Article is available online at http://www.webio.hu/por/2004/10/3/0174

SEMINAR

Chondrosarcoma Cell Differentiation

Experimental data and possible molecular mechanisms

Joseph G SINKOVICS

Cancer Institute, St. Joseph's Hospital; Departments of Medicine and Medical Microbiology-Immunology, The University of South Florida College of Medicine, Tampa, FL, USA

A mixed population of lymphocytes from a healthy donor co-existed with an established culture of allogeneic chondrosarcoma cells, during which time the tumor cells changed from malignantly transformed to benign fibroblast-like morphology; from multilayered to a monolayered growth pattern; lost their potency to grow in colonies in soft agar; and showed signs of senescence. A discussion of possible molecular mechanisms for this event is offered. If there are as yet undiscovered lymphokines that can induce reversal of the malignant geno/phenotype, the cognate gene(s) should be cloned for genetic engineering and for the mass production of the corresponding molecular mediators for clinical trials. (Pathology Oncology Research Vol 10, No 3, 174–187)

Keywords: chondrocytes, chondrosarcoma cells, immune T cell, NK cell, cytotoxic lymphocytes, tumor cell differentiation, telomerase

Introduction

Chondrocytes. Physiological differentiation of chondrocytes advances in stages.[9,109] The uncommitted mesenchymal cells develop vimentin-positive and collagen type 2-producer chondroprogenitor cells. These cells stain with alcian blue. Mature chondrocytes produce collagen type 10 and alkaline phosphatase. Prior to that, hypertrophic chondrocytes arise. These cells mineralize their matrix and induce neoangiogenesis; in response, capillary sprouts invade the matrix. Hypertrophic chondrocytes lose their fibroblastic phenotype, produce the proteoglycan aggrecan, but no fibronectin. They show metachromasia when stained with toluidine blue. Their laminin receptor, switches from $\alpha 6B\beta 1$ to $\alpha 6A\beta 1$.[10,86] Some of these cells utilize the bone morphogenic protein and its receptor (BMP-R) in an autocrine circuit and assume transdifferentiation along osteocytic lineages. BMP, activins and inhibins are members of the transforming growth factor β (TGFβ) family.[1,30,33,61,76,115,117] TGFβ and BMP interact with the Smad complex (*vide infra*) and with the mitogen-activated protein kinases (MAPK). [115,124] Recombinant human BMP-2 (rh-BMP-2) acting through Indian hedgehog (Ihh, *vide infra*) gene transactivation promotes chondrocyte differentiation toward mature cartilage and/or along osteocyte-osteoid pathways.[116]

Chondrocyte differentiation is further regulated by the interaction of chondrocyte inductive growth factors (GF) and their antagonists. Insulin-like GF and its receptor (IGF-R) promote chondrocyte differentiation, induce proteoglycan production and upregulate $p21^{waf1/cip1}$, a growth inhibitory protein (wild type p53 activated protein/cyclin-dependent kinase interacting protein).[38,63,64,75,112] Chondrocyte-specific protein-10 elicits enchondral ossification; thereafter some of the mature (senescent) chondrocytes die apoptotic deaths.[1,57]

Parathyroid hormone-related peptide (PTHrP)[20,56,58,68] initiates enchondral bone formation. BMP-2 is generated by Smad 9; BMP-R crossactivates Smads 1, 5 and 9. The Smads are signaling mediator proteins that shuttle from cytoplasm to nucleus and recruit histone deacetylases. The human *smad* genes are related to *Drosophila* genes *mad* and proteins MAD: "mothers against deca-pentaplegic" (DPP). MADR1 is the human homologue of *Drosophila* protein MAD. BMP4 is the vertebrate homologue of the DPP protein.[34,38,51,80]

Received: April 5, 2004; *accepted:* August 10, 2004

Correspondence: Joseph G SINKOVICS MD, Cancer Institute, St. Joseph's Hospital; Departments of Medicine and Medical Microbiology-Immunology, The University of South Florida College of Medicine, 3001 W Dr Martin Luther King Jr Blvd, Tampa, FL, USA. Tel: (813) 870-4225, fax: (813) 870-4825

The c-*maf* gene product protein c-Maf brings about terminal differentiation of osteopontin-positive chondrocytes. The oncogene v-*maf* fused with the viral *gag* gene was discovered in the avian retrovirus AS42. This retrovirus transduced the ancient c-*maf* gene and induces *m*usculo-*a*poneurotic *f*ibrosarcomas in chicken.[65] The Maf protein is active in multiple myeloma (for references, see[95]).

Cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) (for references, see [95]) are stimulators of chondrocyte differentiation. Yet these mediators are also expressed in de-differentiated chondrocytes.[39,52,53,69]

Ihh gene product proteins through their receptor (R) "patched", induce cartilage nodule formation. Ihh-to-R "patched" interactions result in suppression of the initiation of chondrogenesis and in down-regulation of Sox9. Ihh accelerates terminal differentiation of chondrocytes producing type 10 collagen.[23,108] The chondrocyte differentiation transactivation factor, which mediates the maintenance of nonhypertrophic chondrocytes for PTHrP, is also suppressed by Ihh. Transcription factor activating transcription factor (ATF2) suppresses cyclin D1 and upregulates decorin expression in chondrocytes.[11,40]

Basic fibroblast GFs (bFGF) antagonize chondrocyte differentiation by transactivating the gene encoding the matrix G1a protein (MGP). This effect is abolished by actinomycin D. bFGF antagonize IGF, which upregulates MGP. TGFβ induces Smads, the suppressors of osteochondrogenic differentiation.[5,22,26,43,62,78,82,110] Tumor necrosis factor-α (TNFα) activates the anti-apoptotic nuclear factor κB (NFκB), suppresses Sox9 and antagonizes chondrogenesis.[111] The Sox genes are members of the mammalian sex determining gene family termed SRY-related HMG-box genes and encode DNA-binding motifs of the homeobox proteins.[119] Activated signal transduction and transcription factors (STAT) and upregulated $p21^{waf1/cip1}$ inhibit chondrocyte proliferation.[6,36,83] In transformed cells, STAT contributes to the malignant pheno/genotype (references cited [95]).

The Wnt family of proteins acting through their receptor (R) "frizzled", signal the translocation of βcatenin into the nucleus (Wnt4), or induce phosphatidyl-inositol-3 kinase (PI3K) and protein kinase C (PKC).[27,53,121] PKCα and ξ suppress de-differentiation of chondrocytes backward toward mesenchymal progenitor cells of fibroblast-like morphology. IGF-1 also inhibits de-differentiation through PI3K/Akt and PKC activation by antagonizing NFκB.[52,54] PI3K, PKC and IGF significantly contribute to the malignant behavior when mutated or constitutively overactivated in tumor cells (references cited[95]). The RevT reticuloendotheliosis retrovirus of turkeys carries the v-*rel* gene, which derived from an ancient cellular c-*rel* gene. The locus of the human c-*rel* gene is 2p14-15. The gene product protein is the anti-apoptotic transactivator NFκB. The AKR mouse thymoma-inducing retroviral v-*akt* gene derived from c-*akt*; the gene product protein Akt2 is a proto-oncogene in the PI3K/Akt pathway (references cited[95]).

The term Wnt stands for the fused genes "wingless" from the fruit fly and *int* from the mouse. These genes encode cognate ligands for the "fizzled" R. The not fused Wnt genes are essential for normal embryonic development including the formatting of cartilagenous tissues. One Wnt pathway suppresses the enzyme glycogen synthetase kinase 3β, which eliminates βcatenin through proteasomic degradation. βCatenin mediates the linking of cadherin to the cytoskeleton. When Wnt is mutated and βcatenin is overexpressed due to faulty elimination, the malignant behavior of transformed cells is enhanced. The cystein-rich glycoproteins, Wnt1 and 5b are co-expressed in hypertrophic chondrocytes. Wnt4 accelerates, Wnt5 delays chondrocyte hypertrophy; Wnt1 and 7 block chondrogenesis. The mediator of Wnt signaling, βcatenin, suppresses chondrocyte differentiation. PTHrP also negatively regulates this process. For chondrogenesis of mesenchymal stem cells, PKC activity is required. The PKC proteins also function as proto-oncogenes (for references, see [95]). Homeobox gene product proteins promote chondrocyte maturation through the pathway leading to postmitotic hypertrophy of chondrocytes. The homeobox-containing gene D1x5 is active during the conversion of immature proliferating chondrocytes into postmitotic hypertrophying chondrocytes and in periosteal bone formation, thus this gene may also be an osteoblast maturation regulator.[28]

Connective tissue GFs (CTGF) are members of the platelet-derived GF ligand-to-receptor (PDGF-R) family. Proto-oncogene v-*sis* of the **si**mian **s**arcoma retrovirus derives from c-*sis,* which encodes the B chain of the ligand. These are mitogenic ligands switched on and off, except when mutated and constitutively overactivated in malignant cells (references cited[93]). CTGF expression in chondrosarcoma cells decreases as tumor grade rises: in grade 1 tumors 84%, in grade 2 tumors 53% and in grade 3 tumors 27% of the tumor cells express CTGF.[25,41,71,73,87,123]

Chondrosarcoma cells. The two major types of chondrosarcomas originate centrally in a bone (central chondrosarcoma) or in the cartilaginous cap of an osteochondroma (peripheral chondrosarcoma).[14] The *ext*1, 2 genes regulate FGF and Ihh/PTHrH-to-R signaling. When the suppressor function of these genes is lost due to mutations, the autosomal dominant multiple exostosis syndromes with enchondromas set in. In hereditary multiple chondrosarcomas, these genes suffer germ cell mutations. There are no detectable somatic mutations of these genes in sporadic chondrosarcomas.[15,35,120] Expressions of FGF2, FGF-R1, PTHrP, Bcl-2 and p21 genes and proteins increase with the grade of malignancy in chondrosarcomas.[8,58]

Mesenchymal chondrosarcomas[2,37] originate from focally differentiating pre-chondrogenic cells. The low-grade clear cell chondrosarcomas may de-differentiate into an immature aggressive cell population.[3,4,42] Chondrosarcomas may de-differentiate along various mesenchymal lineages. In the myxoid variant of de-differentiated chondrosarcoma, there is a t(9;22)(q22-31;q11-12) translocation.[7,24,113] Increased Abl protein expression inhibits apoptosis of chondrosarcoma cells.[74] The ancient c-*abl* proto-oncogene was transduced by the Abelson murine leukemia retrovirus (v-*abl*). The proto-oncogene c-*erb*2 is overexpressed in 90% of chondrosarcomas. The normal cartilage is negative for the Erb2 protein. Erb2 protein expression is inversely related with grade: low-grade chondrosarcomas express more Erb2 protein than high-grade de-differentiated tumors.[60,77] The avian erythroblastosis viruses transduced the ancient cellular proto-oncogenes c-*erb*A and c-*erb*B; the gene product proteins are members of the epidermal growth factor receptor (EGF-R) family. In high-grade de-differentiated chondrosarcomas assuming malignant fibrous histiocytoma-like appearance, mutated p53 is overexpressed, whereas low-grade tumors or cartilage are negative for p53 deficiency.[13,88]

The FGF-inducer protein Sox9 regulates differentiation of multipotent stem cells along chondrogenic pathways.[83,119] Decorin and type 2 collagen mRNA levels rise. The large 300 kDa isoform of the oligomeric glycoprotein of extracellular matrix, tenascin, is overexpressd in chondrosarcoma cells.[29] When chondrosarcoma cells differentiate toward more mature chondrocytic phenotype, they assume metachromasia upon staining with toluidine blue. Polyphenotypic differentiation[72] may continue toward rhabdo-myoblastic phenotypes positive for desmin. Clear cell extraskeletal myxoid chondrosarcomas are S100-negative, enolase-positive and chromogranin A-positive.[3,42] Extraskeletal myxoid chondrosarcomas undergo gene translocations resulting in neuroectodermal/endocrine differentiation.[7,24] FGF1, 2 induce type 2 collagen production, whereas inflammatory cytokines IL1β and TNFα suppress this effect. FGF9 counteracts cytokine-mediated repression of Sox9: FGF is a Sox agonist.[83] The anti-inflammatory cytokine IL-4 suppresses chondrosarcoma cell proliferation.[32,84] Dexamethasone also decreases chondrosarcoma cell proliferation and induces maturation of tumor cells.[44]

When multipotential mesenchymal stem cells become chondrocytes, the process is referred to as differentiation.[55,81] Resumption of the fibroblast-like morphology of the mesenchymal stem cells is termed de-differentiation. Under changed cultural conditions, de-differentiated chondrocytes re-differentiate and form cartilage.[85] In the case of chondrosarcoma cells, could a retrograde change toward chondrocytic and mesenchymal pre-chondrocytic fibroblast-like stem cells, which are or are not yet malignantly transformed, be considered to be in the realm of de-differentiation?[17] A resulting cell population resembling that of malignant fibrous histiocytoma would display significant gains in grade and malignancy. When progenitor chondrosarcoma cells form myoblast-like cells, the process is considered to be differentiation along lineages deriving from immature mesenchyme toward more mature cell types.[4,72] The polyphenotypic differentiation of a malignantly transformed mesenchymal stem cell can be envisioned as occurring simultaneously in several lineages, of which one reaches dominance. The sarcoma subtype is determined by this dominant lineage, for example: lipoblasts for liposarcoma. When the second and the third sublineages emerge (for example: myoblasts or fibrohistiocytoblasts) and overgrow the hitherto dominant lineage, the term de-differentiation along these mesenchymal lineages is applied. The original tumor cells do not retro-differentiate to the level of the transformed multipotent mesencymal stem cell to start a new differentiation process. Instead, the original progenitor cell was initiating concurrently several cell lineages along mesenchymal differentiation pathways and these lines expressed varying degrees of dominance. However, when a chondrosarcoma cell population converts into an orderly monolayered sheet of resting large fibroblast-like cells devoid of morphological and biological features of mesenchymal stem cells and is undergoing senescence, differentiation into an entirely new direction is occurring.[89,99]

An episode of *in vitro* lymphocyte-mediated apparent differentiation of human chondrosarcoma cells was reported in the mid-1970s.[89,99] In the elapsed thirty years, no comments were raised in the literature, neither in support for, nor in objection to, this observation. In the present era of molecular immunology, this phenomenon should be re-investigated, because its mechanism could now be elucidated. The earliest observations (1969-70) on the interactions between autologous or allogeneic lymphocytes and sarcoma cells are briefly recited in order to present this remarkable occurrence in context.

Materials and Methods

The Patient. In 1968 a 58-year-old man, (#73587) (*Figure 1*), was admitted to the Solid Tumor (later: Melanoma/Sarcoma) Service at the Department of Medicine, The University of Texas M.D. Anderson Hospital, Houston, TX.[103,104] The patient developed a very large tumor within the bones of his right hemipelvis. An extraosseous mass of the tumor eventually invaded the ascending colon. By biopsy it was a chondrosarcoma with infrequent mitoses of the tumor cells and with lymphocytic infiltrations at the periphery of the tumor. No distant metastases were detectable. The patient was managed with an ileostomy and catheterization of his urinary bladder;

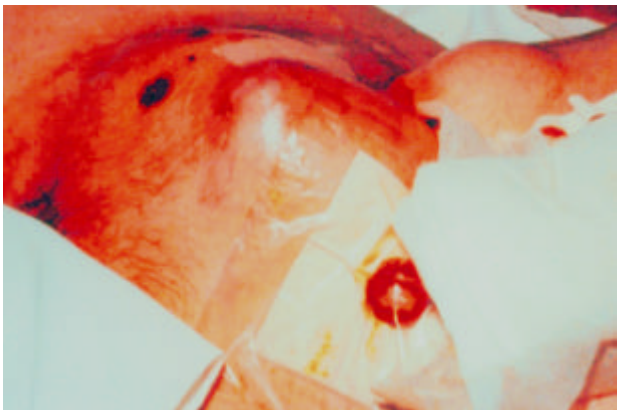

Figure 1. *Tumor of a male patient (MDAH #73587) 58 years of age*

received radiotherapy and was treated with chemotherapy (doxorubicin was not yet available), and was relieved with a subarachnoid block and a prefrontal lobotomy for control of pain, anxiety and suffering. X-ray irradiated (10,000 r) cultured autologous tumor cells were used to immunize the patient by repeated vaccinations. His lymphocytes collected from the buffy coat were directly injected into his tumor. No clinically measurable responses to these treatment modalities were observed. He lived two years and died with repeated gram-negative septicemias and endotoxin shock. A postmortem examination revealed no metastases. He and his wife allowed several biopsies of his tumor and he contributed several blood samples for medical research.

Tissue Cultures. The cell line #1459 was established[98] in November 1968 from a tumor sample obtained before chemo-radiotherapy (*Figure 2*). Transmission electron microscopy of the #1459 cell line was described before.[102] Several primary cultures were grown later from the patient's tumor. His normal fibroblasts were grown once in 1969 in a primary (not established) culture from a subcutaneous site not involved with tumor. Tissue cultures were grown in T flasks, Leighton tubes and Sykes-Moore chambers and were fed with fetal calf serum-containing media in laminar air-flow hoods.

Blood Samples. The patient's lymphoid cells were concentrated from the buffy coat by centrifugation or by the ficoll-hypaque technique.[114] His blood serum or plasma samples were tested with or without heating at 56ºC against his primary and established tumor cell lines with and without lymphocytes added. Fluorescein isothiocyanate-conjugated rabbit anti-human globulin immune sera were used for immunofluorescence stains. Lymphocyte and serum samples of a healthy donor's were tested as controls against the patient's tumor cells and fibroblasts. In assays for lymphocyte-mediated cytotoxicity against tumor cells, the number of lymphocytes exceeded tumor cells 100-500 to 1.

Results

Cytotoxic lymphocytes. The patient's lymphocytes immediately surrounded his tumor cells *in vitro* and in 24 hours the lymphocytes killed the tumor cells (*Figure 3*).[96] These lymphocytes exerted no cytotoxicity on the patient's normal fibroblasts. Tumor cell death occurred by nuclear clumping or by cytoplasmic lysis. The attacker lymphocyte population consisted of small cells with compact nuclei. Occasionally, larger lymphocytes with granular cytoplasm also participated in tumor cell lysis. Most lymphocytes survived and preserved tinctorial characteristics of live cells and could be extracted from cultures of killed tumor cells, and when transferred into new cultures of the patient's tumor cells, the lymphocytes exerted cytotoxicity once again against the tumor cells. Occasionally, single individual cells of the attacker lymphocyte population appeared to disintegrate with nuclear clumping. The patient's serum samples rather inhibited, than promoted lymphocyte-mediated cytotoxicity. By indirect immuno-

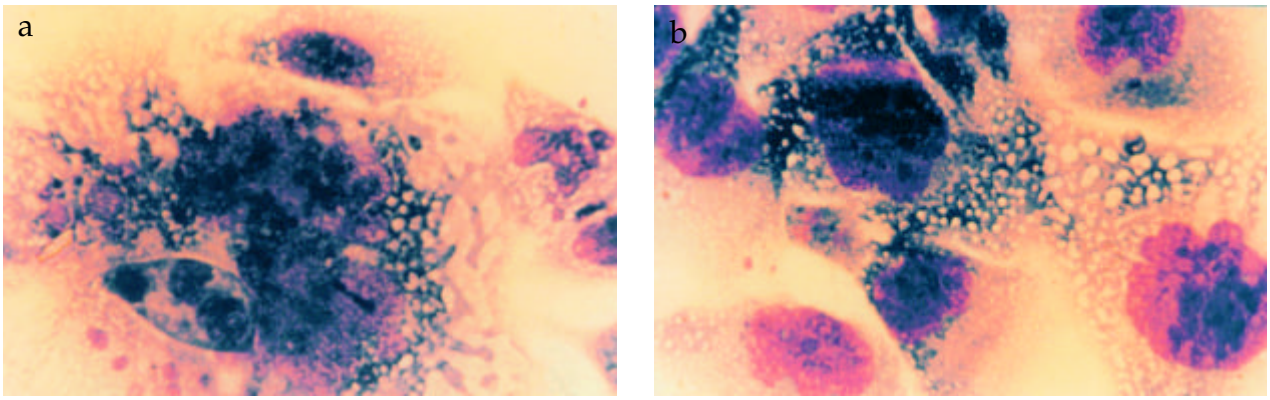

Figure 2. *Chondrosarcoma cell line #1459. Early passage 3* ***(a)*** *and late passage 25* ***(b).*** *The cultures consist of tumor cells devoid of stromal cells. (Note: the highest passage number of #1459 cell line was 35).*

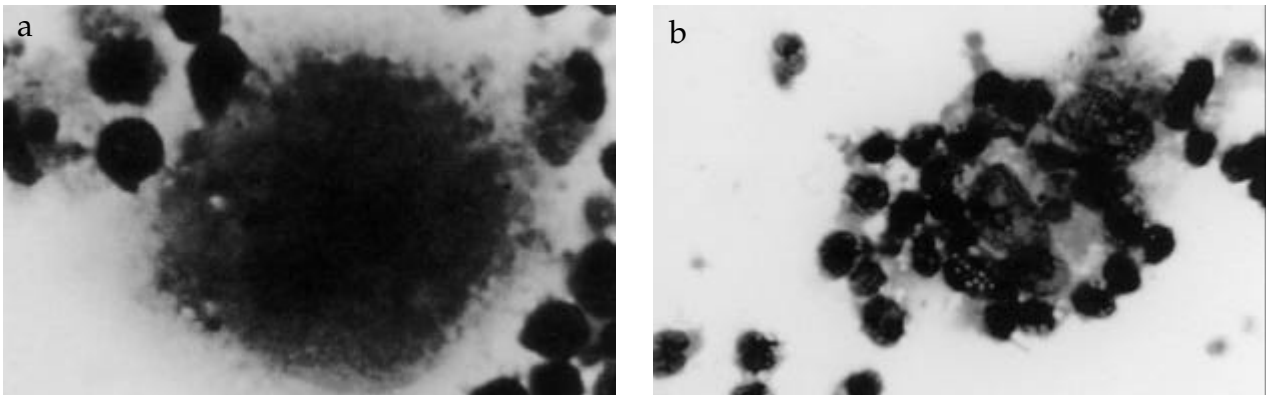

Figure 3. ***(a)*** *Chondrosarcoma cell of culture #1459 is attacked by the patient's (autologous) "small lymphocytes with compact nuclei".* ***(b)*** *Chondrosarcoma cells of culture #1459 die with cytoplasmic lysis and nuclear clumping under the attack of the patient's autologous small lymphocytes with compact nuclei. (Ektachrome #337; Sept 8, 1969)*

fluorescence staining, the patient's serum samples reacted with cytoplasmic antigens expressed by the patient's tumor cells;[103,104] this reactivity appeared to be intensified after repeated administrations of the autologous tumor cell vaccine.

Lymphoid cell preparations of the healthy donor exerted not prompt, but somewhat delayed cytotoxicity on the patient's tumor cells; some of these lymphocytes also reacted with the patient's fibroblasts. Serum samples of the patient rather intensified, than inhibited the cytotoxicity that the healthy donor's lymphocytes exerted against the patient's tumor cells. The attacker lymphocyte population of the healthy donor was dominated by large cells with granular cytoplasm, but a few small lymphocytes with compact nuclei also participated in the reaction (*Figure 4*).

Differentiation-inducer lymphocytes. By late 1969 and early1970, the 33rd passage of the established cell line #1459 was deemed to be free of nontransformed stromal cells.[98] The healthy donor's lymphocytes extracted from the buffy coat and/or ficoll-hypaque purified were added to the 33rd passage of these tumor cells. This lymphocyte population consisted of a mixture of large cells with granular cytoplasm (in majority) and small cells with compact nuclei and less granulated cytoplasm (in minority). Contrary to expectations, the allogeneic lymphocytes exerted cytotoxicity on the tumor cells only occasionally. Instead, this lymphocyte population co-existed with the tumor cells practicing emperipolesis and browsing over the tumor cells (*Figure 5*). On rare occasions, some lymphocytes were observed to die with nuclear clumping. During a coexistence of 4 to 10 weeks, the number of lymphocytes

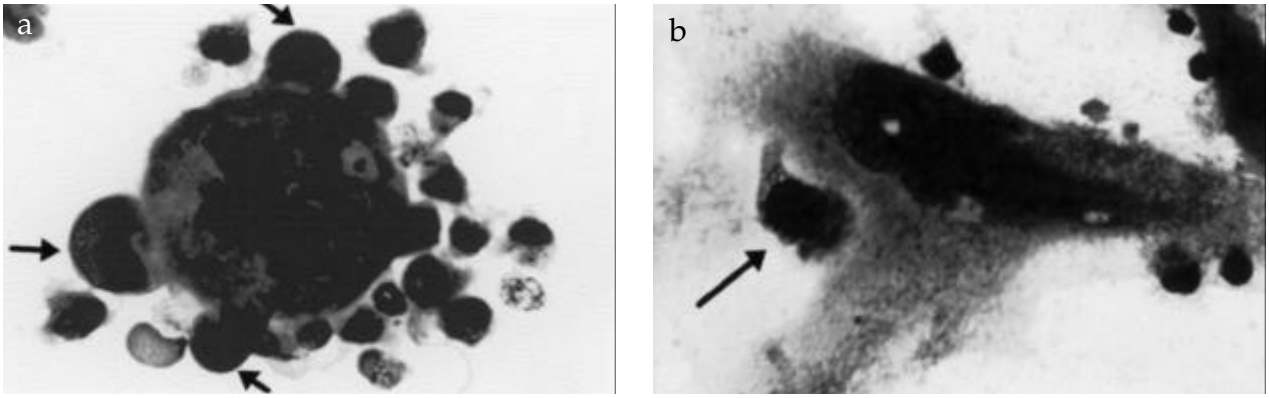

Figure 4. *Chondrosarcoma cell of established culture line #1459 advanced passage[98] exposed to allogeneic lymphocytes of healthy donor's.* ***(a)*** *"Large lymphocytes with granular cytoplasm" (later: NK cells) attach themselves to the membrane of the tumor cell (arrows); "small lymphocytes with compact nuclei" (later: immune T cells) congregate around the tumor cell without launching an attack. In contrast to* ***figure 3a:*** *in the autologous setting, small lymphocytes with compact nuclei dominate, whereas in the allogeneic setting large lymphocytes with granular cytoplasm lead the attack.* ***(b)*** *Comparison of large allogeneic lymphocyte (later: NK cell, arrow) and small lymphocytes with compact nuclei (later: immune T cell) of the healthy donor JGS next to a tumor cell. These probably are the very first pictures published on human NK cells (photographed on Dec 3, 1969)[45, 102] attacking tumor cells.*

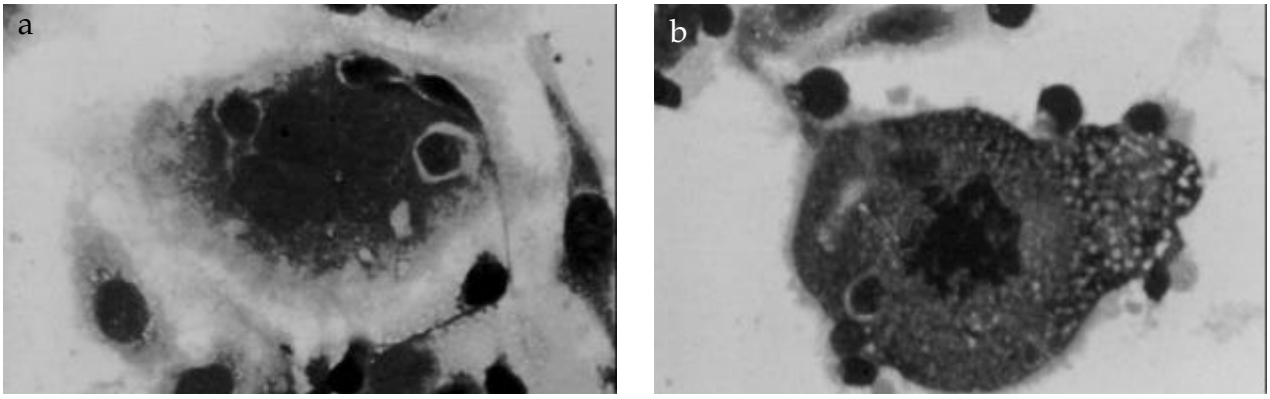

Figure 5. *Chondrosarcoma cells #1459 from passage 33 is surrounded and browsed ("emperipolesis") but not attacked by allogeneic small lymphocytes with compact nuclei of the healthy donor's. (Film #371; April 12, 1970)*

gradually decreased in the co-cultures; by the 6th week, lymphocytes almost completely disappeared from the cultures leaving behind an orderly monolayered sheet of large cells of fibroblast-like morphology (*Figure 6*). These cultures were almost completely devoid of the originally explanted large tumor cells with lobulated nuclei, frequent multipolar divisions and disorderly growth pattern (cells piling up on each other). The remaining large fibroblast-like cells appeared to be in a resting phase with rare mitotic figures and showed no metachromasia when stained with toluidine blue or alcian blue. Previously, cells from the 22nd passage of culture #1459 grew in colonies in soft

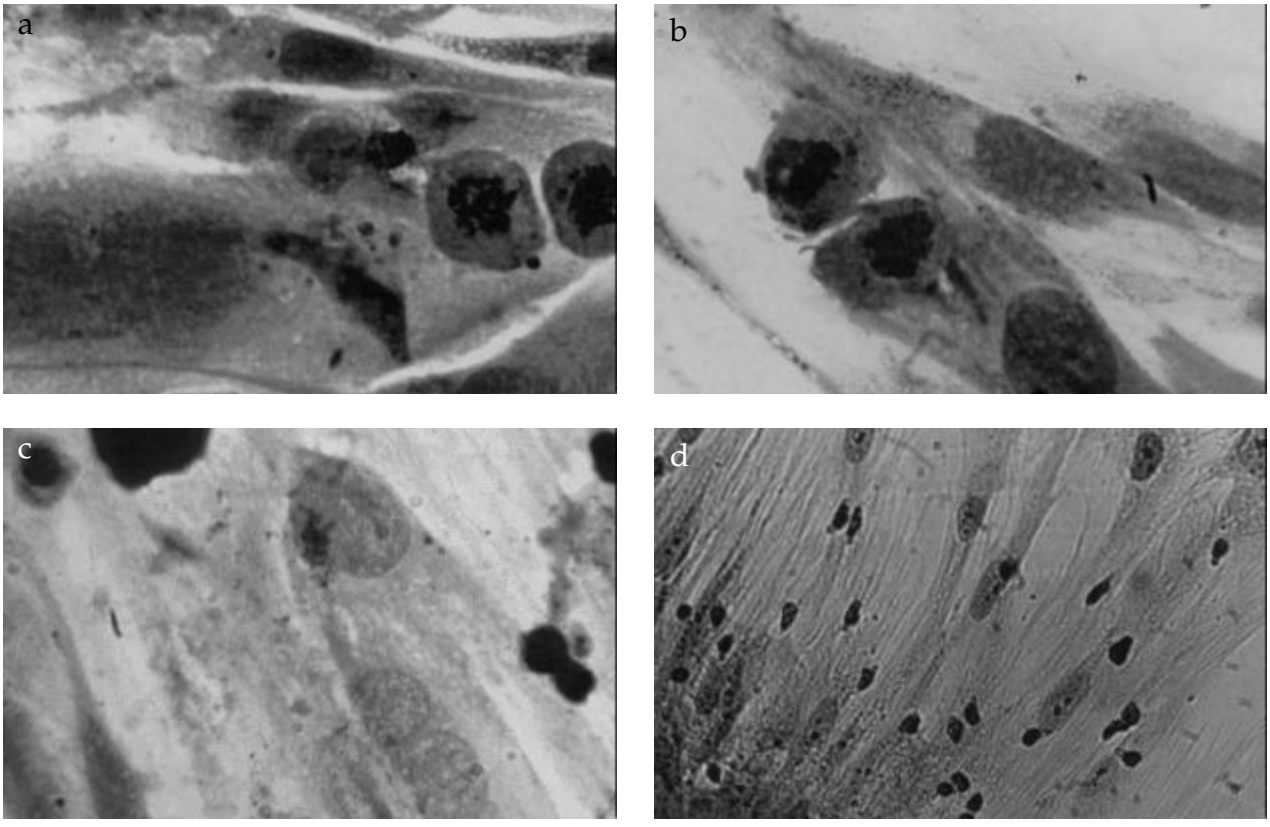

Figure 6. (a, b, c) *Chondrosarcoma cells #1459 from passage 33 showing gradual change of morphology toward "fibroblast-like" appearance after prolonged exposure to lymphocytes of the healthy donor's.* ***(d)*** *Small lymphocytes of the healthy donor persist in remnants of a #1456 cell culture consisting of fibroblast-like cell islands that later underwent senescence and perished. Photograph of a stained T flask culture representing a passage of cells from an original culture vessel in which #1459 cells and healthy donor's lymphocytes co-existed. (a, b, c on film #495; Dec 15, 1973)*

agar. Tumor cells of culture #1459 did not grow in newborn TIMCO Swiss or AKR mice after intravenous inoculations.[103] Samples of the fibroblast-like cells remaining after the diminution in number of the lymphocytes from the 33rd passage of culture #1459 were collected for *in vitro* passages and for inoculation into soft agar. There was no colony formation in soft agar. During attempted passages in culture, the large fibroblast-like cells showed signs of senescence and eventually perished.

Discussion

Cytotoxicity assays. In 1969 and up to the mid-1970s, lymphocyte-mediated cytotoxicity assays performed in the hundreds in chamber-slide or Leighton tube cultures frequently failed to distinguish the tumor-specific reactions of immune T cells (allowing cross-reactions between related tumors) from the indiscriminate cytotoxicity of NK cells. *Figure 7*. shows how the young male patient (MDAH #87551) with primary osteogenic sarcoma and well-preserved pre-therapy immune status yielded mixed lymphocyte populations suppressing equally the growth of soft tissue and bone sarcoma cells and that of two carcinoma cell lines.[99] Within the short observation period of hundreds of similar assays,[92,96] either target cell death or emergence of target cell colonies resistant to lymphocyte-mediated cytotoxicity could regularly be observed, while tumor cell differentiation, if it occurred, could have escaped recognition.

Possible laboratory errors. This report is based on a preliminary publication.[89] The following possible experimental errors were considered but are regarded as most unlikely: 1. Inadvertent exchange of culture vessels. The culture vessels were clearly marked with indelible ink, and technicians were highly skilled to avoid cross-contamination of tissue culture cell lines. 2. Morover, the morphological appearance of the cultures did not change suddenly ("overnight") but set in slowly and gradually in quadruplicate cultures. Fibroblast-like cells undergoing senescence and small islands of cells resembling the original tumor cells co-existed. 3. The buffy coat and even the ficoll-hypaque purified donor lymphocyte preparations contained small numbers of monocytes-macrophages.[114] Could it be that conversion of these cells into fibroblast-like cells occurred? Both the patient and the donor were of the male gender, thus simple karyotyping could not have settled the issue. If the experimental cultures were accidentally contaminated with healthy donor fibroblasts, the normal fibroblasts obeying Hayflick's rules could not have overgrown the immortalized chondrosarcoma cells. The same vein of reasoning applies to inadvertent cross-contamination with the patient's fibroblasts. However, if donor monocytes formed stromal cells in these cultures, organ-specific differentiation of cancer cells induced by stromal cells could remotely be considered. Multiple genetic and epigenetic signaling aberrations that drive malignant cells could be corrected by stromal cells of the microenvironment both *in vivo* and *in vitro* in tissue cultures (for references, see [21,50]). However, allogeneic healthy lymphocytes have not been listed among the stromal cells that induced "tumor reversion".

Possible errors of interpretation. It is difficult to arrive by conjecture at the correct conclusion as to how chondrosarcoma cells were induced to undergo differentiation.

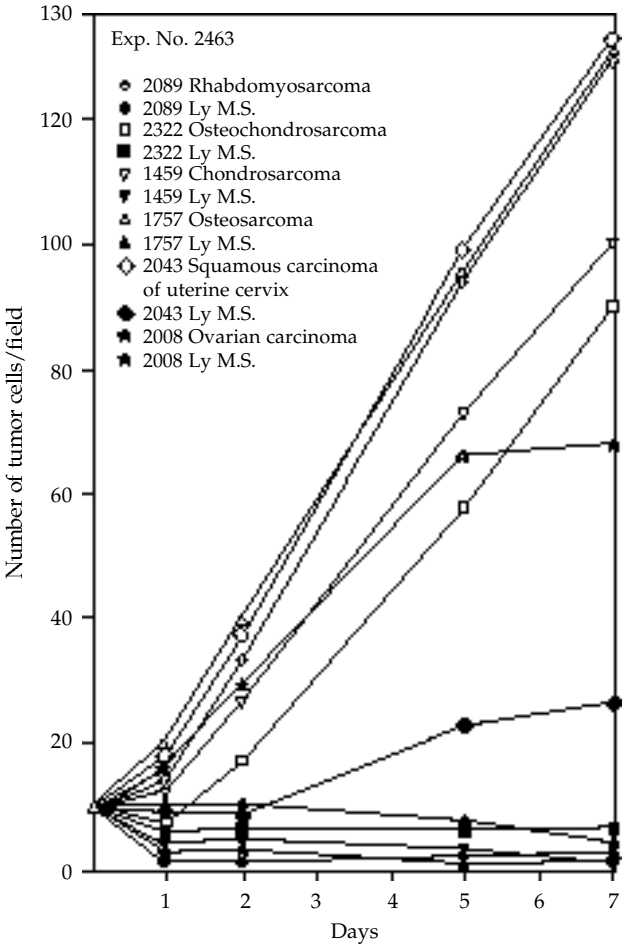

Figure 7. *Effect of peripheral lymphocytes on the growth of human sarcoma cells in vitro. A young male patient (MDAH#87551) with primary osteogenic sarcoma yielded buffy coat lymphocytes: $3.36x10^5$ lymphocytes were set against $4x10^3$ tumor cells per Leighton tube. The top 3 curves show squamous cell carcinoma of the uterine cervix cell line #2043; osteogenic sarcoma cell line #1757; and rhabdomyosarcoma cell line #2089. The mid 3 curves show chondrosarcoma cell line #1459; osteochondrosarcoma cell line #2322; and ovarian carcinoma cell line #2043 (for reference, see[98]). The patient's lymphocyte population inhibited the growth of all tumor cell lines against which it was tested. Stained preparations revealed that small compact lymphocytes acted against bone sarcoma cells; a mixed lymphocyte population consisting of small compact and large granular cells attacked rhabdomyosarcoma cells; and large granular lymphocytes dominated in the attack against carcinoma cells.*

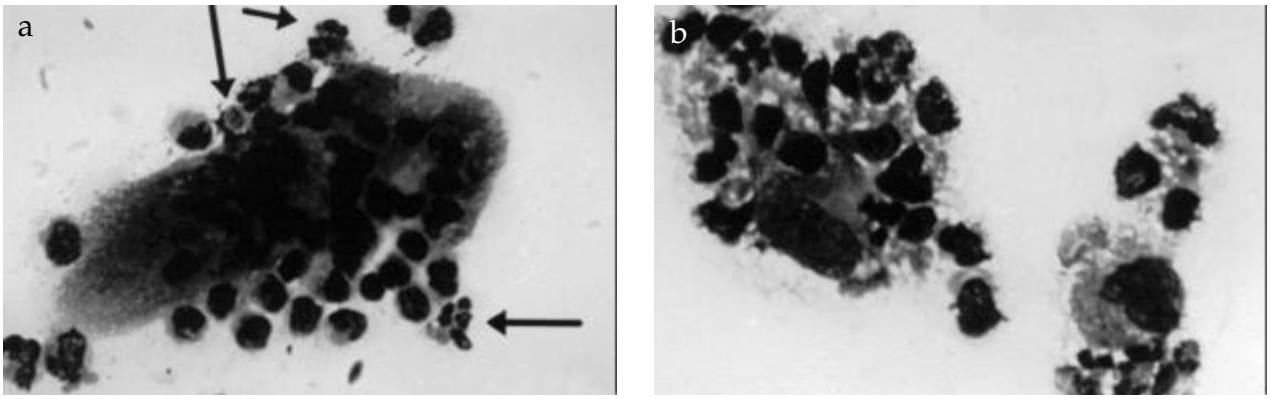

Figure 8. (a) *Chondrosarcoma cells from culture #1459 are attacked by autologous lymphoctes of patient MDAH73587. The attacker cells are small lymphocytes with compact nuclei (later: immune T cells). Some lymphocytes show nuclear clumping (arrows). Film #352; Oct 6, 1969).* ***(b)*** *McAllister's rhabdomyosarcoma cell line (received from American Culture Collection on December 14, 1970) is attacked by large lymphocytes with granular cytoplasm (later: NK cells) from patient (MDAH#85779) with rhabdomyosarcoma; some small lymphocytes with compact nuclei (later: immune T cells, arrows) participate in the attack .*

The genes that promote chondrocyte differentiation from the fibroblast-like pluripotential mesenchymal cell forward (Ihh; IGF; p21;$^{waf1/cip1}$ CTGF; D1x5; PGE_2) may not be those that can arrange regression from the immature transformed chondroblastic stage backward to the fibroblastic stage.[12, 107,122]

Chondrocytes react to inflammatory (IL-1α, IL-6, TNFα, IFNγ, IL-8/CXCL8) and anti-inflammatory (IL-4, IL-10) cyto-, lympho- and chemokines in rheumatoid and osteoarthritis,[32,67,84] and IFNα was claimed to have suppressed micro-metastases of osteosarcoma (references cited[91]), but it remained untested how chondrosarcoma cells in culture would have reacted to these molecular mediators.

Lymphocytes. Human chondrocyte-senescence in osteoarthritis[66] may hold a key to the understanding of the phenomenon described herein. The levels of the senescence-associated enzyme β-galactosidase correlate inversely with telomere length: the higher are the levels, the shorter are the telomeres. Shortening of telomeres comes with cessation of mitoses, declining synthetic activities and loss of responsiveness to growth factors. Dormant clones of lymphocytes may exist in healthy individuals (or in patients suffering with osteoarthritis), and may undergo clonal expansion upon encounter with autologous or allogeneic transformed cells overexpressing telomerases. If a healthy blood donor happens to be in the process of rejecting an incipient clone of malignantly transformed cells (practicing Burnet's "immunosurveillance") at the time of blood withdrawal and the lymphocytes thus obtained are used in an assay against allogeneic malignant

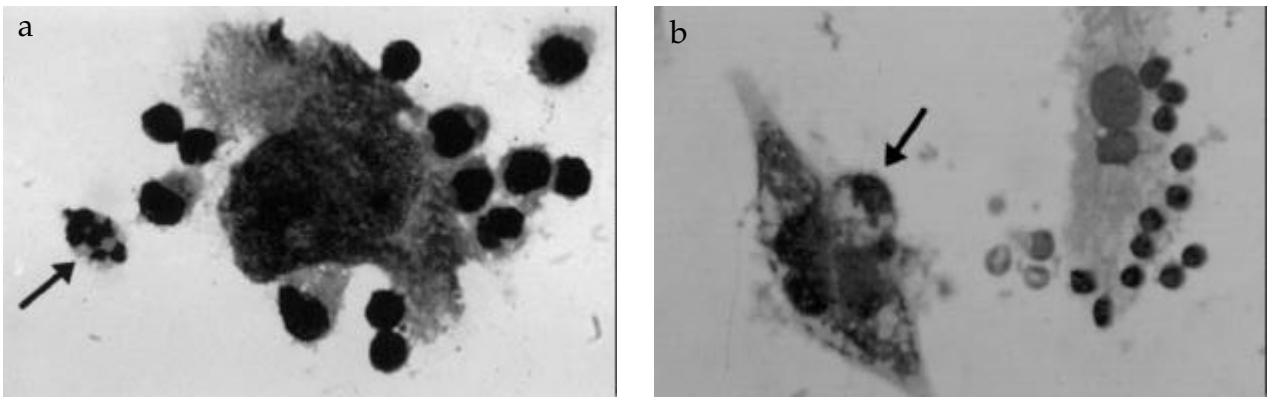

Figure 9. (a) *Small autologous lymphocytes with compact nuclei (later: immune T cells) of patient MDAH73587 induce nuclear disintegration of a chondrosarcoma cell from culture #1459. The lymphocytes preserve the good tinctorial features of viable live cells except for one that undergoes nuclear clumping (arrow).* ***(b)*** *Early passage (passage 2) of rhabdomyosarcoma culture #1449 from a female patient is exposed to lymphocytes of another patient with rhabdomyosarcoma[101]. Small lymphocytes with compact nuclei line up at, but do not attack, an allogeneic fibroblast-like cell. One large lymphocyte with granular cytoplasm (later: NK cell, arrow) attacks and lyses an allogeneic tumor cell. (Film #352; Oct 6, 1969)*

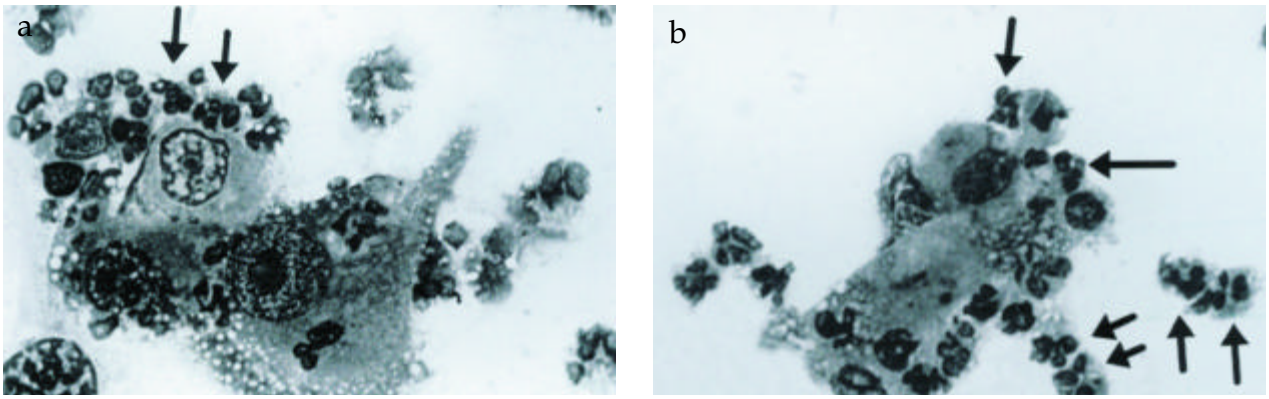

Figure 10. ***(a)*** *Cells from chondrosarcoma cell line #2454 from a 75-year-old male patient (MDAH #87288)*[98] *set in culture on October 14, 1971 are attacked by autologous lymphocytes inducing nuclear disintegration of the tumor cells, while many attacker lymphocytes also suffer nuclear clumping (arrows).* ***(b)*** *Established cell line #3743 set in culture on February 27, 1973 from a malignant cystosarcoma phylloides tumor of the female breast from patient (MDAH #95749)*[98]*. A tumor cell shows nuclear disintegration (arrow), some lymphocytes undergo nuclear clumping (arrow). (Film #417; June 6, 1973)*

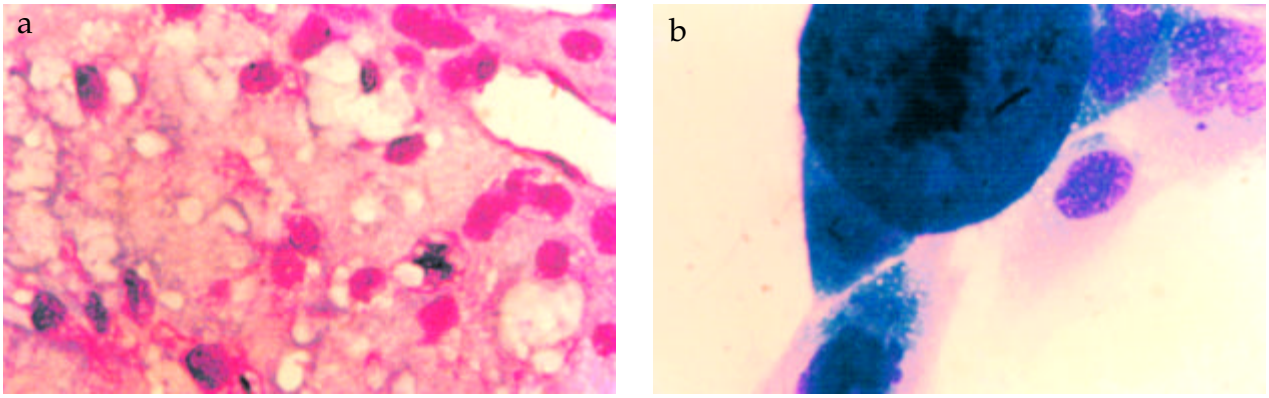

Figure 11. ***(a)*** *Tissue section of the chondrosarcoma of patient (MDAH #73587).* ***(b)*** *Passage 25 of #1459 cells.*[98]

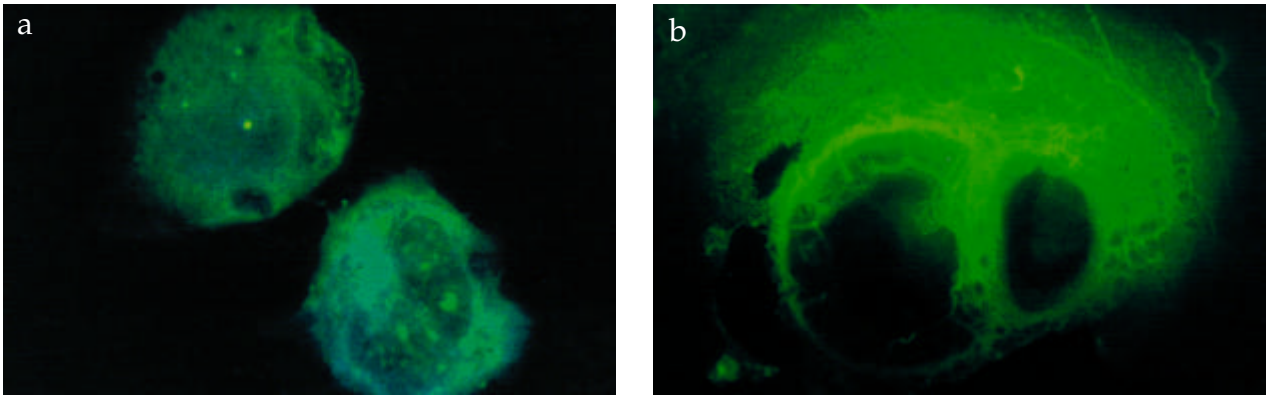

Figure 12. *Indirect immunofluorescence assay with the patient's sera against washed cells of culture #1459 (preparations of Dr. Eiichi Shirato).* ***(a)*** *Pre-immunization.* ***(b)*** *After repeated immunizations*[103] *with X-ray irradiated autolgous tumor cells from culture #1459. Antibodies reacting with cell surface and/or cytoplamic antigenic epitopes circulated in the patient's blood.*[103,104] *Presumably, antibody-coated epitopes were not recognized by the patient's immune T cells ("blocking serum factors"). Conceivably, the cytotoxicity of "large granular lymphocytes" was intensified by the antibodies ("unblocking serum factors") due to interactions with Fc receptors (not known to be functional in the late 1960s).*

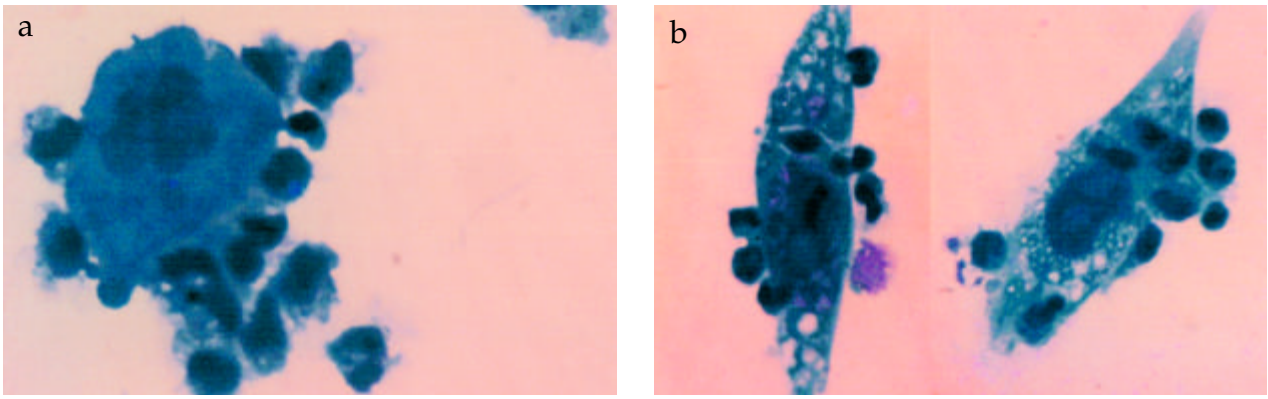

Figure 13. (a, b) *Autologous "small lymphocytes with compact nuclei" destroy #1459 tumor cells. In these cases, immune T cells exert more cytoplasmic lysis than nuclear clumping on the tumor cells. (Film #328; Sept 12, 1969)*

cells, the expanding anti-telomerase subclone of the lymphocyte population may induce telomere shortening and senescence in the malignant cells. Whereas such lymphocyte populations of tumor-bearing patients, akin to defective tumor-infiltrating lymphocytes in melanoma, for example,[59] are suppressed by molecular mediators released from the tumor, or by "blocking" serum factors concealing antigenic epitopes from immune T cells, thus the tumor prevails. In absence of such antibodies, in tissue cultures, autologous lymphocytes may re-exert their cytotoxicity. Indeed, T cell mediated anti-telomerase immune reactions can be generated in patients.[118] Analogously, tumor cells overexpressing survivin induce the expansion of an autologous clone of immunoreactive lymphocytes (for references, see[95]).

Anticancer cell surveillance is not restricted to NK cells.[70] Point-mutated (Ras, HER2/neu) or fusion oncoproteins (Abl/Bcr) are recognized by T cells and are attacked in the tumor-bearing host; the list includes p53.[125] Examples of such autoreactive clones are those that recognize B-cell differentiation antigens CD19 and CD20; or CEA epitopes.[16,31] It is now indubitable that allogeneic adoptive immunotherapy (for CML and kidney carcinoma) induces tumor cell death and remissions at the price of severe graft-*vs*-host disease. Hidden in its efficacy, adoptive immunotherapy with healthy allogeneic lymphocytes (T cells; NKT cells) may also be inductive to tumor cell differentiation in tumor-bearing recipients.

In the early 1970s lymphocyte subtyping was based on morphological criteria.[92] The "small lymphocytes with compact nuclei" were recognized later as immune T cells. The unusual "large lymphocytes with granular cytoplasm" were first (in 1969) misidentified as representatives of a nonphagocytic monocytic lineage;[102] or that they were blastic reactive forms of the smaller lymphocytes acquiring immune reactivity to allogeneic cells anew *in vitro*; or that the healthy donor being a medical oncologist through repeated exposure to "cancer viruses" at the bedside generated "cancer-immune lymphocytes" *in vivo*.[45,46] However, by the early 1970s the large lymphocytes with granular cytoplasm and indiscriminate cytotoxicity to cancer cells were referred to as "the lymphocytes practicing Burnet's

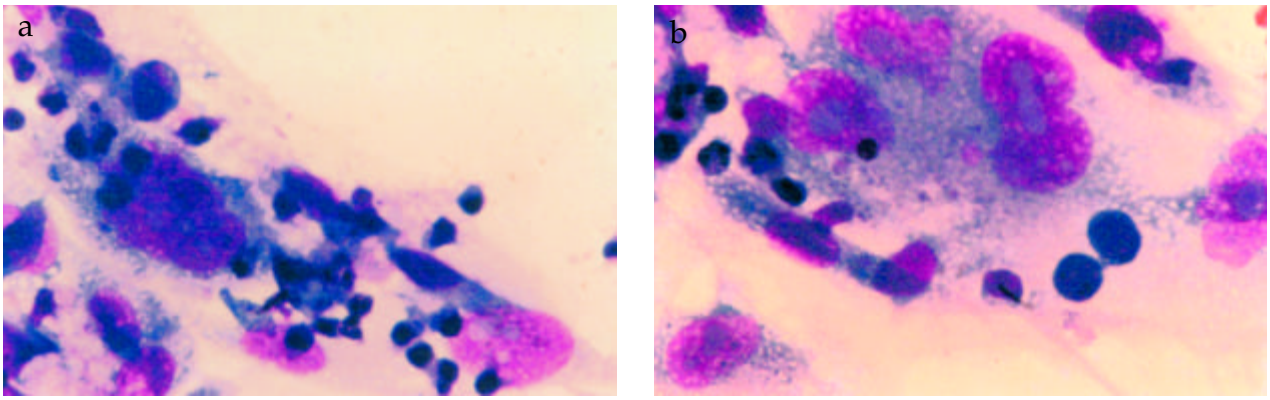

Figure 14. *Allogeneic (from the healthy donor) mixed lymphocyte populations, consisting of "large granular lymphocytes" and "small compact lymphocytes," co-exist with the patient's #1459 tumor cells. No lymphocyte-mediated cytotoxicity to tumor cells is evident* ***(a).*** *Some of the attacker small and large lymphocytes succumbe to nuclear clumping* ***(b).*** *(Film #334; Jan 15, 1970)*

immune surveillance".[47-49] In other laboratories a few years later these very same lymphocytes became designated to be natural killer (NK) cells.[79] Nevertheless, the photographs taken in 1969-73 at the Section of Clinical Tumor Virology and Immunology at M.D. Anderson Hospital and shown in this report and elsewhere remain the very first to depict human NK cells attacking allogeneic tumor cells.[90,92,96,97] Due to the possession of FcR, not yet recognized to be functional in 1970, it could be observed, but not explained at that time, that the cytotoxicity of the large lymphocytes with granular cytoplasm (NK cells) was thus intensified by antibodies. As to the tumor-specificity of the small lymphocytes with compact nuclei (later: immune T cells), they cross-reacted between soft tissue sarcoma cells or chondro- and osteosarcoma cells but not with Ewing's sarcoma cells, keeping in line with a subsequent recognition that Ewing sarcoma cells arise from a stem cell lineage different from that of osteochondrocytes.[105, 106] The large lymphocytes with granular cytoplasm (later: NK cells) exerted indiscriminate cross-reactivity between various types of tumor cells not distinguishing sarcoma, melanoma and cancer cell targets. There were no reliable laboratory procedures available in the early 1970s to separate immune T cells from NK cells. Acting in unison and always overlapping (*Figure 7*), these two major classes of cytotoxic lymphocytes could be distinguished by morphological criteria, since the dominant lymphocyte population was the immune T cell in the autologous settings, and the NK cell in the allogeneic settings (*Figure 8*). Their biological differences appeared as inhibition of immune T cells *versus* intensification of NK cells by antibodies. Immune T cells preferred to kill by nuclear clumping,[94,100] not known then that it was by programmed cell death (apoptosis) exogenously induced by cognate ligands through death receptors; whereas NK cells favored cytolysis,[94,97,100] but not exclusively so, not known then that it was due to the release of granzymes and perforins (*Figure 9*). In the vicinity of tumor cells more immune T cells appeared to die by nuclear clumping than NK cells[94] (*Figure 9,10*). For what was a puzzle then, now Fas ligand released by tumor cells can be incriminated for the destruction of Fas receptor-positive immune T cell clones of the host.[100]

Recapitulation. Figure 11 show the patient's tumor cells in a histological section and in tissue culture; his sera reacting with his tumor cells in an indirect immunofluorescence assay before and after active tumor-specific immunizations (*Figure 12*); the patient's "small lymphocytes with compact nuclei" (immune T cells) attacking his tumor cells *in vitro* (*Figure 13*); and coexistence of the healthy donor's mixed lymphocyte population ("large lymphocytes with granular cytoplasm", later: NK cells; and "small lymphocytes with compact nuclei", later: immune T cells) with the patient's tumor cells (*Figure 14*).

Acknowledgements

The author expresses his gratitude to Dr. Joseph C. Horvath for his help in identifying and transferring 30-years-old negative films and prints to the computer with the identity of the material preserved; and for reading and discussing the manuscript. The author is grateful for intellectual and technical help in his work with human lymphocytes to Drs. Don Dreyer, Ferenc Györkey†, H. David Kay, Eiichi Shirato, C. C. Shullenberger, Cameron Tebbi, Harikishan Thota†and Jose Trujillo†and to medical technologists Jim J. Romero and Jerry R. Cabiness. This work was supported by the Kelsey-Leary Foundation (Houston, TX) and by the Clifton D. Howe Fund (Houston, TX). Professor of pathology, Carlos Muro-Cacho M.D. PhD. of the Sarcoma Service, H.L. Moffitt Cancer Center at the University of South Florida College of Medicine, Tampa, FL read the completed manuscript. The author is grateful to Dr. Muro-Cacho for his endorsement recommending the publication of this material in its entirety.

References

1. *Adams CS, Shapiro IM*: The fate of the terminally differentiated chondrocyte: evidence for microenvironmental regulation of chondrocyte apoptosis. Crit Rev Oral Biol Med 13**:**465-473, 2002.
2. *Aigner T, Loos S, Müller S et al.:* Cell differentiation and matrix gene expression in mesenchymal chondrosarcomas. Am J Pathol 156:1327-1335, 2000.
3. *Aigner, T., Dertinger, S., Belke, J., Kirchner, T:* Chondrocytic cell differentiation in clear cell chondrosarcoma. Hum Pathol 27:1301-1305,1996
4. *Aigner T, Unni KK:* Is dedifferentiated chondrosarcoma a 'dedifferentiated' chondrosarcoma? J Pathol 189:454-462, 1999.
5. *Aikawa T, Segre GV, Lee K*: Fibroblast growth factor inhibits chondrocyte growth through induction of p21 and subsequent inactivation of cycline E-cdk2. J Biol Chem 275:29347-29352, 2001.
6. *Akiyama B, Chaboissier MC, Martin JF et al:* The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. Genes Dev 16:2813-2828, 2002.
7. *Algros MP, Collonge-Rame MA, Bedgejian T et al:* Neuroectodermal differentiation of extraskeletal myxoid chondrosarcoma: a classical feature? Ann Pathol 23:244-248, 2003.
8. *Amling M, Posl M, Hentz M et al:* PTHrP and Bcl-2: essential regulatory molecules in chondrocyte differentiation and chondrogenic tumors. Verh Dtsch Ges Pathol 82:160-169, 1998.
9. *Archer C W, Francis-West P*: The chondrocyte. Int J Biochem Cell Biol 35:401-404, 2003.
10. *Aszodi A, Hunziker EB, Brakebusch C, Fassler R*: β1 Integrins regulate chondrocyte rotation, GH1 progression and cytokinesis. Genes Dev 17:2465-2479, 2003.
11. *Beier F, Lee RJ, Taylor AC, et al:* Identification of the cyclin D1 gene as a target of activating transcription factor 2 in chondrocytes. Proc Natl Acad Sci USA 96:1433-1438, 1999.
12. *Benz K, Breit S, Lukoschek M, et al:* Molecular analysis of expansion, differentiation, and growth factor treatment of human chondrocytes identifies differentiation markers and growth-related genes. Biochem Biophys Res Commun 293:284-292, 2002.
13. *Blasenbreu S, Baretton GB, Bender C et al:* TP53 gene aberrations in chondromatous neoplasms: correlation with immunohistochemical p53 accumulation and MDM2 expression. Verh Dtsch Ges Pathol 82:284-289, 1998.

14. *Bovee JV, van den Broek LJ, Cleton-Jansen AM, Hogendoorn PC:* Up-regulation of PTHrP and Bcl-2 expression characterizes the progression of osteochondroma toward peripheral chondrosarcoma and is a late event in central chondrosarcoma. Lab Invest 80:1925-1934, 2000.
15. *Bovée JVMG, Cleton-Jansen A-M, Wuyts W et al*: EXT-mutation analysis and loss of heterozygosity in sporadic and hereditary osteochondromas and secondary chondrosarcomas. Am J Hum Genet 65:689-698, 1999.
16. *Campi G, Crosti M, Consogno G et al*: $CD4^+$ T cells from healthy subjects and colon cancer patients recognize a carcinoembryonic antigen-specific immunodominant epitope. Cancer Res 63:8481-8486, 2003.
17. *Casorzo L, Chiecchio L, Pisacane A et al*: Cytogenetic findings in a case of dedifferentiated chondrosarcoma. Cancer Genet Cytogenet 144:61-64, 2003.
18. *Chang SH, Oh CD, Yang MS et al:* Protein kinase C regulates chondrogenesis of mesenchymes via mitogen-activated protein kinase signaling. J Biol Chem 273:19213-19219, 1998.
19. *Church V, Nohno T, Linker C, et al:* Wnt regulation of chondrocyte differentiation. J Cell Sci 115:4809-4818, 2002.
20. *Cormier S, Delezoide A-L, Benoist-Lasselin C et al*: Parathyroid hormone receptor type 1/indian hedgehog expression is preserved in the growth plate of human fetuses affected with fibroblast growth factor type 3 activating mutations. Am J Pathol 161:1325-1335, 2002.
21. *Cunha GR, Hayward SW, Wang YZ, Ricke WA:* Role of the stromal microenvironment in carcinogenesis of the prostate. Int J Cancer 107:1-10, 2003.
22. *Dailey L, Laplantine E, Priore R, Basilico C:* A network of transcriptional and signaling events is activated by FGF to induce chondrocyte growth arrest and differentiation. J Cell Biol 161:1053-1066, 2003.
23. *Deckelbaum RA, Chan G, Miao D et al*: Ihh enhances differentiation of CFK-2 chondrocytic cells and antagonizes PTHrP-mediated activation of PKA. J Cell Sci 115:3015-3025, 2002.
24. *Domanski HA, Carlen B, Martens F, Akerman M*: Extraskeletal myxoid chondrosarcoma with neuroendocrine differentiation: a case report with fine-needle aspiration biopsy, histopathology, electron microscopy, and cytogenetics. Ultrastruct Pathol 27: 363-368, 2003.
25. *Eguchi T, Kubota S, Kondo S et al*: Regulatory mechanism of human connective tissue growth factor (CTGF/Hcs24) gene expression in a human chondrocytic cell, HCS-2/8. J Biochem 130:79-87, 2001.
26. *Ellsworth JL, Berry J, Bukowski T et al:* Fibroblast growth factor-18 is a trophic factor for mature chondrocytes and their progenitors. Osteoarthr Cartil 10:308-320, 2002.
27. *Enomoto-Iwamoto M, Kitagaki J, Koyama E et al:* The Wnt antagonist Frzb-1 regulates chondrocyte maturation and long bone development during limb skeletogenesis. Dev Biol 251:142-156, 2002.
28. *Ferrari D, Kosher RA*: D1x5 is a positive regulator of chondrocyte differentiation during enchondral ossification. Dev Biol 252:257-270, 2002.
29. *Ghert MA, Qi WN, Erickson HP et al:* Tenascin-C expression and distribution in cultured human chondrocytes and chondrosarcoma cells. J Orthop Res 20:834-841, 2002.
30. *Grimaud E, Heymann D, Redini, F:* Recent advances in TGF-β effects on chondrocyte metabolism. Potential therapeutic roles of TGF-β in cartilage disorders. Cytokine Growth Factor Rev 13:241-257, 2002.
31. *Grube M, Rezvani H, Sconocchia G et al*: Autoreactive, cytotoxic T lymphocytes specific for peptides derived from normal B-cell differentiation antigens in healthy individuals and patients with B-cell malignancies. Clin Cancer Res 10: 1047-1056, 2004.
32. *Guicheux J, Palmer G, Relic B et al:* Primary human articular chondrocytes, dedifferentiated chondrocytes, and synoviocytes exhibit differential responsiveness to interleukin-4: correlation with the expression pattern of the common receptor gamma chain. J Cell Physiol 192: 93-101, 2002.
33. *Guo W, Gorlick R, Ladanyi M et al:* Expression of bone morphogenetic proteins and receptors in sarcomas. Clin Orthop 365:175-183, 1999.
34. *Hatakeyama Y, Nguyen J, Wang X, et al:* Smad signaling in mesenchymal and chondroprogenitor cells. J Bone Joint Surg Am 3:13-18, 2003.
35. *Hecht JT, Hogue D, Wang Y et al:* Hereditary multiple exostoses (EXT): mutational studies of familial EXT1 cases and EXT-associated malignancies. Am J Hum Genet 60:80-86, 1997.
36. *Hiraoka K, Zenmyo M, Komiya S et al:* Relationship of p21 (waf1/cip1) and differentiation in chondosarcoma cells. Virchows Arch 440:285-290, 2002.
37. *Hoang MP, Suarez PA, Donner LR et al*: Mesenchymal chondrosarcoma: a small cell neoplasm with polyphenotypic differentiation. Int J Surg Pathol 8:291-301, 2000.
38. *Hoodless PA, Haerry T, Abdollah S et al*: MADR1, a MAD-related protein that functions in BMP2 signaling pathways. Cell 85:489-500, 1996.
39. *Huh YH, Kim SH, Kim SJ, Chun JS:* Differentiation status-dependent regulation of cyclooxygenase-2 expression and prostaglandin E2 production by epidermal growth factor via mitogen-activated protein kinase in articular chondrocytes. J Biol Chem 278:9691-9697, 2003.
40. *Ionescu AM, Schwartz EM, Zuscik MJ et al:* ATF-2 cooperates with Smad3 to mediate TGFβ effects on chondrocyte maturation. Exp Cell Res 288:198-207, 2003.
41. *Ivkovic S, Yoon BS, Popoff SN et al*: Connective tissue growth factor coordinates chondrogenesis and angiogenesis during skeletal development. Development 130: 2779-2791, 2003.
42. *Kalil RK, Inwards CY, Unni KK et al:* Dedifferentiated clear cell chondrosarcoma. Am J *Surg Pathol 2:1079-1086, 2000.*
43. *Kato Y, Iwamoto M:* Fibroblast growth factor is an inhibior of chondrocyte terminal differentiation. J Biol Chem 265:5903-5909, 1990.
44. *Kawashima H, Ogose A, Hayami T et al*: Effect of dexamethasone on growth inhibition and chondrogenesis of human chondrosarcoma. J Orthop Sci 8:341-345, 2003.
45. *Kay HD, Cabiness JR, Ervin F et al*: Do lymphocytes presensitized to tumor antigens occur in normal individuals? Proc Ann Meeting Am Soc Microbiol p 111, #M227, 1973.
46. *Kay HD, Cabiness JR, Kitowski T, Sinkovics JG*: Cytotoxicity to, and immune stimulation of, cultured tumor cells by normal human lymphocytes and serum factors. Proceeding 65th AACR 15:64, #254, 1974.
47. *Kay HD, Sinkovics JG, Cabiness JR et al:* The concept of immune surveillance and its experimental testing. Clin Res 22/1: 44A, 1974.
48. *Kay HD, Sinkovics JG:* Cytotoxic lymphocytes from normal donors. Lancet 2: 296-297, 1974.
49. *Kay HD, Thota H, Romero JJ, Sinkovics JG*: Lymphocytes from normal donors are frequently cytotoxic to tumor cells *in vitro*. Clin Res 23/3:340A, 1975.
50. *Kenny PC, Bissell MJ:* Tumor reversion: Correction of malignant behavior by microenvironmental *cues*. Int J Cancer 107:688-695, 2003.
51. *Kim* DW, *Lassar AB:* Smad-dependent recruitment of a histone de-acetylase/Sin3A complex modulates the bone morphogenetic

protein-dependent transcriptional repressor activity of Nkx3.2. Mol Cell Biol 23:8704-8717, 2003.
52. *Kim SJ, Chun JS*: Protein kinase Cα and ξ regulate oxide-induced NFêB activation that mediates cyclooxygenase-2 expression and apoptosis but not dedifferentiation in articular chondrocytes. Biochem Biophys Res Commun 303:206-211, 2003.
53. *Kim SJ, Hwang SG, Kim IC, Chun JS:* Actin cytoskeletal architecture regulates nitric oxide-induced apoptosis, dedifferentiation and cyclooxygenase-2 expression in articular chondrocytes via mitogen-avtivated protein kinase and protein kinase C pathways. J Biol Chem 278:42448-42456, 2003.
54. *Kim SJ, Kim HG, Oh CD et al*: p38 kinase-dependent and –independent inhibition of protein kinase Cξ and α regulates nitric oxide-induced apoptosis and dedifferentiation of articular chondrocytes. J Biol Chem 277:30375-30381, 2002.
55. *Kinisada T, Miyakazi M, Mihara K et al:* A new human chondrosarcoma cell line (OUMS-27) that maintains chondrocytic differentiation. Int J Cancer 77:854-859, 1998.
56. *Kobayashi T, Chung, UI, Schipani, E et al:* PTHrP and indian hedgehog control differentiation of growth plate chondrocytes at multiple steps. Development 129:2977-2986, 2002.
57. *Koshizuka Y, Yamada T, Hoshi K et al:* Cystatin 10, a novel chondrocyte-specific protein, may promote the last steps of the chondrocyte differentiation pathway. J Biol Chem 278:48259-48266, 2003.
58. *Kunisada T, Moseley JM, Slavin Jl et al*: Co-expression of parathyroid hormone-related protein (PTHrP) and PTH/PTHrP receptor in cartilaginous tumours: a marker for malignancy? Pathology 34:133-137, 2002.
59. *Ladányi A, Somlai B, Gilde K et al:* T-cell activation marker expression on tumor-infiltrating lymphocytes as prognostic factor in cutaneous malignant melanoma. Clin Cancer Res 15:521-530, 2004.
60. *Li N, Shen LH, Zhu QF:* Overexpression of c-erb-2 proto-oncogene product in chondrosarcoma. Zhoghua Bing Li Xue Za Zhi 23:37-39, 1994.
61. *Li X, Ionescu AM, Schwartz EM et al:* Smad6 is induced by BMP-2 and modulates chondrocyte differentiation. J Orthop Res 21:908-913, 2003.
62. *Liu Z, Xu J, Colvin JS, Ortiz DM:* Coordination of chondrogenesis and osteogenesis by fibroblast growth factor 18. Genes Dev 16:859-869, 2002.
63. *Loeser RF, Pacione CA, Chubinskaya S:* The combination of insulin-like growth factor 1 and osteogenic protein 1 promotes increased survival of, and matrix synthesis by, normal osteoarthritic human articular chondrocytes. Arth Rheum 48:2188-2196, 2003.
64. *Longobardi L, Torello M, Buckay C et al:* A novel insulin-like growth factor (IGF)-independent role for IGF binding protein-3 in mesenchymal chondroprogenitor cell apoptosis. Endocrinol 144:1695-1702, 2003.
65. *Maclean HE, Kim JI, Glimcher MKJ et al:* Absence of transcription factor c-*maf* causes abnormal terminal differentiation of hypertrophic chondrocytes during enchondral bone development. Dev Biol 262:51-63, 2003.
66. *Martin JA, Buckwalter JA:* Human chondrocyte senescence and osteoarthritis. Biorheology 39:145-152, 2002.
67. *Merz D, Liu R, Johnson K, Terkeltaub R:* IL-8/CXCL8 and growth-related oncogene α/CXCL1 induce chondrocyte hypertrophic differentiation. J Immunol 171:4406-4415, 2003.
68. *Minina E, Kreschel C, Naski MC, et al:* Interaction of FGF, Ihh/PTHrP and BMP signaling integrates chondrocyte proliferation and hypertrophic differentiation. Dev Cell 3:439-449, 2002.
69. *Miyamoto M, Ito H, Mukai S, Kobayashi T et al:* Simultaneous stimulation of EP2 and EP4 is essential to the effect of prostaglandin E2 in chondrocyte differentiation. Osteoarthr Cartil 11:644-652, 2003.
70. *Nagorsen D, Scheibenbogen C, Marincola FM, Letsch, A, Keilholz U*: Natural T cell immunity against cancer. Clin Cancer Res 9:4296-4303, 2003.
71. *Nakanishi T, Nishida T, Shimo T et al:* Effects of CTGF/Hcs24, a product of a hypertrophic chondrocyte-specific gene, on the proliferation and differentiation of chondrocytes in culture. Endocrinology 141:264-273, 2000.
72. *Nielsen GP, Keel SB, Dickersin GR et al:* Chondromyxoid fibroma: a tumor showing myofibroblastic, myochondroblastic, and chondrocytic differentiation. Mod Pathol 12:514-517, 1999.
73. *Nishida T, Kubota S, Nakanishi T et al:* CTGF/Hcs24, a hypertrophic chondrocyte-specific gene product, stimulates proliferation and differentiation, but not hypertrophy of cultured articular chondrocytes. J Cell Physiol 192:55-63, 2002.
74. *O'Donovan M, Russel JM, O'Leary JJ et al:* Abl expression, tumor grade, and apoptosis in chondrosarcoma. Mol Pathol 52:341-344, 1999.
75. *Oh CD, Chun JS:* Signaling mechanisms leading to the regulation of differentiation and apoptosis of articular chondrocytes by insulin-like growth factor-1. J Biol Chem 278:36563-36571, 2003.
76. *Ohbayashi N, Shibayama M, Kurotaki Y et al:* FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis. Genes Dev 16:870-879, 2002.
77. *Park H-R, Kim Y-W, Jung WW et al:* Evaluation of HER-2/neu status by real-time quantitative PCR in malignant cartilaginous tumors. Int J Oncol 24:575-580, 2004.
78. *Raucci A, Laplantine E, Mansukhani A, Basilico C:* Activation of the ERK1/2 and p38 mitogen-activated protein kinase pathways mediates fibroblast growth factor-induced growth arrest of chondrocytes. J Biol Chem 279:1747-1756, 2004
79. *Raulet DH:* Chapter 12. Natural killer cells. In: Fundamental Immunology. W. E. Paul, editor. 5th edition. Lippincott Williams & Wilkins, Philadelphia, PA. Pp 365-391, 2003.
80. *Reguly T, Wrana JL*: In or out? The dynamics of Smad nucleocytoplasmic shuttling. Trends Cell Biol 13:216-220, 2003.
81. *Reiter I, Tzukerman M, Maor G:* Spontaneously differentiating primary chondrocytic tissue culture; a model for enchondral ossification. Bone 31:333-339, 2002.
82. *Rozenblatt-Rosen O, Mosonego-Ornan E, Sadot E et al:* Induction of chondrocyte growth arrest by FGF: transcriptional and cytoskeletal alterations. J Cell Sci 115:553-562, 2002.
83. *Schaefer JF, Millham M, de Crombrugghe B, Buckbinder L*: FGF signaling antagonizes cytokine-mediated repression of Sox9 in SW1353 chondrosarcoma cells. Osteoarthr Cartil 11:233-241, 2003.
84. *Schuerwegh AJ, Dombrecht EJ, Stevens WJ et al*: Influence of pro-inflammatory IL-1α, IL-6, TNFα, IFNγ and anti-inflammatory (IL-4) cytokines on chondrocyte function. Osteoarth Cartil 11: 681-687, 2003.
85. *Schulze-Tanzil G, de Souza P, Villegas Castrejon H et al:* Redifferentiation of dedifferentiated human chondrocytes in high density cultures. Cell Tissue Res 308:371-379, 2002.
86. *Segat D, Comai R, Di Marco E et al:* Integrins α6Aβ1 and α6Bβ1 promote different stages of chondrogenic cell differentiation. J Biol Chem 277:31612-31622, 2002.
87. *Shakunaga T, Ozaki T, Ohara N et al:* Expression of connective tissue growth factor in cartilaginous tumors. Cancer 89:1466-1473, 2000.
88. *Sims WW, Ordonez NG, Johnston D et al*: p53 expression in dedifferentiated chondrosarcoma. Cancer 76:223-227, 1995.

89. *Sinkovics JG:* Acquisition of resistance by human tumor cells to lymphocyte-mediated cytotoxicity. Proc AACR 17: 99, #393, 1976.
90. *Sinkovics JG:* Cytotoxic lymphocytes. Ann Clin Lab Sci 16:488-496, 1986.
91. *Sinkovics JG:* Interferon. In: Medical Oncology, an Advanced Course. 2nd edition, vol. I & II. Sinkovics JG, author. Marcel Dekker, New York, N. Y. Pp 1455, 1986.
92. *Sinkovics JG:* A reappraisal of cytotoxic lymphocytes in human tumor immunology. In: Cancer Biology and Therapeutics, ed. Cory JG, Szentivanyi A, Plenum Press, New York, N.Y., pp 225-253, 1987.
93. *Sinkovics JG:* Oncogenes and growth factors. CRC Crit Rev Immunol 8:217-298, 1988.
94. *Sinkovics JG:* Malignant lymphoma arising from natural killer cells: report of the first case in 1970 and newer developments in the FasL→FasR system. Acta Microbiol Immunol Hung 44:295-307, 1997
95. *Sinkovics JG:* New biological therapeutics: competitors or collaborators of the viral therapy for human cancers. In: Viral Therapy of Human Cancers. Horvath JC, Sinkovics JG, editors. Marcel Dekker, New York, N.Y., in print, 2004.
96. *Sinkovics JG, Cabiness JR, Shullenberger CC*: Monitoring in vitro of immune reactions to solid tumors. Front Rad Ther Oncol 7:141-154, 1972.
97. *Sinkovics JG, Dreyer DA, Shirato E et al:* Cytotoxic lymphocytes. I. Destruction of neoplastic cells by lymphocytes in cultures of human origin. Texas Rep Biol Med 29:227-242, 1971.
98. *Sinkovics JG, Györkey F, Kusyk C, Siciliano MJ:* Growth of human tumor cells in established cultures. Methods Cancer Res 14:243-323, 1978.
99. *Sinkovics J, Györkey F, Shullenberger CC:* Project M23/gm7. The etiologic agents, immunology and spread of human leukemia and sarcoma. In: Research Report. The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, TX. Printing Division, The University of Texas, Austin, TX. Pp 321-322, 1978.
100. *Sinkovics JG, Horvath JC:* Virological and immunological connotations of apoptotic and anti-apoptotic forces in neoplasia. Int J Oncol 19:473-488, 2001.
101. *Sinkovics JG, Shirato E, Cabiness JR, Martin RG*: Rhabdomyosarcoma after puberty: clinical, tissue culture and immunological studies. J Med Exp Clin 1:313-326, 1970.
102. *Sinkovics JG, Shirato E, Gyorkey F et al*: Relationship between lymphoid neoplasms and immunologic functions. In: Leukemia-Lymphoma. 14th Annual Clinical Conference, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX, 1969. Year Book, Medical Publishers, Chicago, IL. Pp 53-92, 1970.
103. *Sinkovics JG, Shirato E, Martin RG, et al:* Chondrosarcoma. Immune reactions of a patient to autologous tumor. Cancer 27:782-793, 1971.
104. *Sinkovics JG, Shirato E, Martin RG, White EC*: Chondrosarcoma. I. A brief review of eighty-three patients. J Med Exp Clin 1:15-25, 1970.
105. *Sinkovics JG, Tebbi K, Cabiness JR:* Cytotoxicity of lymphocytes to established cultures of human tumors: evidences for specificity. Natl Cancer Inst Monogr 37:9-18, 1973.
106. *Sinkovics JG, Thota H, Romero JJ, Waldinger R*: Bone sarcomas: etiology and immunology. Can J Surg 20:494-503, 1977.
107. *Soderstrom M, Bohling T, Ekfors T et al:* Molecular profiling of human chondrosarcomas for matrix production and cancer markers. Int J Cancer 100:144-151, 2002.
108. *St-Jacques B, Hammerschmidt M, McMahon RT:* Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. J Cell Sci 110:2691-2701, 1997.
109. *Stanton LA, Underhill TM, Beier F:* MAP kinases in chondrocyte differentiation. Dev Biol 263:165-175, 2003.
110. *Stheneur C, Dumontier MF, Guedes C et al:* Basic fibroblast growth factor as a selective inducer of matrix G1a protein gene expression in proliferative chondrocytes. Biochem J 369: 63-79, 2003.
111. *Sitcheran R, Cogswell PC, Baldwin Jr AS:* NFêB mediates inhibition of mesenchymal cell differentiation through a posttranscriptional gene silencing mechanism. Genes Dev 17:2368-2373, 2003.
112. *Takigawa M, Okawa T, Pan H et al:* Insulin-like growth factors I and II are autocrine factors stimulating proteoglycan synthesis, a marker of differeniated chondrocytes, acting through their respective receptors on a clonal human chondrosarcoma-derived chondrocyte cell line, HCS-2/8. Endocrinology 138:4390-4400, 1997.
113. *Tarkkanen M, Wiklund T, Virolainen M et al:* Dedifferentiated chondrosarcoma with t(9;22)(q34;q11-12). Genes Chromosomes Cancer 9:136-140, 1994.
114. *Tebbi CK:* Purification of lymphocytes: a new technique. Lancet 1:1392, 1973.
115. *Tuli R, Tuli S, Nandi S et al:* Transforming growth factor β-mediated chondrogenesis of human mesenchymal progenitor cells involves N-cadherin and mitogen-activated protein kinase and Wnt signaling cross-talk. J Biol Chem 278:41227-41236, 2003.
116. *Uyama Y, Yagami K, Hatori et al*: Recombinant human bone morphogenetic protein-2 promotes Indian hedgehog-mediated osteochondrogenic differentiation of a human chondrocytic cell line in vivo and in vitro. Differentiation 72:32-40, 2004.
117. *Valcourt U, Gouttenoire J, Moustakas A, et al.:* Functions of transforming growth factor type I receptors and Smad proteins in the hypertrophic maturation and osteoblastic differentiation of chondrocytes. J Biol Chem 277:33545-33558, 2002.
118. *Vonderheide RH, Domchek SM, Schultze JL et al:* Vaccination of cancer patients against telomerase induces functional antitumor $CD8^+$ T lymphocytes. Clin Cancer Res 10:828-839, 2004.
119. *Wehrli BM, Huang W, de Crombrugghe D, et al.* Sox9, a master regulator of chondrogenesis, distinguishes mesenchymal chondrosarcoma from small blue round cell tumors. Hum Pathol 34: 263-269, 2003.
120. *Wuyts W, Van Hul W, De Boulle K et al:* Mutations in the EXT1 and EXT2 genes in hereditary multiple exostoses. Am J Hum Genet 62:346-354, 1998.
121. *Yang Y, Topol L, Lee H, Wu J:* Wnt5a and Wnt5b exhibit distinct activities in coordinating chondrocyte proliferation and differentiation. Development 130:1003-1015, 2003.
122. *Yates KE, Forbes RL, Glowacki J:* New chondrocyte genes discovered by representational difference analysis of chondroin-duced human fibroblasts. Cells Tissues Organs 176:41-53, 2004.
123. *Yosimichi G, Nakanishi T, Nishida T et al:* CTGF/Hcs24 induces chondrocyte differentiation through a p38 mitogen-activated protein kinase (p38MAPK), and proliferation through a p44/42MAPK/extracellular signal-regulated kinase. Eur J Biochem 268:6058-6065, 2001.
124. *Zhang L, Duan, CJ, Binkley C et al:* A transforming growth factor β-induced Smad3/Smad4 complex directly activates protein kinase A. Mol Cell Biol 24:2169-2180, 2004.
125. *Zwaveling S, Vierboom MP, Ferreira M et al:* Antitumor efficacy of wild-type p53-specific $CD4^+$ T-helper cells. Cancer Res 62:6187-6193, 2002.