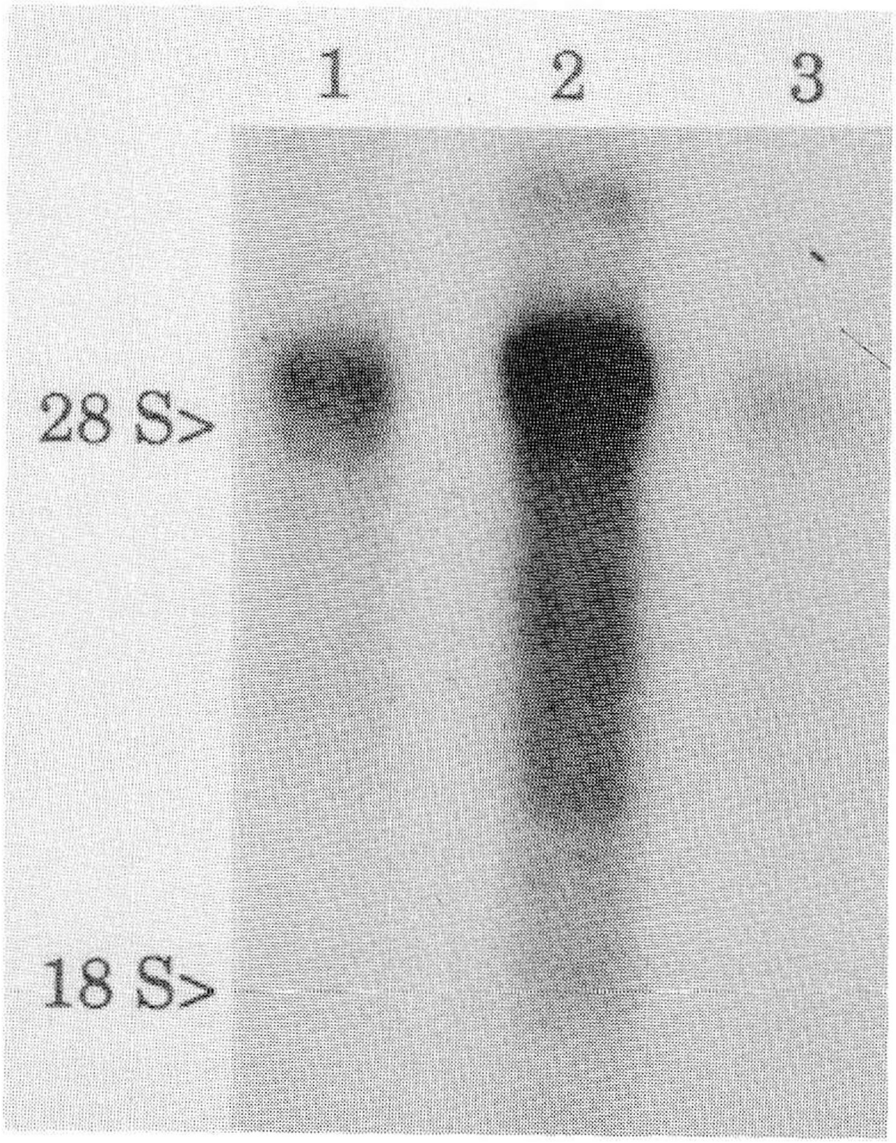

Figure 6.

Northern blot of two individuals with giant cell tumors. Two micrograms of tumor RNA were used. Lane 1 = HT 1080. Lane 2 = GCT from patient 11. Lane 3 = GCT from patient 12. The radiolabeled TGF$\beta 2$ probe was prepared as previously described by Miller et al. [23].

Table 1

Clinical data for patients with giant cell tumor of bone

Case	Gender	Age (years)	Location at presentation	Procedure	Resection margin[a]
1	F	38	Distal femur	Curettage and bone grafting	Intralesional
2	M	60	Proximal humerus	Hemiarthroplasty	Wide
3	F	43	Distal radius	Fibular arthroplasty	Marginal
4	F	12	Distal tibia, lung	Arthrodesis Thoracotomy	Contaminated wide
5	M	34	Proximal humerus	Curettage and cementation	Intralesional
6	F	58	Proximal tibia	Osteoarticular allograft	Wide
7	F	61	Distal femur	Curettage and cementation	Intralesional
8	M	41	Proximal tibia	Curettage and bone graft	Intralesional
9	F	40	Proximal tibia Patella	Transfemoral amputation	Wide
10	F	22	Distal tibia	Curettage and bone graft	Intralesional
11	M	54	Distal femur	Curettage and cementation	Intralesional
12	F	12	Sacrum	Curettage	Intralesional
13	F	41	Proximal tibia	Curettage and cementation	Intralesional
14	F	33	Distal radius	Curettage and cementation	Intralesional
15	M	20	Distal femur	Curettage and cementation	Intralesional

[a]As defined by Enneking (Enneking WF, Spanier SS, and Goodman MA: A System for the Surgical Staging of Musculoskeletal Sarcoma. Clin Orthop Rel Res 153:106–120, 1980).

Table 2

Summary of cytogenetic data in 15 patients with giant cell tumor of bone

	Involvement in ≥ 2 TAs[a]		Clonal abnormalities		
Case	Chromosome telomere	No. of cells[b]	Abnormality	No. of cells	Nonclonal abnormalities
1	2qter	2	t(5;10)(p13;q22)	3	t(14;18)(q24;q21)
	9qter	2			del(5)(q13q33)
	12pter	3			der(11)(t(11;?)(q13;?)
	19qter	6			der(19)t(19;?)(q13;?)
	20qter	5			
2	15pter	3		Ø	t(l5;21)(p11;q11)
					t(14;21)(p12;p13)
					t(8;22)(ql3;ql3)
3	5pter	5	der(20)t(13;20)(q12;q13)	3	del(15)(q13q26)
	8qter	2	del(9)(p13p22)	2	der(16)t(13;16)(q12;q22)
	10pter	2	−X, −8, −15, −20	4	
	11pter	3	−9	5	
	16pter	2	+16	2	
	20qter	3	−19	3	
4	19qter	3		Ø	t(6;19)(q15:q13)
					t(14;19)(q22;q13)
					t(3;4)(q25;q21)
					del(3)(p21p23)
					del(18)(q12.2)
5	3pter	2	−22	4	der(5)t(5;?)(p15;?)
	9pter	2			del(11)(q22q23)
	11pter	4			der(3)t(3;?)(p11;?)
	16pter	2			
	19qter	2			
6	7qter	2		Ø	der(12)t(8;12)(q11;p11)
7		Ø		Ø	del(2)(p13)
					del(19)(q11)
					der(11)t(11;?)(p13;?)
8	8pter	3	dic(11;19)(p15;q13)	2	
	11pter	3			
	19qter	6			
	22pter	3			
	22qter	2			
9	19qter	14	der(19)t(19;?)(q13;?)[c]	14	der(2)t(2;?)(?q37;?)
			−X,t(1;3)(p32;q21)	15	
10		Ø		Ø	del(8)(p11.2)
11	5qter	2		Ø	double ring = 1

	Involvement in ≥ 2 TAs[a]		Clonal abnormalities		
Case	Chromosome telomere	No. of cells[b]	Abnormality	No. of cells	Nonclonal abnormalities
	14qter	5			double minute = 1
	21qter	3			marker 15 = 1
12		Ø		Ø	
13	10pter	2	ring 17	2	
14		Ø		Ø	
15	16pter			2	2 markers
					−22 = 2

[a] TA, telomeric association, see text.

[b] Some cells may contain 2 TA's involving the same terminus.

[c] 3 different slides.

Table 3

Comparative summary of relative expression of transforming growth factors in giant cell tumor of bone

Case	Patient	Cell line	TGF$_{\alpha}$	TGF$_{\beta 1}$	TGF$_{\beta 2}$	TGF$_{\beta 3}$
1	7	GCT	+	+	+ + +	+
2	8	GCT	+ +	+	+ + +	+
3	11	GCT	—	+	+ + +	—
4	12	GCT	+	+ +	+ + +	—
5	13	GCT	—	+ +	+ + + +	—
6	11	FB	—	—	—	—

Abbreviations: GCT, giant cell tumor of bone; FB, fibroblasts.