Morphological Studies on the Resistance of Cartilage to Invasion by Osteosarcoma Cells in Vitro and in Vivo

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ABSTRACT

Cartilage-bone explants from human ribs and phalanges were simultaneously cultured on Millipore membranes with either human osteosarcoma cells (TE-85) or human primary foreskin fibroblasts. The explant-tumor cell interfaces were histologically compared with biopsies from an osteogenic sarcoma. In both specimens, bone spicules were embedded in tumor cell clusters. Irregularities and lacunae in the spicule surfaces with closely attached tumor cells were considered morphological evidence of direct bone erosion by osteosarcoma cells. In contrast to bone, articular and epiphyseal cartilage resisted tumor invasion. Osteosarcoma cells infiltrated the cartilage matrix only to areas that were occupied by vascularized mesenchyme, such as the capillary loops in the growth plate and the nutrient cartilage canals. Inhibition of tumor cell growth underneath the explant on the bottom of the Petri dish and the delayed outgrowth on Millipore membrane areas occupied previously by cartilage explants suggest the release of substances that inhibit growth and proliferation of tumor cells. No such effects have been observed in control cultures with fibroblasts.

These observations are similar to those obtained in previous studies on the resistance of cartilage to vascular invasion. Since collagenolytic activities have been demonstrated in both endothelial and osteosarcoma cells, it has been suggested that these cells share the mechanisms of invasion involving matrix destruction by collagen breakdown. Collagenolytic activity can be inhibited by a cartilage-derived collagenase inhibitor. The capacity of tumor cells to invade a tissue may therefore depend not only on their ability to elaborate collagenase but also on local regulation of such enzyme activity by inhibitory substances.

INTRODUCTION

Invasion is defined as the passing, interpenetration, or infiltration of a cell or tissue into adjacent tissues. Although simple, this definition not only characterizes the classical tissue invasion by neoplastic cells but also includes more controlled invasive processes, i.e., inflammation, wound repair, and vascularization of tissues during embryogenesis or histogenesis. Tissues such as postnatal hyaline cartilage, however, are rarely invaded by inflammatory or neoplastic cells and resist invasion by capillary sprouts (2, 5, 30, 32, 33). The absence of an intrinsic capillary blood supply and the property of cartilage by which it resists penetration by capillary endothelial sprouts has been shown to be at least partially mediated by biologically active compounds that can be extracted from hyaline cartilage by guanidinium hydrochloride (5, 34). These cartilage-derived substances dramatically decrease the proliferation of endothelial cells in vitro (4, 10). It has been established that the bioactivity within these cartilage extracts resides in molecules with a molecular weight of less than 50,000 daltons (4) and not in the macromolecular proteoglycans (41). Among these lower-molecular-weight substances, a protease inhibitor has been isolated and identified that has the ability to inhibit mammalian collagenases (18, 20, 21). Therefore, it has been hypothesized that the collagenase inhibitor may prevent endothelial cell-derived collagenase from degrading the collagen fibers of the cartilage matrix and thereby may inhibit the capillary endothelial cell sprouts from invading hyaline cartilage (20).

Evidence of collagenase activity in a variety of human and animal tumors and a correlation between collagenase activity and the biological behavior of the tumor have indicated that the invasiveness of tumor cells may be based on their collagenolytic activity (1, 3, 11–15, 25, 31, 37, 42). Recently, we have shown that collagenolytic activity associated with a human osteosarcoma and a metastatic mammary carcinoma cell line can also be inhibited by the cartilage-derived collagenase inhibitor (21). In the present report, in vitro experiments were designed to demonstrate the resistance of cartilage explants to osteosarcoma cell invasion.

For these studies a combined organ-cell culture system was developed that allowed the simultaneous culture of human tissue explants and human tumor cells for sequential examination of the explant-tumor cell interface. Explants of bone, cartilage, and cartilaginous growth plates from human ribs and phalanges were cultured in the presence of human osteosarcoma cells or human fibroblasts. The interface between the explants and the cultured cells was studied by light microscopy. Our observations confirm that bone was eroded by malignant cells, whereas cartilage was shown to be resistant to tumor invasion in vitro. Comparison of similar tumor cell-cartilage or tumor cell-bone interfaces from a patient with advanced osteogenic sarcoma of the distal metaphysis of the femur showed that these in vitro observations mimicked the in vivo situation.

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MATERIALS AND METHODS

Roswell Park Memorial Institute Culture Medium 1640 was made up from commercially supplied powder (Grand Island Biological Co., Grand Island, N. Y.) and then passed through 0.22-μm (pore size) Millipore filters for sterilization. The medium was supplemented with 10% fetal calf serum (Reheis Chemical Co., Kankakee, Ill.), which was not heat inactivated (24). Increased buffering capacity was provided by 25 mM N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (Calbiochem, La Jolla, Calif.), and antibiotics were added to yield concentrations of 50 μg gentamicin (Scher- ing Corp., Kenilworth, N. J.) per ml and 5 μg amphotericin B (E. R. Squibb & Sons, New York, N. Y.) per ml in the complete medium. The final osmolality of the medium at pH 7.2 to 7.4 was 304 mOsmoles/kg.

Human rib growth plates from the costochondral junctions of children aged 11 to 13 years were obtained at the time of therapeutic surgery for scoliosis. Phalanges were obtained from a 3-year-old child at the time of therapeutic amputation. After removal of muscle, fascia, perichon- drium, and periosteum, these specimens were cut longitudi- nally into 6- to 9-mm bars with a twin-blade knife that had a 1-mm blade interspace (22). Each specimen thus contained both cartilaginous growth plate and bone, except in the case of the phalanges, in which articular cartilage was also part of the explant, and in the cases in which explants consisted of only cartilage or bone. All specimens containing bone were trypsinized briefly and agitated mildly to remove marrow and endothelial cells. The tissue preparation was done under aseptic conditions in cold serum-free medium, and the specimens were immediately cultured. In preliminary experiments the plasma clot technique was used for the attachment of explants in the culture dishes, as described previously for the organ culture of dog epiphyses (22). The clots were, however, dissolved regularly by the tumor cells. Therefore, the culture system originally described by Fell and Thomas (6) was applied with the following modifications. The cartilage-bone explants were placed on Millipore membranes (filters; 13 mm in diameter; with 1.2- or 5-μm pore size; Millipore Corp., Bedford, Mass.), which served as a growth surface for both the explants and the cells. These membranes were elevated with the following modifications. The cartilage-bone explants were placed on Millipore membranes (filters; 13 mm in diameter; with 1.2- or 5-μm pore size; Millipore Corp., Bedford, Mass.), which served as a growth surface for both the explants and the cells. These membranes were elevated with Falcon stainless steel grids 2 to 3 mm above the bottom of 35-mm Falcon tissue culture dishes (Falcon Plastics, Oxnard, Calif.). For sustaining the viability of the explants by diffusion, the medium was added until the fluid level wet the membrane (Chart 1). The explants were cultured initially 2 to 3 days during which the growth and viability of the tissue was monitored microscopically. Slight changes in the pH of the medium also indicated metabolic activity and therefore viability of the explants (22). A minimum of 10 explants were used in each of the experimental series.

After the initial culture of the explants for 2 to 3 days, osteosarcoma cells (TE-85), obtained from the Naval Biomedical Research Laboratories, Oakland, Calif., were introduced into the organ culture system. This cell line, established by McAllister et al. (24), has cytological and karyological characteristics similar to those of the parent tumor (by growing to high saturation density and by being anaploid) and was chosen for this study since it can be stimulated in culture to release a collagenase that is inhibited by the cartilage-derived protease inhibitor (21). Primary human foreskin fibroblasts served as control cells. Cells in logarithmic growth phase were removed from Falcon flasks by incubation with a trypsin-EDTA solution. A 0.1-ml aliquot of the suspension was counted in a hemocytomoter by the trypan blue dye exclusion test, and then a 0.1-ml aliquot of the cell suspension containing 2 × 10⁶ cells/ml was pipetted onto the explanted tissues (Chart 1). Control cultures were also established in which the osteo- sarcoma cells and fibroblasts were cultured in the absence of explants. All dishes were incubated at 37° in a humidified 5% CO₂-air atmosphere. The cultures were refed every third day, and the explants were processed for histology after 1 or 2 weeks of culture. In some experiments, the cells from the Millipore membranes were processed for histological evaluation immediately after the removal of the explants from the membrane, whereas in others the cells were cultured further for different time intervals before processing. In selected experiments the cartilage was cultured on the grid in the absence of the Millipore membrane, and the cells on the bottom of the culture dish underneath the explant were examined.

For histological evaluations the explants were fixed in situ or, after removal from the Millipore membranes, were fixed in 10% phosphate-buffered formalin (pH 7.3) overnight at 4°. The explanted cartilage-bone junctions were then removed from the Millipore membranes and placed into a staining dish. Specimens containing bone were decalcified in a solution consisting of 40 ml 10% aqueous nitric acid, 30 ml absolute alcohol, and 30 ml 0.5% aqueous chromic acid. Specimens were then dehydrated in graded ethanol solutions and embedded in paraffin. Sections 5 μm thick were stained with hematoxylin and eosin, Gomori's trichrome, or periodic acid-Schiff. Control specimens of un- cultured tissue and explants cultured in the absence of both tumor and control cells were similarly processed.

The Millipore membranes used as a support for the organ-cell cultures were fixed and stained with hematoxylin and eosin either immediately after explant removal or after an additional culture period of the cells in the absence of the explants. Some membranes were mounted onto cover
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RESULTS

Expiants Cultured in the Absence of Cells

Cartilage and bone explants were examined histologically after 3 days, 1 week, 2 weeks, and 3 weeks. At all times the cartilage showed viability, as indicated by the preservation of shape and size, the stainability of its matrix, and the morphology of the chondrocytes. Similarly, the mesenchyme of the cartilage canals was well preserved. After the first week of culture, a characteristic mesenchymal outgrowth from the cartilage was observed. The lateral surfaces of the explants were usually covered with a single layer of spindle-shaped, fibroblast-like cells. At the interface of explant and Millipore membrane, the medium accumulated by capillary action provided excellent growth conditions in which these cells formed a loose network containing periodic acid-Schiff-positive matrix.

In contrast to cartilage, bone proved to be rather inappropriate for survival in long-term organ cultures of 2 and 3 weeks. At the end of the second week of culture, necrosis of osteocytes was indicated by the appearance of empty spaces in the spicular matrix. No osteocytes were observed at the end of the third week of culture. Disintegration of the matrix was indicated by differences in stainability; the spicule cluster was more intensively stained than were its surface zones. Frequently, the surface of the spicules appeared fuzzy but not eroded or invaginated. Therefore, explants containing bone were harvested after 1 week or, maximal, 10 days of culture to guarantee viability of this tissue. Outgrowth from the bone was not observed since most cells at the spicule surface had been removed during the incubation with trypsin.

Cells Cultured in the Absence of Explants

Osteosarcoma cells formed multiple colonies on the Millipore membranes (Fig. 1). These colonies showed a slow radial expansion and were composed of 1 to 5 layers of spindle-shaped or polygonal cells. Osteosarcoma cells were characterized by numerous slender cytoplasmic processes and by their large hypochromatic nuclei containing multiple nucleoli. Mitotic figures were rather rare, although the doubling time of this cell line was approximately 24 hr (28). In vertical sections through the Millipore membranes, basally located osteosarcoma cells were deeply anchored within the membrane by their cytoplasmic processes (Fig. 2). During the entire experimental period, these cells appeared healthy, although cytoplasmic vacuolization was increased at the end of the third week of culture.

Human foreskin fibroblasts formed a confluent contact-inhibited monolayer on the Millipore membranes. The cells were typically characterized by an elongated dipolar cell body and cigar-shaped nucleus, usually containing 2 nucleoli at the poles (Fig. 3).

Expiants Cultured in the Presence of Cells

Morphology of Explants. The surface of the cartilage was covered with solitary osteosarcoma cell colonies consisting of 1 to 2 layers of less than 10 tumor cells. Neither tumor cells nor their cytoplasmic processes penetrated into the cartilaginous matrix. The lateral surfaces of the cartilage explants, which were usually covered by outgrowing mesenchyme, were devoid of osteosarcoma cells (Fig. 4). However, tumor cells were observed within cartilage canals that are connective tissue invaginations containing blood vessels (26, 40). These tumor cells formed clusters that occasionally occupied the entire canal (Fig. 5). Fibroblasts usually covered the entire cartilage top surface with a coherent monolayer (Fig. 6) and, at the lateral surfaces, intermingled with and became morphologically indistinguishable from outgrowing cartilage cells. At the growth plate, tumor cells reached only as far into the cartilage as the vascular loops extended to the area of the last hypertrophic chondrocyte and its calcified matrix (Fig. 7). Fibroblasts were observed in a similar location, as were osteosarcoma cells.

Multiple osteosarcoma cell clusters were observed in bone explants (Fig. 8). Tumor cells surrounded bone spicules in a tight girdle of 1 to 5 cell layers. The contact between tumor cells and bone was usually so intimate that tumor cells followed the outline of the bone surface exactly (Fig. 9). Spicules surrounded by 1 layer of osteosarcoma cells were characterized by a smooth surface; those surrounded by multiple cell layers or embedded in tumor cell clusters had rough and irregular surfaces (Figs. 9 and 10). These irregularities in the spicule surface (comparable to "moth-eaten" surfaces and to the creeping of tumor cells in these invaginations) suggested an active erosion of bone by osteosarcoma cells. Some viable osteocytes but no osteoclasts were observed in these explants. Erosion of bone was further indicated by the loss of cultured phalangeal bone when it was exposed to osteosarcoma cells (Fig. 12). The bone located between the articular cartilage and the epiphyseal growth plate showed erosive cavities and discontinuations of the spicules. Normal human foreskin fibroblasts grew into the bone cavity and, in contrast to the osteosarcoma cells, formed strands between the bone spicules that lacked close association to the bone. The surface of the spicules was rarely covered with these cells and remained smooth (Fig. 11). Phalanges cultured in the presence of fibroblasts showed no loss of bone (Fig. 13).

Interface of Cells with Explants. As described above, cartilage explants cultured on Millipore membranes exhibited an outgrowth of mesenchymal cells that were most prominent at the cartilage-membrane interface but that also covered the membrane beneath the explants (Fig. 4). Osteosarcoma cells in the same cultures grew in multiple
layers on the Millipore membrane, but they failed to overgrow the mesenchymal cell outgrowth from cartilage. There was no evidence of mixing or interdigitating of the 2 cell types. In contrast, fibroblasts growing on the membrane adjacent to the explants intermingled with and became morphologically indistinguishable from the outgrowing cartilage cells.

Cells after Removal of Cartilage Explants. Osteosarcoma cells or human foreskin fibroblasts were cultured in the presence of either cartilage-bone junctions, cartilage, or bone for extended periods of 1, 2, and 3 weeks. In some experiments the cells were observed immediately after the explants were removed from the Millipore membrane, whereas in others the cells were cultured further for different time intervals. After immediate removal of explants, areas previously occupied by the cartilage explants were practically devoid of cells, especially osteosarcoma cells (Fig. 14). At the border of these areas of "no growth," cells grew together to different densities; few osteosarcoma cells appeared necrobiotic (Fig. 14). In contrast, in areas in which bone explants had been cultured, cells grew beneath the explants, although their density was somewhat less than that outside the explant. In other experiments, osteosarcoma cells were cultured beyond the data of explant removal. The area at which the cartilage explant was originally positioned on the Millipore membrane resisted the migration of tumor cells for approximately 5 days (Fig. 15). In contrast, human foreskin fibroblasts immediately overgrew the area occupied previously by the cartilage explant and covered it with a continuous monolayer. Areas in which bone explants had been cultured were overgrown without a time delay by both osteosarcoma cells and human foreskin fibroblasts. After covering these areas, osteosarcoma cells and fibroblasts exhibited growth characteristics similar to those outside the explant, showing multifocal colonies for osteosarcoma cells and a continuous contact-inhibited monolayer for human foreskin fibroblasts.

Explants Cultured in the Absence of Millipore Membranes

In experiments in which osteosarcoma cells were cultured in the presence of cartilage explanted directly onto the stainless steel grids, the bottom of the Petri dish directly underneath the cartilage piece was found to be devoid of osteosarcoma cells. On removal of the cartilage explant, this zone of no growth was then overgrown within 5 days. No such effect was observed beneath bone explants or with human foreskin fibroblasts cultured in the presence of either cartilage or bone. Similar to the "halo" on the Millipore membrane, cells at the interface between the zone of no growth and the normal growth areas on the bottom of the Petri dish frequently exhibited numerous cytoplasmic vacuoles. Some cells were necrotic.

Histopathology of an Osteogenic Sarcoma

In the present patient the osteogenic sarcoma was located in the distal metaphysis of the femur. A dense gray-white tumor mass, approximately 10 cm in diameter, was found to fill the marrow cavity in the metaphyseal region. The cortex was laterally eroded, and the penetrating tumor had characteristically lifted the periosteum. Residual cortical and metaphyseal cancellous bone was embedded in tumor masses. Surface irregularities and numerous lacunae in bone spicules with closely attached tumor cells were interpreted as morphological evidence for bone resorption in the absence of osteoclasts (Figs. 16 and 17). Osteocytes and osteocyte ghosts were present, as they were in tissue explants described previously. The epiphyseal cartilage, however, was preserved for the most part (Fig. 18), except for the central area of the growth plate cartilage that was penetrated by tumor that invaded the entire distal epiphyseal bone. The extension of tumor from the metaphysis to the epiphysis through preexisting connective tissue canals was promoted by microfractures and associated hematoma. The articular cartilage appeared to represent a barrier to further tumor spread (Fig. 19). The tumor cells closely followed the normal contours of the articular cartilage, spreading as far as the vascular loop extended to the area of the calcified cartilage matrix.

DISCUSSION

Osteogenic sarcomas are highly malignant tumors that almost always occur singly, usually in long tubular bones. About 70% of these neoplasms occur in the lower end of the femur and the upper end of the tibia. Although bone is almost always eroded (2, 30), growth plate or articular cartilage, unless calcified or locally violated, resists further spread of this sarcoma. These observations may indicate that hyaline cartilage either acts as a physical barrier to tumor invasion or contains matrix compounds that are translated into a specific antinvasion system. In this study the interaction of human cartilage-bone explants and human osteosarcoma has been examined in an organ-cell culture system.

The morphological data provide evidence that bone spicules from both explants and biopsies of osteogenic sarcoma were eroded by osteosarcoma cells. Surface irregularities and lacunae of bone spicules with closely attached tumor cells were indicative of a direct bone erosion and resorption in the absence of osteoclasts. Nevertheless, the ability of tumor cells to erode and resorb bone was not without controversy, as the number of studies done with tissue culture may indicate (7-9, 29, 35-37, 39). In some studies, enhanced bone resorption by multiple explants of human tumors placed around the rat calvaria bone has been explained by the release of "resorption-stimulating factors" that should mainly act on osteoclasts (8, 9). In other studies, intraosseously implanted tumor caused an early proliferation of osteoclasts; once the intraosseous tumor explants were large enough to envelope residual bone spicules, the osteoclasts disappeared, but the bone resorption continued (7). The ratio between tumor cells and bone seems to be decisive for the bone resorption by this neoplasm. A similar effect was observed in our organ-cell culture system. Spicules surrounded by a single layer of osteosarcoma cells usually showed a smooth bone surface, whereas those wrapped in tumor cell clusters or those enveloped by multiple layers of tumor cells had irregular surfaces with numerous lacunae, suggesting bone erosion (Figs. 9 and 10). These observations were similar to those in human osteogenic sarcomas in vivo. Erosion of
bone spicules in the epiphysis (Fig. 16) and metaphysis (Fig. 17) was associated with the wrapping of bone spicules in tumor cells. Neither osteoclasts nor osteoblasts and other potential bone-resorbing cells were found between tumor cells and spicules. This observation that cells other than osteoclasts are able to resorb bone is not without precedence. Recently, Mundy et al. (27) reported that cultured human peripheral blood monocytes without morphological differentiation into osteoclasts are capable of eroding killed-fetal-rat bones. Since cortisol significantly inhibited this mononuclear cell-stimulated bone erosion, it was speculated that this effect was due to inhibition of lysosomal enzyme release from these cells. Lysosomal enzymes have been implicated in bone resorption (38), and cortisol has been shown to inhibit the release of lysosomal enzymes by cultured bones (6). However, we believe that, in the system described here, the bone, at least in the biopsy specimen, was alive and directly eroded by the tumor cells both in vivo and in vitro.

Cartilage was able to resist the invasion by osteosarcoma cells in the organ-cell culture system as well as in the biopsies of an osteogenic sarcoma. When cartilage explants were cultured in the presence of osteosarcoma cells, the top surfaces of the explants were covered with solitary tumor colonies. The lateral surfaces and the bottom surface were devoid of tumor cells, and only in the cartilage canals were osteosarcoma cells infiltrating the blood vessel-containing connective tissue matrix. At the growth plate, tumor cells reached only as far as the columnar cartilage as the vascular loops had extended, prior to their removal by trypsin incubation of the explants, to the area of the last hypertrophic chondrocyte and its calcified matrix. Since tumor cells in vivo and in vitro will only occupy spaces of the cartilage in which previously blood vessels had been located, it is suggested that the mechanisms by which cartilage resists invasion are similar for both tumor and endothelial cells. In experiments with vascular endothelium, the capillary sprouts of the chick chorioallantoic membrane were unable to reach a cartilage block explanted with a Millipore membrane onto the chick chorioallantoic membrane (23). However, calcified tissue or cartilage altered experimentally by selective extraction was readily penetrated by vascular loops of the chick chorioallantoic membrane. Recently, we have shown that cartilage from which these low-molecular-weight substances were extracted was invaded not only by endothelial cells but also by osteosarcoma cells. These findings lead to the conclusion that cartilage contains extractable matrix compounds that inhibit invasion in an experimental system. When these diffusible or extractable compounds were further studied, it was found that the inhibition of endothelial cell proliferation was caused by molecules with a molecular weight of less than 50,000 daltons (4). From this specific cartilage extract, a protease inhibitor has been identified that has the ability to inhibit mammalian collagenases, including that released by endothelial cells (20). Therefore, the theory was forwarded that the resistance of cartilage to blood vessel invasion may be, at least in part, regulated by this cartilage-derived collagenase inhibitor.

In the present study the release of certain factors from the cartilage, which influenced growth and proliferation of tumor cells, was indicated by the limited growth of tumor cells on the cartilage surfaces and, more specifically, by the inhibition of the tumor cell growth underneath the cartilage explants, even when this explant had been removed from the growth-supporting Millipore surface. A similar phenomenon was observed when cartilage explants were placed on the supporting grids without the Millipore membranes. Osteosarcoma cells did not form cell layers beneath the grids and, even after removal of the cartilage explant, the tumor cells overgrew this area of negligible or no growth only after a delay of 5 days. This may indicate that the cartilage has released material that attaches to the surface of the organ culture dish, elaborating its growth-inhibitory activity toward the tumor cells. In control cultures with fibroblasts, no such effect was observed. These observations lead to the possible conclusion that the inability of tumor cells to penetrate cartilage may not be due to a physical barrier that this tissue provides against the invading cells.

Endothelial cells and tumor cells evidently share, mechanistically, the characteristics of invasion by destroying connective tissue matrices, especially collagen. Collagenolytic activity has been demonstrated in both endothelial cells and the osteosarcoma cell line used in these investigations. Enzyme activity from both of these cell lines can be inhibited by a cartilage-derived, low-molecular-weight collagenase inhibitor (20, 21). Therefore, these observations permit the hypothesis to be forwarded that cartilage is resistant to both capillary and tumor cell invasion since it contains a specific collagenase inhibitor (18). The capacity of tumor cells to invade a tissue not only may depend on their ability to produce collagenase but also may be locally regulated by substances that inhibit connective tissue-degradative enzyme activities of invasive cells (16, 17, 19).

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REFERENCES

Fig. 1. Human osteosarcoma cells cultured on Millipore membranes for 8 days, forming multiple colonies that expand radially and are composed of several layers of predominantly spindle-shaped cells. H & E, × 150.

Fig. 2. An osteosarcoma cell colony composed of 4 layers of polygonal cells, shown in a vertical section through the Millipore membrane after a culture period of 10 days. Cytoplasmic processes (arrows) are deeply anchored into the membrane. H & E, × 385.

Fig. 3. A confluent contact-inhibited monolayer of human fibroblasts growing for 8 days on the Millipore membrane. The cells are typically characterized by a dipolar cell body and cigar-shaped nucleus. H & E, × 145.

Fig. 4. Cartilage expiant cultured on a Millipore membrane for 10 days, exhibiting extensive mesenchymal outgrowth (double arrow). Osteosarcoma cells are on the same membrane (arrow). There is no intermingling of the 2 cell lines. Periodic acid-Schiff, × 60.

Fig. 5. Human osteosarcoma cells after 14 days in culture, occupying a cartilage canal that consists of a blood vessel-containing connective tissue. No tumor cells are found within the cartilaginous matrix. Periodic acid-Schiff, × 60.

Fig. 6. A single sheet of fibroblasts (arrows) covering the entire cartilage surface in a 10-day culture. H & E, × 145.

Figs. 7 to 10. Human osteosarcoma cells cultured in the presence of rib costochondral junctions.

Fig. 7. Tumor cell invasion inhibited by growth plate cartilage in a 10-day culture (arrows). Tumor cells reach as far as the vascular loops to the area of the last hypertrophic chondrocyte and its calcified matrix. H & E, × 450.

Fig. 8. Bone spicules embedded in a dense cluster of tumor cells in an 8-day culture. The clefts at the tumor cell-spicule interface are probably artifacts introduced during the processing of the tissue for histology. H & E, × 450.

Fig. 9. Multiple layers of tumor cells in intimate contact with a bone spicule, after 10 days in culture. The spicule shows an irregular surface with deep invaginations, suggesting bone erosion by adjacent tumor cells. H & E, × 450.

Fig. 10. Irregularities of the bone spicule surface at attached tumor cell cluster, suggesting active bone erosion by tumor cells in a 10-day culture. H & E, × 450.

Fig. 11. Human fibroblasts are cultured for 14 days in the presence of rib costochondral junctions. Strands of fibroblasts are formed between bone spicules. The surface of the bone spicule is not covered with cells and remains smooth. H & E, × 450.

Fig. 12. Phalanx cultured in the presence of osteosarcoma cells. The bone mass is reduced, and the spicules are irregular, invaginated, and fragmented. The cartilage shows smooth and unaltered cut surfaces (Culture Period, 1 week).

Fig. 13. Phalanx cultured in the presence of fibroblasts. Size and shape of both bone and cartilage are well preserved (Culture Period, 1 week).

Fig. 14. Millipore membrane area previously occupied by cartilage expiant for 10 days, devoid of osteosarcoma cells. At the border of these areas of no growth, cells grew together to different densities; few osteosarcoma cells appear necrobiotic (arrows). H & E, × 65.

Fig. 15. Osteosarcoma cells cultured for 4 days beyond the date of cartilage removal from the Millipore membrane. Osteosarcoma cells at the border of no growth appear fibroblast-like and are migrating into the area of no growth. H & E, × 165.

Figs. 16 to 19. Osteogenic sarcoma arising in the distal metaphysis of the femur of a 10-year-old girl.

Fig. 16. Bone spicule in the metaphysis, surrounded tightly by osteosarcoma cells. The irregularities in the bone surface suggest bone-resorptive activity mediated by tumor cells. Osteoclasts are absent. H & E, × 190.

Fig. 17. Remnant of bone spicule embedded in tumor of the epiphysis. Tumor cells are in close contact with an irregular bone surface. H & E, × 470.

Fig. 18. Columnar cartilage of the growth plate, resisting invasion by blood vessels and tumor cells. H & E, × 190.

Fig. 19. Articular cartilage resisting invasion by osteosarcoma cells. H & E, × 190.
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growth plate

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bone
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