Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 333 (2005) 216–222
BBRC
www.elsevier.com/locate/ybbrc

Phenotypic instability of Saos-2 cells in long-term culture

Heinz-Juergen Hausser *, Rolf E. Brenner

Division for Biochemistry of Joint and Connective Tissue Diseases, University of Ulm, D-89081 Ulm, Germany

Received 11 May 2005
Available online 31 May 2005

Abstract

The human osteosarcoma cell line Saos-2 is widely used as a model system for human osteoblastic cells, though its phenotypic stability has not been ascertained. We therefore propagated these cells over 100 passages and compared relevant phenotypic properties. In general, higher passage cells exhibited higher proliferation rates and lower specific alkaline phosphatase activities, though mineralization was significantly more pronounced in cultures of late passage cells. Whereas expression of most genes investigated did not vary profoundly, some genes exhibited remarkable differences. Decorin, for instance, that has been discussed as a regulator of proliferation and mineralization, is strongly expressed only in early passage cells, and two receptors for pleiotrophin and midkine exhibited an almost mutually exclusive expression pattern in early and late passage cells, respectively. Our observations indicate that special care is required when results obtained with Saos-2 cells with different culture history are to be compared.

Keywords: Saos-2 cells; Osteoblasts; Cell culture; Phenotype evolution; Mineralization; Decorin; Anaplastic lymphoma kinase

Since their first description in 1975 [1], Saos-2 cells have been widely used as a model system for osteoblastic cells. Promising advantages for using this cell line are (1) its world wide availability, (2) its good and well-documented characterization, (3) the possibility to obtain large amounts of cells in short time, and (4) the fact that Saos-2 cells exhibit the entire differentiation sequence of osteoblastic cells. The latter point, particularly the ability of Saos-2 cells to deposit a mineralization-competent extracellular matrix [2], makes these cells a valuable model for studying events associated with the late osteoblastic differentiation stage in human cells, as nodule formation can usually not be observed in cultures of primary human osteoblasts isolated from spongiosa of adult donors [3]. Furthermore, working with primary osteoblast preparations always bears the possibility that contaminating non-osteoblastic cells contribute to or even dominate the effects under investigation, apart from the fact that the osteoblastic cells by themselves cannot be expected to be homogeneous, for instance with regard to their differentiation stage.

Important known disadvantages of cell lines derived from tumors, on the other hand, are associated with their origin from a pathological tissue of a single individual, raising the possibility that observed effects may be related to the pathological state of the original tumor rather than being representative for the respective untransformed cell, or might represent peculiarities of the individual donor. Indeed, many tumor derived cell lines have been initially established in order to investigate phenomena that are characteristic for the tumor from which the cells have been derived.

Apart from the fact that cells isolated from tumor samples can be expected to be genetically heterogeneous, another possible point of concern arises from observations that established cell lines may be genetically unstable during long-term cultivation [4,5]. Though systematic studies on phenotype evolution are scarce, karyotype evolution has been reported in HMT-3522 cells cultured over 205 passages that was attributed to

* Corresponding author. Fax: +49 731 500 23797.
E-mail address: heinz-juergen.hausser@medizin.uni-ulm.de (H.-J. Hausser).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.05.097

genetic instability, and heterogeneity was accompanied by changes in the growth capacities of the cells [5]. Indicative of phenotype evolution, two variants of the monocytic cell line U937 have been described, one being responsive to tumor necrosis factor and the other one being resistant [6]. And even for Saos-2 cells isolated observations on passage-dependent alkaline phosphatase activity [7] and relative responsiveness of adenylyl cyclase to stimulation by parathyroid hormone and forskolin, respectively, have been reported [8].

Based on these observations we wanted to study more systematically the phenotypic stability of Saos-2 cells during long-term culture. To this aim we cultured Saos-2 cells over approximately 100 passages and looked in selected passages for a set of properties relevant for their use as a model system for osteoblastic cells. Here, we describe that although many of the properties studied were not obviously dependent on passage number, some of them exhibited pronounced passage dependence.

Materials and methods

Materials. Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), and trypsin/EDTA solution were purchased from Biochrom (Berlin, Germany). Ascorbic acid 2-phosphate, β-glycerophosphate, Sigma Fast *p*-nitrophenyl phosphate tablet sets, and alizarin Red-S were from Sigma (Taufkirchen, Germany), and thiazolyl blue tetrazolium bromide was purchased from Fluka (Taufkirchen, Germany). All other chemicals used were of analytical grade. The human osteosarcoma cell line Saos-2 was from the American Type Culture Collection (Rockville, MD).

Cell culture. For propagation of Saos-2 cells, cells were maintained in DMEM containing 44 mM $NaHCO_3$, 2 mM L-glutamine, and 10% FCS (growth medium) at 37 °C in 10% CO_2 in air. After reaching approximately 80% confluency, cells were subcultured by rinsing the cell layer with 0.05% (w/v) trypsin and 0.53 mM EDTA, and subsequent incubation at 37 °C for 10 min. Splitting ratios were approximately 1:5. From every fifth to 10th passage, aliquots were stored in liquid nitrogen in 5% (v/v) dimethyl sulfoxide in DMEM containing 10% FCS for later use in experiments. After propagation over 100 passages, cells from selected passages were thawed and initially plated into 75 cm^2 culture flasks (approximately 1.5×10^4 cells/cm^2) in growth medium. Unless otherwise stated cells were plated for experiments at an initial density of 1.0×10^4 cells/cm^2 and maintained in growth medium for 24 h. Then fresh growth medium containing additionally 0.2 mM ascorbic acid 2-phosphate was added and replaced twice a week. For mineralization studies, cultures were further supplemented with β-glycerophosphate at a final concentration of 10 mM during the last 24 h.

MTT assay. In the proliferation studies, viable cells were determined by the MTT assay that relies on the ability of mitochondrial dehydrogenases to oxidize thiazolyl blue tetrazolium bromide (MTT; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to an insoluble blue formazan product. Cells were incubated with growth medium containing 0.3 μM MTT at 37 °C for 1 h. After removing the culture supernatant and washing the cells, isopropanol containing 40 mM HCl was added and the optical density of the solution was read at 530 nm in an enzyme-linked immunosorbent assay (ELISA) plate reader.

Alkaline phosphatase activity. Alkaline phosphatase activity was assayed in cell lysates by determining the release of *p*-nitrophenol from *p*-nitrophenyl phosphate using Sigma Fast *p*-nitrophenyl phosphate tablet sets according to the instructions of the manufacturer. Cells were lysed with 0.1 mM $ZnCl_2$, 1 mM $MgCl_2$, and 0.1% (v/v) Triton X-100 in 20 mM Tris/HCl, pH 7.4, and release of *p*-nitrophenol was determined by measuring the absorbance at 405 nm over a period of 4 min. In parallel triplicate cell cultures, cell numbers were determined using a Casy 1 cell counter (Schärfe System, Reutlingen, Germany), and alkaline phosphatase activity was normalized to cell number.

Matrix mineralization. For quantification of matrix mineralization, cell cultures were stained with alizarin Red-S essentially as described [9]. Cells were washed with PBS at room temperature and fixed with 4% formaldehyde in PBS. Fixed cell cultures were stained with 40 mM alizarin Red-S (pH 4.2) for 10 min using an orbital shaker. Due to the solubility of amorphic mineral deposits at pH 4.2, staining under these acidic conditions is considered to be specific for hydroxyapatite crystals. To remove non-specifically bound stain, cultures were washed five times with deionized water and once with PBS for 15 min at ambient temperature. Bound dye was solubilized in 10 mM sodium phosphate (pH 7.0) containing 10% cetylpyridinium chloride and quantitated spectrophotometrically at 562 nm. Quantitation by this method has a degree of accuracy similar to the quantitation of hydroxyapatite by binding assay [10].

RT-PCR. Total RNA was extracted from cultured cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and digested with RNase-free DNase (Qiagen) to remove any contaminating genomic DNA. cDNA synthesis from total RNA was performed with Omniscript reverse transcriptase (Qiagen) using $(dT)_{15}$ (1 μM) and random hexanucleotide primers (5 μM; Roche Diagnostics, Mannheim, Germany) simultaneously. Aliquots of the cDNAs were incubated with HotStarTaq DNA polymerase (Qiagen) and the primers specified in Table 1. All primers were synthesized by MWG Biotech (Munich, Germany) and were of high purity salt-free (HPSF) quality. The polymerase was activated (15 min at 96 °C) and then 32 (unless otherwise stated) cycles (45 s at 94 °C, 45 s at 60 °C, and 45 s at 72 °C) were performed on a RoboCycler Gradient 96 (Stratagene, Amsterdam, The Netherlands). Amplification products were visualized by agarose gel electrophoresis after staining with ethidium bromide. Images were acquired using the ImageMaster VDS system (Amersham Biosciences, Freiburg, Germany) and the accompanying software (version 2.0).

Statistical analysis. All experiments were repeated at least twice, and similar results were obtained in all these independent experiments. Quantitative proliferation and mineralization assays were performed in quadruplicate in these independent experiments, and means and standard deviations of typical experiments are shown. Data were analyzed using Student's *t* test. A value of $P < 0.05$ was considered statistically significant.

Results

Saos-2 cells from late passages exhibited increased proliferation rates and decreased alkaline phosphatase specific activity

In order to study the phenotypic stability of Saos-2 cells during long-term culture, we propagated Saos-2 cells over more than 100 passages. As under these conditions selection of cells with higher growth capacity might be expected, we compared the proliferation of cells that had been stored frozen upon different times of propagation. It can be seen in Fig. 1 that cells from later passages exhibited higher proliferation rates than early passage cells, the maximal effect being an increase by approximately 65%. Concomitantly, there was a de-

Table 1
Primers used for RT-PCR

Target	Accession No.	Product size	Upstream primer	Downstream primer
Col I	NM_000088	200	GGCCCAGAAGAACTGGTAC	CGCTGTTCTTGCAGTGGTAG
ALP	NM_000478	189	ACCTCGTTGACACCTGGAAG	CCACCATCTCGGAGAGTGAC
BSP	NM_004967	229	ACACTGGGCTATGGAGAGGA	CTGCCTCTGTGCTGTTGGTA
OC	NM_199173	194	GGCAGCGAGGTAGTGAAGA	AGCAGAGCGACACCCTAGA
DCN	NM_001920	189	GCTCTCCTACATCCGCATTG	GAGCCATTGTCAACAGCAGA
BGN	NM_001711	213	ACAGTGGCTTTGAACCTGGA	TCATCCTGATCTGGTTGTGG
BMP-2	NM_001200	211	CTATCCCCACGGAGGAGTTT	TCAAAACTTTCCCACCTGCT
BMP-4	NM_001202	199	TGATACCTGAGACGGGGAAG	CCAGACTGAAGCCGGTAAAG
FGF-1	NM_000800	181	TGCCTCCAGGGAATTACAAG	TGGCCAGTCTCGGTACTCTT
FGF-2	NM_002006	167	TTCTCTGGCAGTTCCTTATGA	GACCTGGCGAAGACTGAAAA
TGF-β1	NM_000660	219	CACGTGGAGCTGTACCAGAA	GAACCCGTTGATGTCCACTT
IGF-2	NM_000612	212	ACACCCTCCAGTTCGTCTGT	GGGGTATCTGGGGAAGTTGT
VEGF	NM_003376	218	GGGCAGAATCATCACGAAGT	ATCTGCATGGTGATGTTGGA
Ptn	NM_002825	199	CTGCCTTCTTGGCATTCATT	TTCATGGTTTGCTTGCACTC
Midk	NM_002391	209	CCTGCAACTGGAAGAAGGA	CTTTCCCTTCCCTTTCTTGG
SDC-3	AF_248634	244	GACATCCCTGAGAGGAGCA	GTGTCCCAGGTGGAGATGAT
ALK	NM_004304	192	GCAACATCAGCCTGAAGACA	GCCTGTTGAGAGACCAGGAG
PTPRZ	NM_002851	181	ATTCTGCAGCCCTAAAGCAA	AGGAGAGGGTGCTGGGTAAT
LRP-5	NM_002335	190	TTCGTCATGGGTGGTGTCTA	CGGAGCTCATCATGGACTTT
LRP-6	NM_002336	192	CCCATGCACCTGGTTCTACT	CTGGAACTGGGACTCTGAGC
GAPDH	NM_002046	188	GAGTCCACTGGCGTCTTCAC	GGTGCTAAGCAGTTGGTGGT
β-Actin	NM_001101	202	TGGGACGACATGGAGAAAAT	CAGAGGCGTACAGGGATAGC

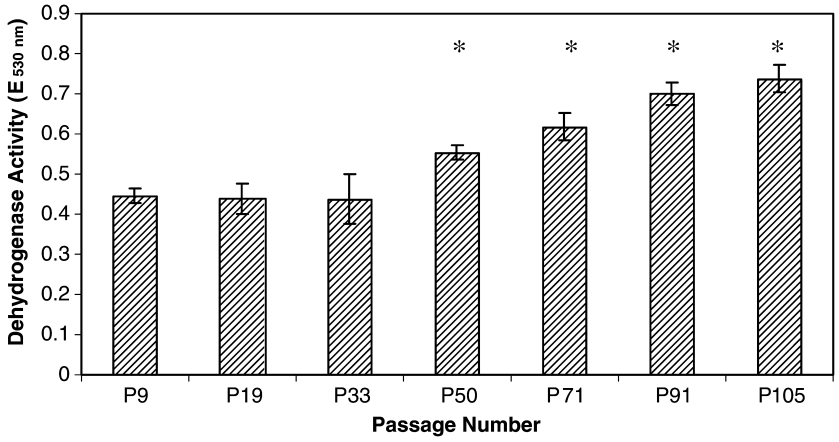

Fig. 1. Increased proliferation rates in late passage Saos-2 cells. Saos-2 cells from different passages were seeded at an initial density of 5000 cells/cm^2. After 4 days, relative cell numbers were determined by assessment of mitochondrial dehydrogenase activity. Results of a typical experiment performed in quadruplicate are shown. Values are given as means ± SD. *Different from control ($P < 0.05$).

crease in the specific activity of alkaline phosphatase (Fig. 2).

Saos-2 cells from late passages exhibited a higher matrix mineralization than early passage cells

The increased proliferative capacity and the decreased specific activity of alkaline phosphatase might be indicative for a reduction of osteoblastic properties in our late passage cells. We therefore decided to compare the capacity of cells from different passages to mineralize their extracellular matrix as an osteoblastic property of a late differentiation stage. As can be seen in Fig. 3, matrix mineralization was not decreased in cultures of Saos-2 cells from higher passages. On the contrary, cultures of higher passage cells even exhibited an up to 5-fold higher matrix mineralization.

Expression of decorin was strongly decreased in late passage cells

The observed higher matrix mineralization in cultures of high passage Saos-2 cells described above was not compatible with the assumption of a generally decreased osteoblastic phenotype in these cells. To further characterize the differentiation status of our Saos-2 cells, we looked for the expression of several osteogenic markers and matrix proteins by means of RT-PCR (Fig. 4). As suggested by the decreased specific activity of alkaline phosphatase, we could observe a corresponding decrease

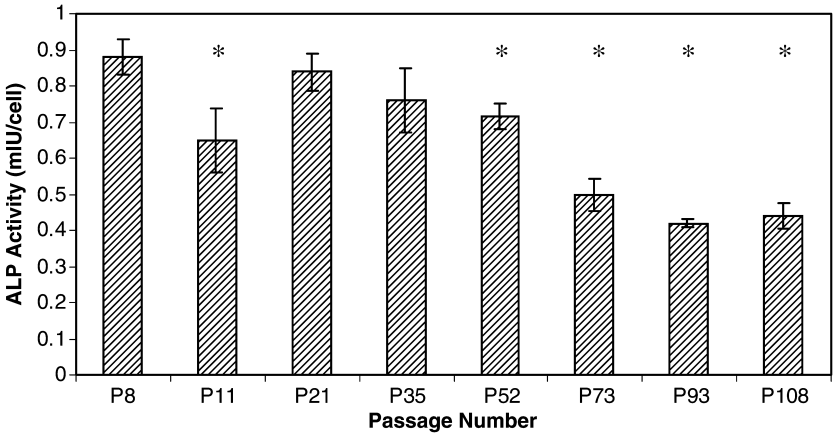

Fig. 2. Decreased alkaline phosphatase activity in late passage Saos-2 cells. Saos-2 cells from different passages were seeded at an initial density of 10,000 cells/cm^2. After 3 days, alkaline phosphatase activity was determined. Results of a typical experiment performed in triplicate are shown. Values are given as means ± SD. *Different from control ($P < 0.05$).

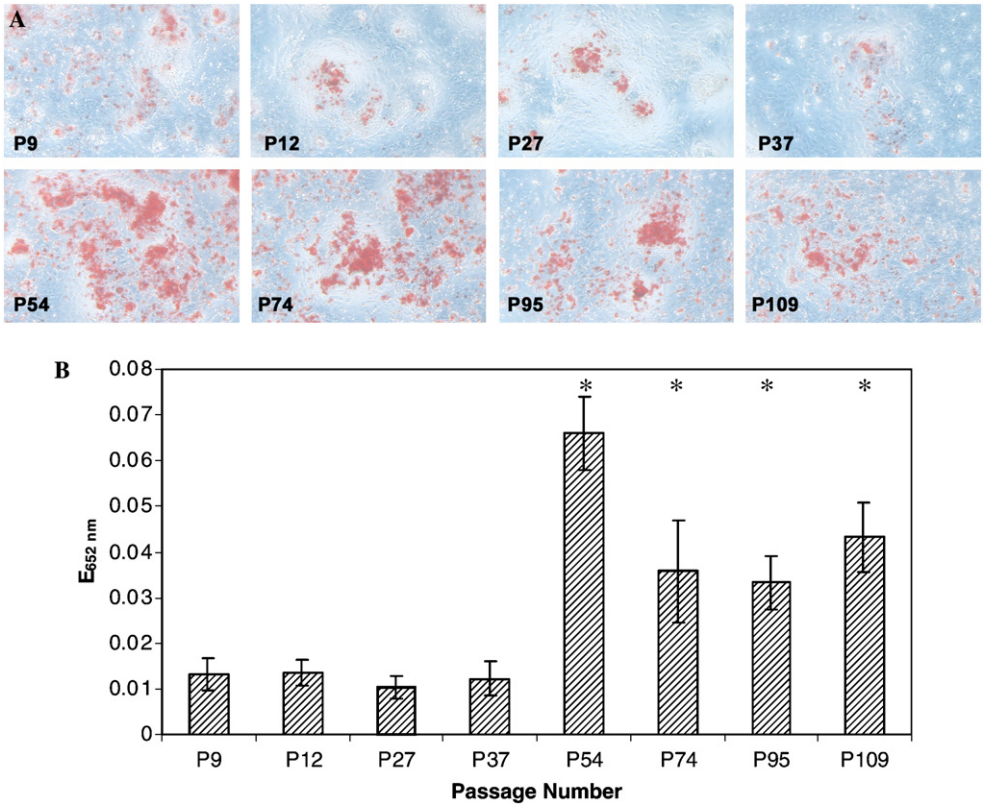

Fig. 3. Increased matrix mineralization in late passage Saos-2 cell cultures. Saos-2 cells from different passages were seeded at an initial density of 10,000 cells/cm^2 and cultured in the presence of ascorbic acid 2-phosphate for 17 days. Mineralization was visualized by staining with alizarin Red-S (A). Bound dye was solubilized and quantitated photometrically (B). Results of a typical experiment performed in quadruplicate are shown. Values are given as means ± SD. *Different from control ($P < 0.05$).

in the relative expression of this enzyme. Furthermore, consistent with the finding of an increased matrix mineralization in cultures of high passage cells, expression of bone sialoprotein (BSP) and osteocalcin (OC) was increased in these cells. However, with the exception of decorin, differences in expression did not exceed a factor of 2 based on the integrated optical densities of the visualized amplification products. The small leucine-rich proteoglycan decorin was strongly expressed in early passage cells, and its expression was profoundly decreased in late passage cells. The same passage dependence was observed for decorin expression already 1

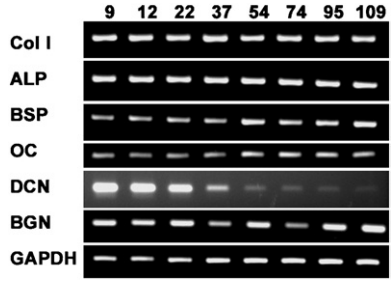

Fig. 4. Expression of matrix proteins and differentiation markers. Saos-2 cells from different passages were seeded at an initial density of 10,000 cells/cm^2 and were cultured in the presence of ascorbic acid 2-phosphate. After 10 days, total RNA was isolated and reverse transcribed. Expression of collagen type I (Col I), alkaline phosphatase (ALP), bone sialoprotein (BSP), osteocalcin (OC), decorin (DCN), and biglycan (BGN) was analyzed by RT-PCR. Note that 40 amplification cycles were needed to obtain the biglycan amplification products. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown for control.

day after subculturing (not shown), ruling out the possibility that the low expression levels in late passage cells are merely caused by downregulation when late passage cells reach confluency earlier due to their increased proliferation rate.

Passage number did not have a pronounced effect on the expression of various growth factors

As autocrine regulation by secreted growth factors might contribute to the observed differences in the behavior of our Saos-2 cells from different passages, we looked for the expression of a panel of growth factors known to influence the properties of osteoblastic cells (Fig. 5). Though for some of these growth factors minor changes in expression could be reproducibly observed, the changes were not very pronounced for any of these growth factors.

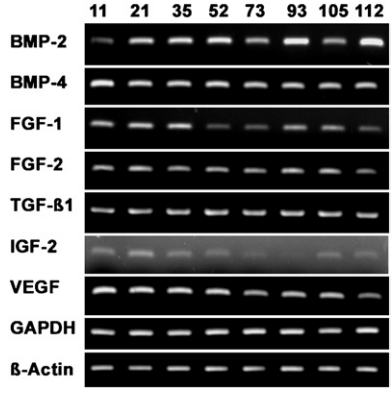

Fig. 5. Expression of growth factors by Saos-2 cells from different passages. Saos-2 cells from different passages were seeded at an initial density of 10,000 cells/cm^2. The next day total RNA was isolated and reverse transcribed. Expression of growth factors was analyzed by RT-PCR. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin is shown for control.

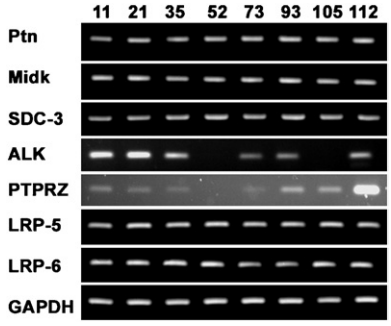

Fig. 6. Expression of pleiotrophin and midkine and their receptors. In the experiment described in Fig. 5, expression of pleiotrophin (Ptn), midkine (Midk), syndecan-3 (SDC-3), anaplastic lymphoma kinase (ALK), receptor-type protein tyrosine phosphatase zeta (PTPRZ), low density lipoprotein receptor-related protein 5 (LRP-5), and low density lipoprotein receptor-related protein 6 (LRP-6) was analyzed by RT-PCR with total RNA obtained 1 day after subculturing. Note that 40 amplification cycles were needed to obtain the amplification products of ALK and PTPRZ.

Two of the receptors for pleiotrophin exhibited a distinct expression pattern in early and late passage Saos-2 cells, respectively

Whereas expression of pleiotrophin and midkine themselves as well as expression of three of their putative receptors appeared to be largely independent of the passage number, two of their receptor molecules were distinctly expressed in early and late passage Saos-2 cells, respectively (Fig. 6). While expression of anaplastic lymphoma kinase (ALK), that we here show for the first time to be expressed in osteoblastic cells, was most prominent in early passage cells, the receptor-type protein tyrosine phosphatase zeta (PTPRZ) was expressed almost exclusively in late passage cells.

Discussion

As Saos-2 cells are widely used as a model system for human osteoblastic cells we were interested in whether these cells are phenotypically stable during prolonged cell culture. To study this question, we propagated Saos-2 cells over approximately 100 passages and analyzed selected passages for a number of phenotypic properties. The two most important findings of our study were: (1) a remarkable stability of most of the phenotypic properties studied, with differences being only quantitative in nature and not exceeding a factor of 2, and (2) a striking passage dependence of some other phenotypic properties.

Phenotypic variability may be the result of either genetic heterogeneity or/and genetic instability of the starting cell population, yielding the basis for subsequent

selection processes under cell culture conditions. Karyotype evolution during prolonged in vitro culture has already been observed in some established cell lines [5,11]. Although the underlying variability has been suggested to be created randomly [5], karyotype evolution in these reported cases was accompanied by changes in the growth capacities of the cells, with a selection of the in vitro most rapidly growing cell types [11]. In line with these reports we too observed an increased proliferation rate in late passage Saos-2 cells. It should be noted, however, that the maximal effect we observed was an increase by approximately 65% during a 4-day incubation period. Indeed, in most cases where we could observe differences in the properties of Saos-2 cells from different passages, the differences were not very much pronounced.

From all the phenotypic properties we studied we saw the most striking differences in the expression of the small leucine-rich proteoglycan decorin and of two receptors for pleiotrophin and midkine. Decorin was strongly expressed in our early passage cells, but after propagation over approximately 20 passages expression of this proteoglycan declined and was hardly detectable anymore in the late passage cells. Importantly, this difference in decorin expression was already present in freshly plated cells, implying that the low decorin expression in late passage cells is an inherent property of these cells and not, for instance, secondary to the increased proliferation potential of these cells. Interestingly, Saos-2 cells have been described in the literature both as cells that synthesize decorin [2] and as cells that barely synthesize decorin [12]. Apart from its involvement in the regulation of collagen fibrillogenesis [13], decorin has additional roles as a negative regulator of cell proliferation [14] and as an inhibitor of matrix mineralization [15,16]. At least consistent with these latter two functions, we observed increased proliferation rates and increased matrix mineralization in our late passage cells, where decorin expression was low.

Though we did not observe any dramatic differences in the expression of a number of growth factors known to be important for the regulation of osteoblastic cells, two receptors recently described as receptors for pleiotrophin and midkine, two structurally related heparin-binding growth factors implicated in bone growth and fracture repair [17–22], exhibited a striking preferential expression in early and late passage Saos-2 cells, respectively. Whereas expression of anaplastic lymphoma kinase [23,24] was most prominent in our early passage cells, expression of the receptor-type protein tyrosine phosphatase zeta [25,26] appeared to be almost restricted to the late passage cells. Though the exact functional role(s) of pleiotrophin and midkine in the regulation of osteoblasts is still incompletely understood, it is conceivable that experiments targeting this question using Saos-2 cells from different passages as a model for human osteoblastic cells may yield different results, depending on which of the two signaling pathways will be activated.

From our studies described in this paper we would like to conclude that although due to the remarkable stability of many phenotypic properties Saos-2 cells may be well suited as a model system for human osteoblastic cells in many cases, it should always be kept in mind that these cells may behave differently in certain experimental situations, depending on their culture history. Such differences in culture history occur, e.g., when the cells are propagated in different laboratories. Special care appears to be appropriate when using clonally selected Saos-2 cells, such as in experiments with stably transfected cells where the properties of the (clonally selected) transfected cells are to be compared with the untransfected controls.

Acknowledgments

This work was financially supported by the Deutsche Forschungsgemeinschaft (Grants HA1652/1-1 and HA1652/1-2). The expert technical assistance of Gabi Mettenleiter and Christiane Schulz is gratefully acknowledged.

References

[1] J. Fogh, G. Trempe, New human tumor cell lines, in: J. Fogh (Ed.), Human Tumor Cell Lines in Vitro, Plenum Press, New York, 1975, pp. 115–159.
[2] D.J. McQuillan, M.D. Richardson, J.F. Bateman, Matrix deposition by a calcifying human osteogenic sarcoma cell line (SAOS-2), Bone 16 (1995) 415–426.
[3] R. Gundle, J.N. Beresford, The isolation and culture of cells from explants of human trabecular bone, Calcif. Tissue Int. 56 (1995) S8–S10.
[4] S.E. Mamaeva, Karyotypic evolution of cells in culture: a new concept, Int. Rev. Cytol. 178 (1998) 1–40.
[5] K.V. Nielsen, M.W. Madsen, P. Briand, In vitro karyotype evolution and cytogenetic instability in the non-tumorigenic human breast epithelial cell line HMT-3522, Cancer Genet. Cytogenet. 78 (1994) 189–199.
[6] L. Kaszubowska, H. Engelmann, M. Gotartowska, M. Iliszko, J. Bigda, Identification of two U937 cell sublines exhibiting different patterns of response to tumour necrosis factor, Cytokine 13 (2001) 365–370.
[7] J.R. Farley, S.L. Hall, S. Herring, N.M. Tarbaux, T. Matsuyama, J.E. Wergedal, Skeletal alkaline phosphatase specific activity is an index of the osteoblastic phenotype in subpopulations of the human osteosarcoma cell line SaOS-2, Metabolism 40 (1991) 664–671.
[8] H. Gao, P.V. Bodine, R. Murrills, F.J. Bex, J.P. Bilezikian, S.A. Morris, PTH-dependent adenylyl cyclase activation in SaOS-2 cells: passage dependent effects on G protein interactions, J. Cell. Physiol. 193 (2002) 10–18.
[9] C.M. Stanford, P.A. Jacobson, E.D. Eanes, L.A. Lembke, R.J. Midura, Rapidly forming apatitic mineral in an osteoblastic cell line (UMR 106-01 BSP), J. Biol. Chem. 270 (1995) 9420–9428.
[10] P.C. Schiller, G. D'Ippolito, B.A. Roos, G.A. Howard, Anabolic or catabolic responses of MC3T3-E1 osteoblastic cells to para-

thyroid hormone depend on time and duration of treatment, J. Bone Miner. Res. 14 (1999) 1504–1512.
[11] R. Kerler, H.M. Rabes, Karyotype evolution of the clonal rat liver cell line CL 52 during progression in vitro and in vivo, Cancer Genet. Cytogenet. 87 (1996) 140–147.
[12] Y. Takeuchi, S. Fukumoto, T. Matsumoto, Relationship between actions of transforming growth factor (TGF)-beta and cell surface expression of its receptors in clonal osteoblastic cells, J. Cell. Physiol. 162 (1995) 315–321.
[13] K.G. Vogel, M. Paulsson, D. Heinegard, Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon, Biochem. J. 223 (1984) 587–597.
[14] A. De Luca, M. Santra, A. Baldi, A. Giordano, R.V. Iozzo, Decorin-induced growth suppression is associated with up-regulation of p21, an inhibitor of cyclin-dependent kinases, J. Biol. Chem. 271 (1996) 18961–18965.
[15] K. Hoshi, S. Kemmotsu, Y. Takeuchi, N. Amizuka, H. Ozawa, The primary calcification in bones follows removal of decorin and fusion of collagen fibrils, J. Bone Miner. Res. 14 (1999) 273–280.
[16] Y. Mochida, W.R. Duarte, H. Tanzawa, E.P. Paschalis, M. Yamauchi, Decorin modulates matrix mineralization in vitro, Biochem. Biophys. Res. Commun. 305 (2003) 6–9.
[17] S. Imai, M. Kaksonen, E. Raulo, T. Kinnunen, C. Fages, X. Meng, M. Lakso, H. Rauvala, Osteoblast recruitment and bone formation enhanced by cell matrix-associated heparin-binding growth-associated molecule (HB-GAM), J. Cell Biol. 143 (1998) 1113–1128.
[18] R.S. Tare, R.O. Oreffo, N.M. Clarke, H.I. Roach, Pleiotrophin/osteoblast-stimulating factor-1: dissecting its diverse functions in bone formation, J. Bone Miner. Res. 17 (2002) 2009–2020.
[19] X. Yang, R.S. Tare, K.A. Partridge, H.I. Roach, N.M. Clarke, S.M. Howdle, K.M. Shakesheff, R.O. Oreffo, Induction of human osteoprogenitor chemotaxis, proliferation, differentiation, and bone formation by osteoblast-stimulating factor-1/pleiotrophin: osteoinductive biomimetic scaffolds for tissue engineering, J. Bone Miner. Res. 18 (2003) 47–57.
[20] X.B. Yang, D.W. Green, H.I. Roach, N.M. Clarke, H.C. Anderson, S.M. Howdle, K.M. Shakesheff, R.O. Oreffo, Novel osteoinductive biomimetic scaffolds stimulate human osteoprogenitor activity—implications for skeletal repair, Connect. Tissue Res. 44 (2003) 312–317.
[21] G. Li, J.R. Bunn, M.T. Mushipe, Q. He, X. Chen, Effects of pleiotrophin (Ptn) over-expression on mouse long bone development, fracture healing and bone repair, Calcif. Tissue Int. (2005) [Epub ahead of print].
[22] S. Ohta, H. Muramatsu, T. Senda, K. Zou, H. Iwata, T. Muramatsu, Midkine is expressed during repair of bone fractures and promotes chondrogenesis, J. Bone Miner. Res. 14 (1999) 1132–1144.
[23] G.E. Stoica, A. Kuo, A. Aigner, I. Sunitha, B. Souttou, C. Malerczyk, D.J. Caughey, D. Wen, A. Karavanov, A.T. Riegel, A. Wellstein, Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin, J. Biol. Chem. 276 (2001) 16772–16779.
[24] G.E. Stoica, A. Kuo, C. Powers, E.T. Bowden, E.B. Sale, A.T. Riegel, A. Wellstein, Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types, J. Biol. Chem. 277 (2002) 35990–35998.
[25] N. Maeda, K. Ichihara-Tanaka, T. Kimura, K. Kadomatsu, T. Muramatsu, M. Noda, A receptor-like protein-tyrosine phosphatase PTPzeta/RPTPbeta binds a heparin-binding growth factor midkine. Involvement of arginine78 of midkine in the high affinity binding to PTPzeta, J. Biol. Chem. 274 (1999) 12474–12479.
[26] K. Meng, A. Rodriguez-Pena, T. Dimitrov, W. Chen, M. Yamin, M. Noda, T.F. Deuel, Pleiotrophin signals increased tyrosine phosphorylation of beta beta-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase beta/zeta, Proc. Natl. Acad. Sci. USA 97 (2000) 2603–2608.