The radiographic stage of giant cell tumor related to stromal cells' proliferation

Tissue cultures in 13 cases

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The clinical behavior of giant cell tumor is related to the radiological appearance. To test the hypothesis that in vitro proliferation of the neoplastic stromal cell population of giant cell tumors is related to the radiological appearance, this study was undertaken. A prospective analysis of the cells migrating from 13 consecutive tumors was conducted. Growth curves and population doubling-times (PDT) for first and fifth passages were calculated and alkaline phosphatase levels were measured and compared to preoperative radiographic staging. A strong negative correlation was found between PDT and the radiographic stage. Tumors in stages I and II (low aggressiveness) were found to have an average cell population doubling-time of 11 (SD 2.2) days, while those in stage III (high aggressiveness) showed a doubling-time of 6 (SD 2.2) days. Low alkaline phosphatase activity was noted in all cultures, a finding consistent with the putative preosteoblastic potential of these stromal cells. This putative origin is also indicated by the differentiation response to retinoic acid. The findings suggest that the in vitro proliferation of the mononuclear stromal cell population of giant cell tumors is related to the radiographic stage and may predict the clinical behavior of these tumors.

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Investigations of the giant cells in giant cell tumors of bone have suggested that they are typical of normal osteoclasts (James et al. 1991, Nelson et al. 1991); the stromal cells, however, have not been well defined. Some authors have identified monocyte-related antigens in the stromal cells (Burmester et al. 1983, Ling et al. 1986, 1988), and it appears that a subpopulation of spindle-shaped cells in the stroma may constitute the essential neoplastic population (Roessner et al. 1987). These spindle cells are not of hematopoietic origin (Aqel et al. 1988), and, unlike the hematopoietic monocytes and giant cells, which do not possess proliferation-associated antigens (Roessner et al. 1987), this population rapidly proliferates.

There is a poor correlation between cellular appearance and clinical behavior of giant cell tumors (Jaffe et al. 1940). A possible explanation is the heterogeneity of the tumor, in which most of the cells in the lesion are reactive and not neoplastic (Roessner et al. 1987). With this knowledge, it would seem that any histological analysis, in order to predict clinical outcome, should be based solely on the neoplastic cells, which probably form a minority.

We have investigated whether cellular characteristics in vitro could be related to the radiographic appearance. Enneking has suggested a correlation between radiographic criteria and clinical behavior of giant cell tumors (Enneking 1986, Present et al. 1986).

Material and methods

Specimens were obtained prospectively from 13 consecutive patients who presented with a biopsy-confirmed diagnosis of giant cell tumor of bone. Monolayer cultures were obtained from all tumors, since each tumor was cultured from several separate pieces simultaneously in several plates.

Cells were subcultured every 10–30 days, depending on the growth characteristics of the culture. Growth curves and population doubling-times (PDT) were obtained for P1 (first passage) and P5 cultures. Comparisons between P1 and P5 cultures were used to assess the consistency of the doubling-times measured ($r = 0.92$). Thus only P1 results will henceforth be reported. While growing the cultures, we saw that two distinct groups of tumors occurred, i.e., those with a fast proliferation rate in vitro and those with a slow proliferation rate in vitro. The tumors were arbi-

trarily subclassified into one of these two groups, fast proliferation rate (doubling time is a week or less) or slow proliferation rate (doubling-time over a week) according to the doubling-time. Growth curves were obtained by seeding cells at 5,000 cells/mL in 24 well dishes in alpha-modified Eagle's medium, supplemented with 10% fetal calf serum. Cells were trypsinized and counted every 2 days during the first week and then every week for 6 weeks. The media were replenished weekly. Cell numbers were calculated using a Coulter Counter ZM (current 0.1 amperes, threshold 5–100, attenuation 32). Cellular alkaline phosphatase levels were measured in vitro (Majeska and Rodan 1982). Retinoic acid (10^{-7} molar) had been added to cell cultures, which were subcultured and grown in a defined serum-free medium (DCCM-1, Biological Industries, Bet-Haemeq, Israel) (Robinson et al. 1995). Cells were exposed for 48 hours. Cell counts and immunohistochemical markers of osteoblast-like differentiation were assayed.

Radiographic staging of the tumors was performed in a blinded fashion with respect to proliferation capacity by one of the authors (DR), and according to the method of Enneking et al. (Enneking 1986, Present et al. 1986). Briefly, stage I lesions (2 cases) are considered latent giant cell tumors. Radiography reveals a lucent lesion with an encircling reactive rim of mature bone. Stage II lesions (5 cases) are active, tend to enlarge steadily with expansion and deformation of bone, maintaining a thin but well-defined shell of reactive bone. Stage III giant cell tumors (6 cases) are aggressive. Radiography demonstrates a poorly defined destructive lesion, lacking obvious margins. Each tumor was then characterized in terms of its radiographic stage and cellular population doubling-time (Table 1). Recurrent tumors were classified according to their preoperative appearance.

Response of the stromal cells to retinoic acid was evaluated in 5 different cultures in order to evaluate better the origin of the proliferating subpopulation and to exclude the cultures being taken over by fibroblasts.

Statistics

Each data point in the cell proliferation and alkaline phosphatase assays was obtained by averaging the results of four wells, and each experiment was repeated 2–3 times. All data are expressed as medians and ranges. Comparisons of radiographic stage to cellular proliferation and activity were made with nonparametric analysis of variance, according to Kruskal-Wallis.

Table 1. Clinical features, radiographic stages and stromal cell population median (range) doubling-time (PDT, days)

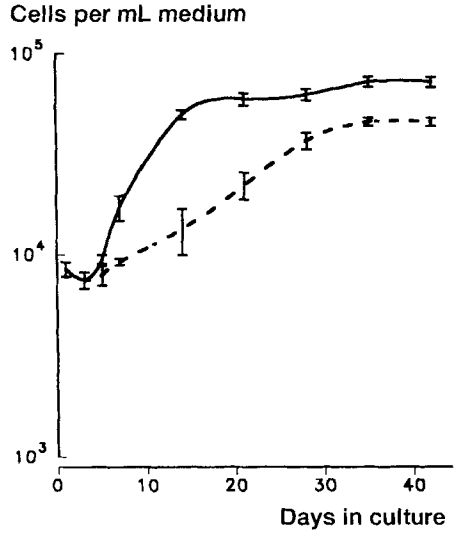
No.	Age	Location of tumor	Prior therapy [a]	Stage	PDT P_1	
1	48	Distal femur	–	II	13	(11–15)
2	51	Proximal tibia	–	II	10	(8–13)
3	35	Distal femur	–	II	12	(10–14)
4	42	Distal femur	B	II	9	(7–11)
5	34	Distal femur	B	I	11	(9–12)
6	18	Proximal femur	–	II	13	(12–14)
7	30	Metacarpus	–	I	10	(9–12)
8	45	Proximal tibia	–	III	5	(4–7)
9	38	Iliac bone	–	III	6	(4–8)
10	5	Metatarsus	B	III	3	(2–4)
11	20	Distal tibia	B	III	4	(3–6)
13	60	Iliac bone	A	III	10	(8–12)

[a] A Curettage; B Curettage and cement

Results

The original explant-derived cell cultures took varying periods of time to reach confluence, ranging from 7–40 days. However, this parameter alone cannot be used to monitor the aggressiveness or the proliferation capacity of the tumors, since the size of the tissue fragments and the rate of migration of cells from the tissues also affect this measurement. All explants developed a uniform appearance in culture with multinucleated giant and cuboidal-shaped mononuclear cells, interspersed with spindle-shaped cells. The latter cell type grew more dominant in subsequent passages. In contrast to fibroblasts, these spindle-shaped cells tended to form a lattice-like arrangement in culture (Gallardo et al. 1970). Giant cells did not survive passage. However, in long-term explant cultures, they persisted in the explants throughout the period of the experiment. Alkaline phosphatase activity was detected in all cultures; the level of activity was relatively low (60–100 mLU/mg protein) explaining the known lack of histochemical staining in slides of giant cell tumors (Roessner et al. 1984). The level of alkaline phosphatase activity did not change with passage of the cells and there was no significant difference in the levels of this enzyme among the various tumors.

Growth curves of the cultures differed from tumor to tumor (Figure). Tumors in lower radiographic stages, i.e., stage I or II vs. stage III, tended to have a much longer population doubling-time during the log phase of growth, and the plateau density appeared to be slightly lower. The population doubling-time did not change during the first five passages. Thus, this parameter appears to represent an inherent characteristic of the stromal cells.



Growth curves of a typical tumor with low aggressiveness (radiographic stage II, patient DS, solid line) versus that a tumor with high aggressiveness (radiographic stage III, patient DV, dashed line). Note significant differences in growth rates without differences in lag times. This absence of difference in lag time apparently indicates that the differences in growth rate reflect actual differences in proliferation potential, and are not affected by seeding and attachment problems during the establishment of the culture.

PDTs between the 7 low aggressiveness (stages I and II) tumors and the 6 high aggressiveness (stage III) tumors were different (Table 2). The difference in PDT between aggressive stage III tumors and stages I and II tumors was significant (p-value Kruskal-Wallis = 0.02). It should be noted that stage III tumors are known to be recurrence prone. In our series, this is reflected by the large proportion of recurrent tumors in this group (4/6), as compared to stage I and stage II tumors (2/7).

The cell proliferation rate was slowed following exposure to retinoic acid, as demonstrated by lowered cell counts (Table 3). Levels of osteopontin, osteonectin, alkaline phosphatase and keratan sulfate were increased, as compared to cultures which were not exposed to retinoic acid. In order to ascertain which cell population is proliferating in vitro and which in vivo, primary explant cultures of giant cell tumors (which in early stages contain multiple cell types) as well as histological slides of these tumors were stained by proliferating cell nuclear antigen (PCNA). It appears that no stain is present in the giant cells, while the spindle-shaped cells are stained intensively. These data are similar to those of Roessner et al. (1984).

Table 2. Stromal cell population median (range) doubling-time (PDT, days) in relation to radiographic aggressiveness

Radiographic stage	n	Stage	PDT P_1
Low aggressiveness group	7	I and II	11 (8–14)
High aggressiveness group	6	III	5 (2–12)

Table 3. Stromal cell proliferation

Treatment group	Cell number
Retinoic acid [a]	21,000 ± 1,500
Control cultures [b]	35,000 ± 1,500

[a] 1×10^{-7} M in absolute ethanol 48 hours' exposure
[b] DCCM media and absolute ethanol alone added
t-test, p = 0.01

Discussion

Despite the abundance of clinical reports which have studied the behavior of giant cell tumors, there have been no attempts to correlate the clinical course with tissue culture characteristics. The histologic grade has proved unreliable as an indicator of the aggressiveness of giant cell tumors, except when atypia of the stromal cells is found. This finding has been correlated to an increased incidence of metastases (Komiya et al. 1986). In contrast, the radiographic appearance has been found to correlate well with the macroscopically evident aggressiveness of resection specimens, including soft tissue extension and cortical perforation (Present et al. 1986).

Primary cultures derived from giant cell tumors, prior to passage, include several types of cells (Aqel et al. 1988, Ling et al. 1988). In these cultures the stromal cell population is predominant which is particularly evident after the second passage. As noted, this predominance comes about because neither the monocyte-related mononuclear cell population nor the giant cells undergo mitotic cell division. Thus, the only subpopulation which proliferates is the non-hematopoietic spindle-shaped stromal cell (Roessner et al. 1984).

The apparent association shown in our study between the proliferation capacity of the stromal cells and the radiographic stage of the tumor may suggest that bone destruction by this tumor may be dependent on the growth of the spindle-shaped stromal cells. The stromal cells were hypothesized to mediate bone destruction through their capacity to recruit macrophages, induce the formation of bone-resorbing giant cells and secrete cytokines capable of activating other cell

types (Kito 1991). As there is an apparent association between in vitro proliferation capacity and an aggressive radiological appearance, we conclude that a casual relationship may exist, although this point is not proven.

The spindle-shaped stromal cells from giant cell tumors possess certain characteristics typical of osteoblast precursors including cAMP response to PTH (Goldring et al. 1978, 1986, Wientroub et al. 1982) and the expression of low levels of alkaline phosphatase. In addition, immunohistochemical studies performed on histologic sections in our laboratory have shown that some of the stromal cells (note that this is a heterogeneous population and many of the stromal cells are related not to the neoplastic cells but rather to the reactive cells), express the bone-specific antigens for osteocalcin, osteopontin and bone sialoprotein (Robinson et al. 1993). In view of these findings (Robinson and Einhorn 1994), as well as the data reported here, we conclude that the neoplastic cell population of giant cell tumors is partly composed of spindle-shaped mononuclear stromal cells which synthesize uniformly low levels of bone-specific products and which have a proliferation capacity associated with the radiological appearance of the tumor. The radiological appearance of the tumors has been linked to their clinical behavior (Present et al. 1986).

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