Osteosarcoma Cells Differentiate into Phenotypes from all Three Dermal Layers

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Abstract

Background Osteosarcomas are the most common solid malignant bone tumors, but little is known of their origin. The embryonal rest hypothesis views cancer cells as arising from committed progenitor stem cells in each tissue. Adult tissue contains primitive stem cells that retain the ability to differentiate across dermal lines, raising the possibility that the stem cell of origin of cancers may be from a more primitive stem cell than a progenitor.

Questions/purposes Can osteosarcoma cells, when cultured under conditions used for multipotent stem cells, be induced to differentiate into multiple phenotypes, including those of the three different dermal lineages: mesodermal, ectodermal, and endodermal?

Methods One rat and one human osteosarcoma cell line were cultured and treated with concentrations of 0, 10^{-10} , 10^{-9} , 10^{-8} , 10^{-7} , and 10^{-6} mol/L dexamethasone for 5 weeks. Seventeen phenotypes were assayed either by tissue-specific histochemical stains or antibodies to tissue-specific proteins. Each phenotype was tested across all dexamethasone concentrations for each cell line and each phenotype was tested in three separate experiments with induction by dexamethasone

Results Rat osteosarcoma (ROS) 17/2.8 and human osteosarcoma cell line U-2 show the appearance of cells that have markers for (1) mesodermal phenotypes such as bone, cartilage, skeletal muscle, and endothelial cells,

(2) ectodermal phenotypes such as astrocytes, oligodendrocytes, neurons, and keratinocytes, and (3) an endodermal phenotype, hepatocytes. This indicates osteosarcomas are composed, at least in part, of primitive stem cells capable of differentiating into tissues from all three dermal lineages.

Clinical Relevance If osteosarcomas arise from primitive stem cells, then treatment of osteosarcomas with exogenous differentiation agents may cause the stem cells to differentiate, thus halting their proliferation and stopping tumor growth.

Introduction

A current hypothesis on cancer cell origin states that neoplasms are formed from progenitor stem cells found in various adult tissues [11, 14, 26, 41, 46, 59]. Stem cells are defined by two parameters: extended proliferation potential and the ability to differentiate into one or more phenotypes [3, 4, 6, 56]. Normal adult tissue contains multipotent adult stem cells that retain the ability to differentiate into several mesodermal phenotypes from all three dermal lineages [32, 50] and progenitor stem cells that have the limited differentiation capability of forming only the cell types in that tissue [51]. Tumors are thought to arise from clonal expansion of a progenitor cell to a group of cells consisting of partially or wholly differentiated cells that no longer divide and a small percentage of cells that continue to divide – cancer stem cells [11, 15, 16, 42, 51, 53, 59]. This hypothesis has been supported by the observations that fully differentiated malignant neoplasms express a distinguishable phenotype of that particular tissue [5, 14, 15, 33] and that undifferentiated neoplasms are monoclonal in origin [5, 7, 16, 34]. These two findings also suggest

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differentiation corresponds to the incremental lineage progression of progenitor stem cells [38].

Some studies indicate that osteosarcoma cells also arise from progenitor stem cells, although the particular stem cell has not been identified but it is thought to be an osteoprogenitor cell capable of differentiating into phenotypes seen in cartilage and bone [9, 17, 19, 20, 55]. We suggest the stem cells giving rise to the cancer may be more primitive than an immediate progenitor cell, ie, a multipotent stem cell. Teratomas form tissue from all dermal layers and thus would seem to arise from cells that are either embryonic stem cells or have the same pluripotent ability to differentiate into phenotypes from all three dermal lineages [6, 52]. Osteosarcomas reportedly contain cells that express markers for mesodermal tissue such as cartilage and muscle [23], and ectodermal tissue markers like cytokeratin [27]. Ewing's sarcoma contains cells with mesenchymal, epithelial, and neural features [9, 10, 46]. Sarcoma cells promote neuronal and glial differentiation [19, 21, 22], which means that the cells with these markers in Ewing's sarcoma could have arisen from nonsarcoma cells. However, none of these studies showed that osteosarcoma cells could differentiate to phenotypes other than cartilage or bone.

We therefore asked whether two osteosarcoma cell lines, when grown under conditions used to isolate and

differentiate multipotent adult stem cells (MASCs), could be induced to differentiate into phenotypes of all three dermal lineages: mesodermal, ectodermal, and endodermal?

Materials and Methods

Cells from two separate osteosarcoma cell lines, one rat and one human, were tested for the ability to differentiate in vitro when induced with dexamethasone. Cells from each cell line were cultured in the wells of five 24-well plates (total = 120 wells for each cell line) of six columns and four rows (total of 20 rows for each cell line). The cells were treated with six dexamethasone concentrations, one concentration per column, for 5 weeks to induce differentiation. Each of the 20 rows was used for the assay of one particular phenotype. Thus, 17 different phenotypic assays and three control rows were performed for each cell line, with each phenotypic assay having one well of all six dexamethasone concentrations (one row of six wells). This constituted one experiment. The experiment was repeated three times for each cell line, thus providing three wells per dexamethasone concentration per phenotypic assay per cell line (Fig. 1).

Rat osteosarcoma (ROS 17/2.8) cells are a clonal cell line derived from a rat osteosarcoma and we obtained them

Fig. 1 A flow chart of one experiment to induce phenotypes in vitro for one cell line is shown. The entire study consisted of six such experiments, three for each cell line.

from the originator of the cell line, Dr. Gideon Rodan. The cell line is alkaline phosphatase-positive and responds to parathyroid hormone, both properties characteristic of osteoblasts [33]. U-2 cells were obtained from ATCC (Manassas, VA, USA). This line originated from a moderately differentiated sarcoma of the tibia of a 15-year-old girl [13, 14, 43]. Both osteosarcoma cell lines have an extended proliferation potential. We obtained them at more than 50 cell doublings and tested them at five to 20 cell doublings (one to four passages) past that. ATCC and Dr. Rodan authenticate their cell lines. Both cell lines were kept separate, with each cell line having media changed separately and being passaged separately. Each cell line had media changed separately using separate pipettes, etc. We checked the karyotype of both cell lines when they were received and then before the experiments.

Both cell lines were obtained frozen and then were grown and tested as described for mammalian MSCs [32, 50]. They were thawed and plated at a density of 100,000 cells per 100-mm culture dish (Corning Glass Works, Corning, NY, USA) that previously had been coated with 1% gelatin (Fisher Scientific, Pittsburgh, PA, USA) in Eagle's minimal essential medium (EMEM) + 10% preselected horse serum. The cells proliferated to confluence after approximately 8 days in culture and were released from the dish with 0.025% trypsin (Invitrogen, Carlsbad, CA, USA) in Dulbecco's phosphate-buffered saline (PBS) with 0.01% EDTA.

The cells then were frozen in aliquots of 1 mL containing 10^6 cells in EMEM + 10% horse serum and 7.5% dimethyl sulfoxide (Sigma-Aldrich Corp, St Louis, MO, USA). Cryopreservation was performed in freezing chambers (Fisher Scientific, Norcross, GA, USA) to slow freeze the cells to -80°C [32, 50]. After being frozen for at least 24 hours, aliquots of the frozen cells were thawed and plated at a density of 5000 cells per 16-mm well in all 24 wells of five 24-well gelatin-coated culture plates (Corning) in EMEM + 10% horse serum to start the experiments.

The 24-well plates have six columns and four rows of wells. Each column was treated with dexamethasone (Sigma-Aldrich) at a concentration of 0, 10^{-10} , 10^{-9} , 10^{-8} , 10^{-7} , or 10^{-6} mol/L for 5 weeks beginning on Day 1. Differentiation was assessed by histologic or immunohistochemical staining for specific phenotypes, and each phenotype assay was performed across one row of a plate (all six dexamethasone concentrations). Each experiment consisted of 17 phenotype assays and three control rows of no primary antibody for the immunohistochemical assays. Three experiments of five plates each were performed for each osteosarcoma cell line. Thus, each phenotypic assay was performed in three wells of each dexamethasone concentration for each cell line (Fig. 1).

The number of positively stained cells or, in the case of cartilage and bone, positively stained nodules, in each well was counted and then averaged over all three wells of each dose of dexamethasone for each phenotype for each cell line. As dexamethasone is a nonspecific and inefficient inducer of differentiation, results are expressed as a range of values: $-$ = no cells stained in a 16-mm well; $+$ = one to 10 cells or nodules stained per well; and $++$ = 10 to 25 cells or nodules stained per well.

Proteoglycans were identified with safranin O staining at pH 7.0 or Alcian BlueTM staining (Sigma-Aldrich) at pH 1.0. Neutral lipid was stained with Sudan Black

The presence of most phenotypes was determined by antibody labeling of the cell cultures in 24-well plates as described above. One row of a plate, consisting of all six dexamethasone concentrations, was tested with a single antibody. Different antibodies were used in different rows. The cells in each well undergoing antibody testing were washed three times in PBS. The cultures were fixed with -20°C methanol for 5 minutes followed by three more PBS rinses. The cells in each well were incubated in 0.25 mL 0.3% H_2O_2 in 0.1% Triton[®] X-100 (Dow Chemical Co, Midland, MI, USA) in PBS for 10 minutes followed by three PBS rinses. The cultures were incubated in 0.4 mL 1% goat serum in 0.5% Tween-20[®] (Croda Inc, Edison, NJ, USA) in PBS for 30 minutes at 37°C followed by three PBS rinses. The cultures were incubated with 0.3 mL of the primary antibody in PBS with 0.1% Triton[®] X-100 for 60 minutes at 37°C and washed three times with PBS with a second blocking step of 1% goat serum in PBS with 0.5% Tween-20[®] for 20 minutes at 37°C . The cultures then were incubated with 0.3 mL of secondary antibody, horseradish peroxidase-labeled goat anti-mouse IgG (KPL Inc, Gaithersburg, MD, USA) diluted 1:2000 in PBS at 37°C for 30 minutes followed by five PBS rinses. TrueBlueTM (KPL), 0.3 mL, was added and the cultures were incubated in the dark for up to 20 minutes followed by five rinses with distilled water. Photographs were taken on a light microscope. Three rows on three separate 24-well plates, containing all dexamethasone concentrations, were used as a negative control without primary antibody. One of us (PAL) evaluated all photomicrographs.

Primary antibodies for phenotypes obtained from Sigma-Aldrich were: muscle-type intermediate filaments with monoclonal anti-desmin clone De-U-10 at 1:400 [13], oligodendrocytes or myelin with monoclonal anti-CNPase [1, 44], monoclonal antibody VM-1 for keratinocytes at 1:200, monoclonal antibody for glial fibrillary acidic protein (GFAP) for astrocytes at 1:400, and smooth muscle α -actin (IA4) (Sigma-Aldrich) at 1:400. Primary antibodies for phenotypes obtained from the Developmental Hybridoma Bank were: striated muscle with antibody to heavy chain of myosin (MF-20), cartilage with Type II collagen

(CIICI), bone with bone sialoproteins I and II (WVIDI 9C5), osteonectin (MPIIB10₁), P2A4 and P2B1 for endothelial cells. Neurons were identified with spinal cord neurons (Rat-401) and phosphorylated neurofilaments (RT-97). All antibodies were used at 1:200 dilution. Hepatocytes were identified by markers. A monoclonal antibody to α -feto-protein (AF), obtained from Sigma-Aldrich and as a generous gift of Dr. Stewart Sell, were used at 1:10 dilution. All antibodies, according to the source, are known to react with rat and human antigens.

Results

U-2 osteosarcoma cultures but not ROS 17/2.8 had safranin O-stained nodules in the controls (Table 1). U-2 cultures had safranin O-stained areas, indicative of the presence of proteoglycan, in the controls (Table 1). This staining was diffuse without discernible nodules (Fig. 2A). The size and shape of the safranin O nodules in the U-2 cultures changed with increasing dexamethasone concentrations. There were distinct nodules in 10^{-10} (Fig. 2B) to 10^{-8} mol/L dexamethasone. At 10^{-7} and 10^{-6} mol/L dexamethasone, the safranin O areas in the U-2 cultures were more diffuse (Fig. 2C) but limited in area to a small portion of the culture (data not shown). ROS 17/2.8 cells had no safranin O-stained nodules in either the controls or 10^{-10} mol/L dexamethasone (Table 1). The ROS 17/2.8 cultures showed distinct nodules whenever they were induced (Fig. 2D). All nodules for ROS 17/2.8 showed staining for Type II collagen, but in the U-2 cultures, only nodules in cultures treated with 10^{-10} to 10^{-8} mol/L dexamethasone showed staining for Type II collagen (Fig. 3A). Nodules of bone appeared only with dexamethasone treatment and were identified by extracellular matrix staining for osteopontin (Fig. 3B) and bone sialoprotein II (10^{-8} mol/L dexamethasone [Fig. 3C]; 10^{-9} mol/L dexamethasone [Fig. 3D]). Several other mesodermal phenotypic markers were observed with dexamethasone treatment but not in controls. These included skeletal myotubes stained with an antibody to sarcomeric myosin (Fig. 4A), adipocytes identified with staining for neutral lipid (Fig. 4B), and smooth muscle cells stained with antibody to smooth muscle α -actin (Fig. 4C). The last two appeared only at 10^{-8} to 10^{-6} mol/L dexamethasone (Table 1). Endothelial cells were identified by uptake of acetylated low-density lipoprotein and with two different antibodies (Fig. 5). Endothelial cells also were present at 10^{-8} to 10^{-6} mol/L dexamethasone.

Ectodermal tissue was identified with staining various neural tissues and keratinocytes. No neuronal markers were seen in control cultures. Cells stained for GFAP

Table 1. Summary of phenotypes observed in the osteosarcoma cell lines

Antibody/stain	Dexamethasone concentration (mol/L)					
	0 (control)	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}
Safranin O	-/+	-/++	++	++	+	+
CIICI	-	-	-	+	++	++
MPIII	-	-	-	+	++	+
WVID1	-	+	++	+	+	+
MF-20	-	-	+	++	+	-
SBB	-	-	-	+	++	++
IA4	-	-	-	+	+	+
LDL	-	-	-	+	++	++
P4A4	-	-	-	+	++	++
P2B1	-	-	-	+	++	++
GFAP	-	+	+	++	++	+
CNPase	-	-	-	++	++	+
RT-97	-	+	++	+	-	-
VM-1	-	-	-	-	+	++
OV-6	-	-	+	+	+	-
H4	-	-	+	+	-	-
AFP	-	-	+	+	+	+

- = no cells stained in a 16-mm well; + = one to 10 cells or nodules stained per well; ++ = 10 to 25 cells or nodules stained per well; -/+ or -/++ = ROS 17/2.8 cells had no cells/nodules stained per well but U-2 cells had cells/nodules stained at the levels indicated; antibodies and stains: safranin O (pH 7.0) = stain for cartilage; CIICI = antibody against Type II collagen; MPIII = antibody against osteopontin; WVID1 = antibody against bone sialoprotein II; MF-20 = antibody against sarcomeric myosin; SBB = Sudan Black B, stain for neutral lipids; IA4 = antibody against smooth muscle α -actin; LDL = rhodamine-labeled acetylated low-density lipoprotein; P4A4 = antibody against human umbilical vein endothelial cells; P2B1 = antibody against platelet/endothelial cell adhesion molecule (PECAM); GFAP = antibody against glial fibrillary acidic protein; CNPase = antibody against 2',3'-cyclic nucleotide-3'-phosphodiesterase; RT-97 = antibody against neurofilaments; VM-1 = antibody against keratinocytes; OV-6 = antibody against hepatocytes; H4 = antibody against hepatocytes and hepatocarcinoma cells; AFP = antibody against α -fetoprotein.

(astrocytes) were seen in all dexamethasone-treated cultures but were more numerous at 10^{-7} (Fig. 6A) and 10^{-8} mol/L dexamethasone (Table 1). Cells stained positive for CNPase were present only at 10^{-8} (Fig. 6B) to 10^{-6} mol/L dexamethasone (Table 1). In contrast, cells stained positive for neurofilaments were seen at 10^{-10} to 10^{-8} (Fig. 6C) mol/L dexamethasone concentrations (Table 1). Cells stained with an antibody against keratinocytes, another ectodermal tissue, were seen with dexamethasone-treated cultures but were not seen in controls. We found these cells only in cultures treated with 10^{-6} (Fig. 6D) and 10^{-7} (Fig. 6E) mol/L dexamethasone (Table 1). One group of cells at 10^{-7} mol/L dexamethasone appeared to be epithelial (Fig. 6E).

Fig. 2A–D The photomicrographs show (A) control U-2 cells (Original magnification, $\times 40$); (B) U-2 cells treated with 10^{-10} mol/L dexamethasone (Original magnification, $\times 40$); (C) U-2 cells treated

with 10^{-6} mol/L dexamethasone (Original magnification, $\times 40$); and (D) ROS 17/2.8 cells treated with 10^{-9} mol/L dexamethasone (Original magnification, $\times 100$) in culture at 35 days stained with safranin O.

Fig. 3A–D The photomicrographs show U-2 cells in (A) culture treated with 10^{-7} mol/L dexamethasone and stained with antibody to Type II collagen (CH2C); (B) a bone nodule in a culture treated with 10^{-7} mol/L dexamethasone and stained with MPIII, an antibody to osteonectin; (C) a bone nodule in a culture treated with 10^{-8} mol/L

dexamethasone and stained with WVIDI, an antibody to bone sialoprotein; and (D) a bone nodule in a culture treated with 10^{-9} mol/L dexamethasone and stained with WVIDI. The cells were treated with dexamethasone for 35 days (Original magnification, $\times 100$).

Fig. 4A–C The photomicrographs show ROS 17/2.8 cells (A) in culture treated with 10^{-8} mol/L dexamethasone and stained with an antibody to myosin heavy chain (arrows point to myotubes with three nuclei); (B) in culture treated with 10^{-6} mol/L dexamethasone and stained with Sudan Black B (arrows point to adipocytes); and (C) in culture treated with 10^{-7} mol/L dexamethasone and stained with an antibody to smooth muscle α -actin. The cells were treated with dexamethasone for 35 days (Original magnification, $\times 200$).

Fig. 5A–B (A) The photomicrograph shows a culture of U-2 cells treated with 10^{-8} mol/L dexamethasone stained with an antibody for endothelial cells (P4A4). The arrows point to stained cells (Original magnification, $\times 60$). (B) This photomicrograph shows a culture

Cells staining with antibodies against hepatocytes, an endodermal phenotype, also were identified in some dexamethasone-treated cell cultures but not in controls. Three different hepatocyte antibodies stained cells in culture. Cells positive for OV-6 (Fig. 7A) were present at 10^{-9} to 10^{-7} mol/L dexamethasone, cells positive for H4 (Fig. 7B) were present at 10^{-9} and 10^{-8} mol/L dexamethasone, and cells positive for α -fetoprotein (Fig. 7C) were present in cultures treated with 10^{-9} to 10^{-6} mol/L dexamethasone (Table 1).

Discussion

Stem cells are defined as cells having an extended proliferation potential and the ability to differentiate into one or more phenotypes [3, 56]. Several studies have suggested cancers arise from progenitor stem cells [9, 17, 20, 55] thought to be progenitors specific to the tissue in which the cancer is located. We presumed the stem cells giving rise to osteosarcoma may be more primitive than an immediate progenitor cell. We therefore tested whether two osteosarcoma cell lines, when grown and induced to differentiate under conditions used to test MSCs, possessed the ability to differentiate into phenotypes representative of tissues of mesodermal, ectodermal, and endodermal origins.

We recognize limitations of our study. First, we tested only two osteosarcoma cell lines. One, ROS 17.2.8, is a clonal rat osteosarcoma cell line that has been used as a model for osteoblasts [33]. The other, U-2, was obtained from an osteosarcoma in a 15-year-old girl. We have not tested every osteosarcoma but focused on two representative osteosarcoma cell lines: one having several characteristics of differentiated osteoblasts and the other from a typical human osteosarcoma exhibiting moderate differentiation. Second, we did not test for the induction of every possible phenotype in the human body but looked at

treated with 10^{-7} mol/L dexamethasone stained with a different antibody (P2B1) for endothelial cells (Original magnification, $\times 100$). The cells were cultured in dexamethasone for 35 days.

Fig. 6A–E The photomicrographs show U-2 cells differentiated into cells displaying ectodermal markers in cultures at 35 days in (A) culture treated with 10^{-7} mol/L dexamethasone and stained with an antibody to GFAP, a marker for astrocytes (Original magnification, $\times 100$); (B) culture treated with 10^{-8} mol/L dexamethasone and stained with antibody to CNPase, a marker for oligodendrocytes (Original magnification, $\times 150$); (C) culture treated with 10^{-8} mol/L

dexamethasone and stained with an antibody to neurofilaments (RT-97) (arrows point to positively stained cells) (Original magnification, $\times 100$); (D) culture treated with 10^{-6} mol/L dexamethasone and stained with VM-1, an antibody to keratinocytes (Original magnification, $\times 200$); and (E) culture treated with 10^{-7} mol/L dexamethasone stained with VM-1 (Original magnification, $\times 200$).

representative phenotypes from each dermal lineage, including phenotypes expected to be in an osteosarcoma, such as cartilage, bone, and vascular phenotypes, and phenotypes not normally associated with osteosarcomas, such as skeletal muscle, fat, neurons, and hepatocytes.

Both osteosarcoma lines exhibited the induction of expected and unexpected mesodermal phenotypes. U-2 cultures had light staining with safranin O at pH 7.0, a marker for glycosaminoglycan in the absence of dexamethasone (controls). However, the same control wells for

the U-2 cultures did not have any Type II collagen-positive cells, which may indicate the safranin O staining was for glycosaminoglycans not associated with cartilage. The ROS 17/2.8 and U-2 cultures treated with dexamethasone contained nodules that stained with antibodies to proteins unique to cartilage and bone (Table 1). This is consistent with studies showing osteosarcoma cells form bone *in vivo* [23, 33, 43]. Phenotypic markers characteristic for smooth muscle and endothelial cells also were seen with dexamethasone treatment. Osteosarcomas are vascular, but the

Fig. 7A–C The photomicrographs show U-2 cells in (A) culture treated with 10^{-9} mol/L dexamethasone stained with OV-6; (B) culture treated with 10^{-8} mol/L dexamethasone stained with H4; and (C) culture treated with 10^{-9} mol/L dexamethasone stained with antibody to α -fetoprotein. The cells were cultured for 35 days; arrows point to stained cells (Original magnification, $\times 150$).

vasculature could arise from the stroma. These results indicate osteosarcoma cells could differentiate to the vascular cells in the sarcoma. Dexamethasone treatment, however, also induced the differentiation of cells expressing phenotypic markers unique to adipose tissue or skeletal muscle, phenotypes not associated with osteosarcomas and not reported in osteosarcomas in vivo.

Both osteosarcoma cells differentiate into phenotypes representing the ectodermal lineages. Dexamethasone induced the appearance of markers for astrocytes, oligodendrocytes, and neurons. Dexamethasone also induced cells that stained with an antibody specific for keratinocytes.

Finally, dexamethasone induced cells expressing markers unique to the endodermal phenotype, hepatocytes. Our results are consistent with those of other studies of non-osteoblast phenotypes, including some ectodermal phenotypes, in osteosarcomas in vivo [2, 8, 12, 23, 27, 30, 39, 40, 45, 46, 49]. However, no studies have reported the presence of staining in osteosarcomas in vivo for neurons, oligodendrocytes, skeletal muscle, or adipocytes.

Both osteosarcoma cell lines showed induction of the endodermal phenotype hepatocytes. There currently are no published studies of endodermal phenotypes in osteosarcomas in vivo.

The osteosarcoma lines responded to dexamethasone treatment in vitro in an identical fashion to MSCs isolated from normal tissue. First, when MSCs are treated with dexamethasone, each phenotype has a characteristic range of dexamethasone concentrations at which phenotypic expression is most marked [31, 32, 50, 58]. Both osteosarcoma cell lines exhibited the same pattern of differentiation in response to dexamethasone as MSCs. Second, the number of positively stained cells for any given phenotype was low, never more than 50 stained cells in a 16-mm culture well. Third, cells positive for markers for several phenotypes were present at any given dexamethasone concentration, which is expected as dexamethasone is a nonspecific inducer and thus will induce multiple phenotypes. When isolating MSCs from normal tissue, we selected for stem cells by the freeze-thaw method which kills committed cells present in the tissue while maintaining undifferentiated cell viability [31, 32, 50, 58]. Both osteosarcoma cell lines were cultured in fetal calf serum by ATCC or Dr. Rodan. Previous work has shown fetal calf serum contains differentiation factors that differentiate progenitor and multipotent stem cells in culture [31, 32]. We therefore applied the same process to the osteosarcoma cell lines to eliminate any progenitor or differentiated cells produced before we obtained the cells. The U-2 cultures are probably clonal, but it is possible that they contain progenitor cells. However, the ROS 17/2.8 cells are clonally derived and were selected for osteoblastic properties [33]. Thus, the ability of the ROS 17/2.8 cells to differentiate in response to dexamethasone in an identical fashion to MSCs cannot be attributed to the presence of progenitor cells but indicate the cells are multipotent.

It is known that 95% or greater of the cells in osteosarcomas do not proliferate whereas only approximately 5% or less of the cells continue dividing [18, 20, 28, 37, 57, 60, 62]. The proliferating cells are called cancer stem cells. Cancer stem cells, then, are identified by only one of the criteria for stem cells – extended proliferation potential. The potential of cancer stem cells to differentiate, and thus their type of stem cell, has not been conclusively shown. Mesenchymal stem cells (MSCs) reportedly can be induced to

become osteosarcoma-like cells in vitro [7, 29, 36]. However, in these studies, genetic manipulation of the MSCs was required for differentiation and no attempt was made to isolate MSCs from osteosarcomas. Thus it is not known whether osteosarcoma cells are derived from MSCs. In addition, MSCs generally are limited to differentiation in the mesodermal lineage. Our observations suggest cultures of osteosarcoma cells consist of multipotent stem cells capable of differentiating into phenotypes of all three dermal lineages. The originating stem cells may be more primitive than previously thought. This is consistent with the formation of teratomas in the adult [24, 25, 35, 47, 48, 52]. These tumors must arise from a primitive stem cell with differentiation potential across all three dermal lineages. That the osteosarcoma cells respond to dexamethasone in a fashion identical to that of MSCs when grown and tested under the same conditions raises the possibility that the primitive stem cell might be MSCs. Studies are underway to determine whether these osteosarcoma cell lines have the cell surface markers associated with MSCs [32, 61].

We propose that all osteosarcomas arise from primitive multipotent stem cells and respond to inductive signals in the local environment to commit to and partially progress down an osteogenic lineage. We further suggest some of the cells in the osteosarcoma remain multipotent with the ability to proliferate – the cancer stem cells. As we did not test all osteosarcomas, we cannot be certain that, in reality, all sarcomas contain multipotent stem cells. However, if this is the case, treatment of osteosarcomas with exogenous differentiation agents may cause the small fraction of multipotent, cancer stem cells in the osteosarcoma to differentiate, thus causing them to cease proliferating [51] and providing a new treatment in the armamentarium against osteosarcomas.

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