Heterogeneity of xenografted osteosarcoma

A human sarcoma transplanted into nude mice

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Human osteoblastic osteosarcoma transplanted into nude mice developed two different subtypes of non-osteogenic sarcoma: one solid and the other cystic. This could reflect the heterogeneity of osteoblastic osteosarcoma; various tumor regions have different characteristics.

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In recent years, the biological characteristics of human osteosarcoma have been the subject of various studies (Amitami and Nakata 1977, Mii et al. 1988), and the histologic characteristics of the osteosarcoma have been used for prognostic purposes (Scranton et al. 1975).

Xenografts of osteosarcoma in nude mice have been studied by many authors (Brosjö et al. 1985, Bauer 1986, Brosjö 1989). The transplanted tumor is usually identical with the original tumor (Povlsen et al. 1975), but instability in xenotransplanted human tumors has been reported (Donhuijsen et al. 1988). We have studied the instability of xenotransplanted osteosarcoma in nude mice.

Material and methods

Original human tumor

An osteolytic process in the proximal femur was found in a 10-year-old boy. During the biopsy and before chemotherapy was administered (no chemotherapy or radiotherapy had been given previously), five sections of nonnecrotic tumor tissue were kept cold in sterile saline until implantation into nude mice, which took place shortly after biopsy. The tissue was taken from the periphery of the tumor, which contained the highest proportion of proliferating cells.

Histology of primary human tumor

The original tumor was characterized by a high cellularity with large amounts of woven bone and osteoid produced by the anaplastic stroma cells, both of which are considered main characteristics of the histologic subtype of osteoblastic osteosarcoma (Figure 1). The tumor cells were pleomorphic with nuclear polymorphism, and prominent nucleoli were also found. The tumor was vascularized, but no telangiectatic or cyst formations were found. The tumor was considered a histologic subtype of osteoblastic osteosarcoma.

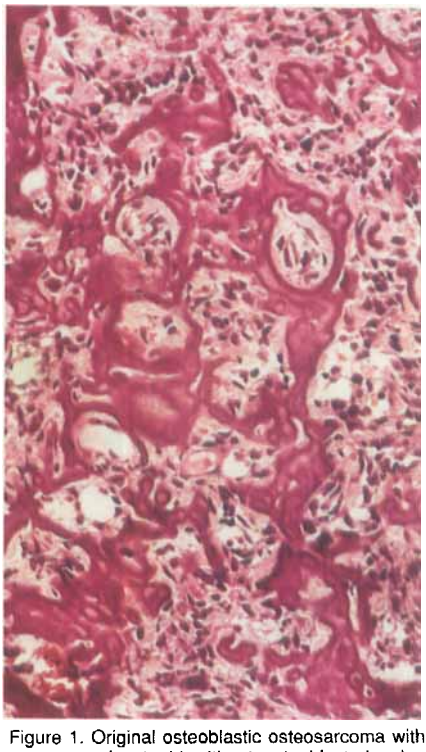

Figure 1. Original osteoblastic osteosarcoma with presence of osteoid without osteoblast rimming and stroma cells with anaplasia (H&E, ×200).

Animals and tumor transplantation

Five fresh nonnecrotic human tumor fragments of 2–3 mm in diameter obtained from the biopsy were implanted subcutaneously in both flanks of five 8 to 10-week-old male athymic (nu/nu) mice obtained from IFFA-CREDO (Lyon, France).

Tumor-bearing animals were killed at 8 weeks, when the tumors reached a volume of around 3 cm^3. At autopsy, one tumor piece was removed for histologic study and another sample of fresh tumor tissue was cut into pieces measuring 2 mm in diameter and were used for the inoculation of new nude mice. Altogether, 28 animals were used.

Tumor size in mice was assessed every week using a slide caliper (Züricher Modell, Dentarium 042–751). The tumor volume was estimated from perpendicular tumor measurements using the formula for an ellipsoid $\pi \times a \times b \times c/6$, where a, b, and c represent the diameters in three planes, respectively.

Growth rate was expressed by the volume doubling time, calculated as log. $2 \times (Tt–To)/log. Vt–log. Vo$, where Vo and To are the volume and time when a measurable tumor was found, and Vt and Tt represent the value when the nude mice were killed (Bauer et al. 1986).

Histologic study of transplanted tumors

The gross morphology of the mass tumor of all the animals was examined. The tumors were fixed in 10 percent buffered formalin and embedded in paraffin. Sections 5 microns thick were cut and stained with hematoxylin and eosin (H&E) and Masson's trichrome.

The number of mitoses of each case was also measured using routinely H&E-stained sections from the same block. Ten high-power (×40) fields were examined for each case, and the mean number of mitoses per high-power field was calculated.

Ag NOR staining technique

The nucleolar organizer regions (NOR) of the nucleoli of cells were demonstrated by means of an argyrophil technique (Ag NOR).

The same specimen from processed blocks of paraffin for histologic studies was cut at 4 microns. These sections were dewaxed in xylene, dehydrated through decrescent ethanols to deionized water, and then submitted to the Ag NOR method as described by Crocker and Nar (1987) and Crocker et al. (1989). Briefly, the incubation medium was prepared by dissolving 2 g/dL of gelatin in 1 g/dL of aqueous formic acid. This was mixed in a ratio of 1:2 volumes with 50 g/dL aqueous (distilled, deionized) silver nitrate solution. The resulting mixture was poured over the sections and incubated for 30 min at room temperature in the dark. After this, the mixture was washed off with deionized water, the sections were immersed in xylene, and then mounted on a synthetic medium (DPX). No counterstain was used.

In each case the Ag NOR "dots" in 100 randomly selected nuclei were counted using a ×100 oil immersion lens. Careful focusing allowed us to view the Ag NOR as black "dots" of varying sizes: large, medium, and small, both in groups and single.

Figure 2. Nude mice with a xenotransplanted osteogenic sarcoma.

Results

Original tumor

Three out of five transplanted tumors proved positive in the nude mice transfer (Figure 2). A firm tumor was found in 2 mice and a cystic tumor was found in 1. No local invasion or metastasis was found.

Transplanted tumor

Xenografts mice. The firm tumor was serially transplanted into 16 nude mice in four passages. The cystic tumor was serially transplanted into 7 nude mice in three passages.

Growth rate. Both tumors grew exponentially in nude mice (Figure 3). The growth rate of the firm

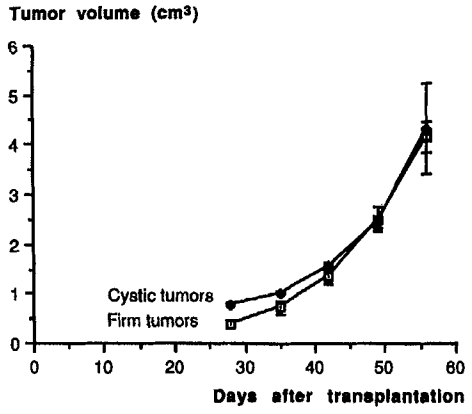

Figure 3. The growth curves from firm tumor (n 15) and cystic tumor (n 3) in all the passages in nude mice. Each line represents the mean value of all the tumors.

Table 1. Histologic characteristics of the xenografted tumors

	Firm tumor	Cystic tumor
Number of mitoses	12.9	6.1
AgNOR[a]	1.6	0.6
AgNOR[b]		
Large	30	30
Medium	50	58
Small	20	12
Grouped	18	31
Single	82	69

[a]Mean value per cell.
[b]Mean value per 100 cells.

tumor (n 15) in all the passages, expressed as the volume doubling time, ranged from 5 to 31 days. The mean volume doubling time of the firm tumor was 12 days. The growth rate of the cystic tumor (n 3) ranged from 12 days to 33 days. The mean volume doubling time of the cystic tumor was 19 days.

Histology

Firm tumor. The gross morphology in all of them exhibited a lobulated tumor with a pseudocapsule. The tumor was firm and whitish (Figure 4A). The histologic studies showed fields with spindle-cell patterns, with cell anaplasia and absence of osseous production by the tumor cells (Figure 5A). The mean number of mitoses was 12.9 by HPF (×40; Table 1).

AgNOR analysis. In all the specimens, clearly defined, silver-stained "dots" were observed in 92 percent of all the nuclei. The pooled mean AgNOR numbers per nucleus was 1.62 (Table 1).

Local invasion and metastatic tumors were not found in autopsies of nude mice.

No histologic or microscopic changes were noticed in this tumor while it was still growing in the nude mice.

Cystic tumor. The gross morphology of all of them showed a brown multilobulated mass with a pseudocapsule. The tumor was soft, hemorrhagic, and cyst-

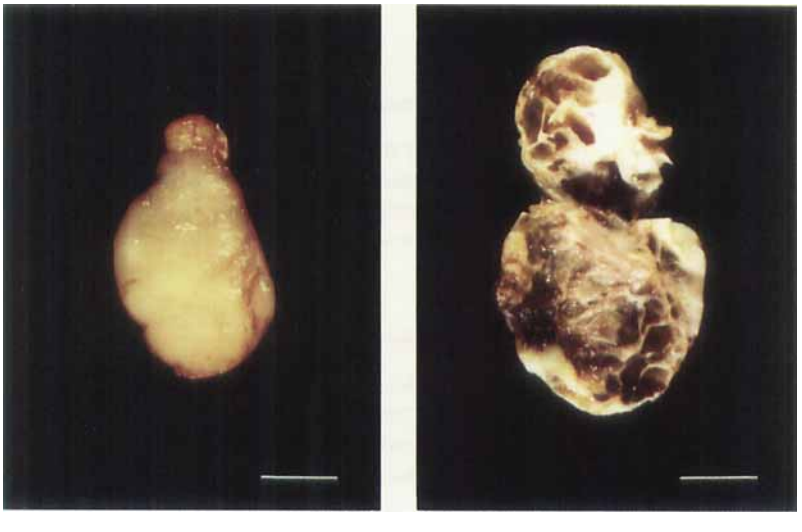

Figure 4. Gross appearance of xenotransplanted osteosarcoma, passage 2. Bar = 1 cm.

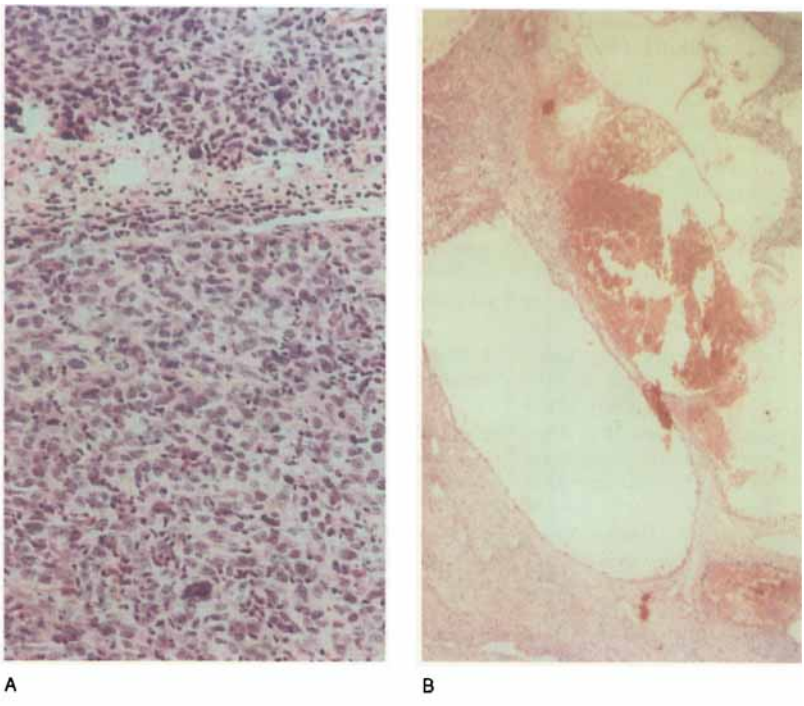

Figure 5. Xenotransplanted osteosarcoma, passage 2.

A. Solid tumor showing spindle cells with the nuclei very closely packed. No osteoid tissue was observed (H&E, ×150).

B. Cystic tumor with proliferation of ominous cells in the stroma surrounding the cyst-like vascular spaces. No masses or streamers of osteoid were observed on the walls of the cyst (H&E, ×35).

like in appearance (Figure 4B). The histologic studies showed large cystic spaces, some of which contained red blood cells (Figure 5B). Surrounding the cystic spaces, spindle cells were observed without any evidence of osseous production by the tumor cells. The mean number of mitoses was 6.1 by HPF (x40).

AgNOR analysis. In all the specimens, clearly defined silver-stained "dots" were observed in 66 percent of all the nuclei. The pooled mean AgNOR number per nucleus was 0.68 (Table 1).

Local invasion and metastatic tumors were not found in the autopsies.

No histologic or macroscopic changes have been noticed in this tumor. This tumor was lost due to the animal's death.

Discussion

Osteoblastic osteosarcoma xenografted into athymic mice developed two different morphologic subtypes: one being a firm tumor and the other a cystic tumor.

Apart from the macroscopic changes that have been mentioned above, we observed in both the macroscopic and the histologic study of the xenografted tumors the disappearance of the osteogenic foci that had existed in the original tumor. Similar changes have been described by Llombart-Bosh et al. (1988). This histologic instability could be related to cell differentiation (Donhuijsen et al. 1988).

Different subpopulations of clonal tumor cells have been distinguished in the osteosarcoma (Chigira and Watanabe 1988, Kubo et al. 1988, Schmidt et al. 1988, Tanooka 1988, Dedhar et al. 1989, Randall et al. 1989). The biological events of tumor progression represent the results of a sequential selection of variant subpopulations within its clone, and this is probably due to genetic instability within the tumor cell population (Nowell 1986).

Our results document the gross morphologic and cellular instability of xenotransplanted osteosarcoma. Tumor heterogeneity might justify a possible failure of systemic clinical or experimental therapy against this type of cancer (Heppner and Miller 1989).

References

Amitani K, Nakata Y. Characteristics of osteosarcoma cells in culture. *Clin Orthop* 1977; 122: 315–24.

Bauer H C, Brosjö O, Broström L A, Nilsson O S, Reinholt F P, Tribukait B. Growth and ploidy of human osteosarcoma xenografts in serial passage in nude mice. *Eur J Cancer Clin Oncol* 1986; 22 (7): 821–30.

Brosjö O. Osteosarcoma and interferon. Studies of human xenografts in the nude mouse. *Acta Orthop Scand* (Suppl 229) 1989: 1–36.

Brosjö O, Bauer H C, Broström L A, Nilsonne U, Nilsson O S, Reinholt F P, Strander H, Tribukait B. Influence of human alpha interferon on four human osteosarcoma xenografts in nude mice. *Cancer Res* 1985; 45 (11): 5598–602.

Chigira M, Watanabe H. Establishment of highly metastatic clones without tumorigenicity derived from Dunn osteosarcoma. *Tumour Biol* 1988; 9 (6): 293–300.

Crocker J, Nar P. Nucleolar organizer regions in lymphomas. *J Pathol* 1987; 151 (2): 111–8.

Crocker J, Boldy D A, Egan M J. How should we count AgNORS? Proposals for a standardized approach. *J Pathol* 1989; 158 (3): 185–8.

Dedhar S, Mitchell M D, Pierschbacher M D. The osteoblast like differentiated phenotype of a variant of MG 63 osteosarcoma cell line correlated with altered adhesive properties. *Connect Tissue Res* 1989; 20 (1–4): 49–61.

Donhuijsen K, Budach V, van Beuningen D, Schmidt U. Instability of xenotransplanted soft tissue sarcomas. Morphologic and flow cytometric results. *Cancer* 1988; 61 (1): 68–75.

Heppner G H, Miller B E. Therapeutic implications of tumor heterogeneity. *Semin Oncol* 1989; 16 (2): 91–105.

Kubo K, Shidou M, Koiwa S, Morita K. Establishment of cell lines from human osteosarcoma. *Proc Jpn Acad* Ser B Phys Biol Sci 1988; 64 (8): 233–236.

Llombart Bosch A, Carda C, Boix J, Pellin A, Peydro Olaya A. Value of nude mice xenografts in the expression of cell heterogeneity of human sarcomas of bone and soft tissue. *Pathol Res Pract* 1988; 183 (6): 683–92.

Mii Y, Tsutsumi M, Shiraiwa K, Miyauchi Y, Hohnoki K, Maruyama H, Ogushi H, Masuhara K, Konishi Y. Transplantable osteosarcomas with high lung metastatic potential in Fischer 344 rats. *Jpn J Cancer Res* 1988; 79 (5): 589–92.

Nowell P C. Mechanisms of tumor progression. *Cancer Res* 1986; 46 (5): 2203–7.

Povlsen C O, Visfeldt J, Rygaard J, Jensen G. Growth patterns and chromosome constitutions of human malignant tumours after long-term serial transplantation in nude mice. *Acta Pathol Microbiol Scand* (A) 1975; 83 (6): 709–16.

Randall J C, Morris D C, Zeiger S, Masuhara K, Tsuda T, Anderson H C. Presence and activity of alkaline phosphatase in two human osteosarcoma cell lines. *J Histochem Cytochem* 1989; 37 (7): 1069–74.

Schmidt J, Strauss G P, Schön A, Luz A, Murray A B, Melchiori A, Aresu O, Erfle V. Establishment and characterization of osteogenic cell lines from a spontaneous murine osteosarcoma. *Differentiation* 1988; 39 (3): 151–60.

Scranton P E Jr, DeCicco F A, Totten R S, Yunis E J. Prognostic factors in osteosarcoma. A review of 20 years' experience at the University of Pittsburgh Health Center Hospitals. *Cancer* 1975; 36 (6): 2179–91.

Tanooka H. Monoclonal growth of cancer cells: experimental evidence. *Jpn J Cancer Res* 1988; 79 (6): 657–65.