Title: Differences in the pattern and regulation of mineral deposition in human cell lines of osteogenic and non-osteogenic origin.

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Short Title: Differences in regulation of mineral deposition in human cell lines

Abstract:

Bone marrow-derived mesenchymal stem cells (MSCs) are widely used as a cellular model of bone formation, and can mineralize *in vitro* in response to osteogenic medium (OM). It is unclear however whether this property is specific to cells of mesenchymal origin. We have analysed the OM response in three non-osteogenic lines, HEK293, HeLa and NTera, compared to MSCs. Whereas HEK293 cells failed to respond to OM conditions, the two carcinoma-derived lines NTera and HeLa deposited a calcium phosphate mineral comparable to that present in MSC cultures. However, unlike MSCs, HeLa and NTera cultures did so in the absence of dexamethasone. This discrepancy was confirmed as BMP inhibition obliterated the OM response in MSCs but not in HeLa or NTera, indicating that these two models can deposit mineral through a mechanism independent of established dexamethasone or BMP signalling.

Keywords: Osteogenic treatment, mineral deposition, mesenchymal stem cell, cancer cell line.

Abbreviations:

ALP	alkaline phosphatase
BMP	bone morphogenetic protein
CLATH	clathrin
CTRL	control medium
DEX	dexamethasone
DORSO	dorsomorphin
ECM	extra-cellular matrix
ELISA	enzyme-linked immunosorbent assay
ESEM	Environmental Scanning Electron Microscopy
MSC	mesenchymal stem cell
OM	osteogenic medium
OS	osteogenic supplements
XRD	X-ray diffraction

Introduction:

Research into bone metabolism represents an important area of research with wide-ranging biomedical applications for conditions including osteoporosis, orthopaedic repair and genetic disorders [Kimelman et al., 2007; Khosla et al., 2008]. The process of bone formation is studied extensively *in vitro*, using cellular models to analyse the mechanisms regulating bone formation and bone repair. Beyond their use to elucidate fundamental aspect of developmental bone biology or to screen pharmacological regulators of bone metabolism, *in vitro* models of osteogenic differentiation are increasingly used for the development of new translational strategies combining cell progenitors and tissue engineering approaches for bone repair [Cancedda et al., 2003; Marie and Fromigue, 2006]. *In vitro* osteogenic differentiation studies routinely involve the use of primary progenitors and cell lines.

Mesenchymal stem cells (MSCs), which can be isolated from bone marrow aspirates, represent the established model of choice for the study of postnatal osteogenesis [Beresford et al., 1993; Cheng et al., 1994; Jaiswal et al., 1997; Pittenger et al., 1999; Sottile et al., 2002]. MSCs can be expanded and differentiated *in vitro* using established an osteogenic medium (OM), which includes dexamethasone, ascorbic acid phosphate and β-glycerophosphate. *In vitro* osteogenesis has also been observed in other cell systems such as embryonic stem cell cultures from both mouse and human origin [Buttery et al., 2001; Sottile et al., 2003; zur Nieden et al., 2003] and osteosarcoma cell lines such as SaOS [Clover and Gowen, 1994; Pautke et al., 2004], despite notable biological differences [Tang et al., 2008; Palmieri et al., 2009].

Although the efficiency of this OM-based differentiation protocol is well established in these models, it is unclear whether the potential to deposit mineral is restricted to osteogenic cell types and multipotent progenitors, or whether common cellular models and cancer cell lines originating from outside the bone lineage could respond to this OM. This has not been formally investigated, and would highlight cell sources which could reliably act as negative control when analysing OM-treated progenitor cultures, in order to adequately appraise their osteogenic potential and rule out artefactual deposition of mineral as previously described in some non-osteogenic cellular models [Khouja et al., 1990]. Here we report the assessment of the mineral deposition obtained in 4 cultures of cells from osteogenic and non-osteogenic origin. The OM response observed in NTera [Andrews et al., 1984], HeLa [Gey et al., 1952] and HEK29 [Graham et al., 1977] cells was compared to that of bone marrow-derived MSCs [Okamoto et al., 2002; Sottile et al., 2003] at molecular and cellular levels. Levels of OM-induced mineral deposition and changes in marker expression were measured over time, and the nature of mineral deposits obtained *in vitro* were analysed. Our study highlights major differences in the pathways regulating the mineralizing response across cell lines *in vitro*.

Materials and Methods:

Reagents were purchased from Invitrogen (Paisley, UK) unless otherwise stated.

Cell Culture:

Human bone marrow-derived mesenchymal stem cells (MSCs) [Okamoto et al., 2002; Sottile et al., 2003], human cervical carcinoma cells (HeLa) [Gey et al., 1952], human embryonic kidney cells (HEK293) [Graham et al., 1977; Thomas et al. 2005], and human embyonal carcinoma cells (NTera-2) [Andrews et al., 1984] were grown on tissue culture plastic in control medium ('CTRL') containing DMEM supplemented with 10% FCS, 1 mM L-glutamine, 1% nonessential amino acids and antibiotics, and maintained at 37 ºC in a humidified atmosphere containing 95% air and 5% CO_2. Cell stocks were passaged with Trypsin/EDTA and split 1:4 for routine culture. For osteogenic assays, cells were plated in control medium at 5×10^4 cells/well in 12-well tissue culture plates and grown to confluence. The medium was then changed (considered as 'day0') and cells were subsequently grown in CTRL or osteogenic medium ('OM') containing 100 nM Dexamethasone ('Dex', Sigma-Aldrich, Gillingham, UK), 10 mM β-glycerophosphate (Sigma-Aldrich) and 0.05 mM L-ascorbic acid-2-phosphate (Sigma-Aldrich) [Jaiswal et al., 1997; Bruder et al., 1998; Pittenger et al., 1999; Jaiswal et al., 2000; Halleux et al., 2001; Sottile et al., 2002].

Quantification of mineralization and alkaline phosphatase activity:

For detection of mineralized nodules, cells were washed with PBS and fixed in 4% (v/v) paraformaldehyde at room temperature for 15 min. The wells were then washed twice with excess dH_2O prior to incubation with 1.5 mL of 1% Alizarin-Red S solution for 15 min. The quantitative measurement of mineral deposition was performed by extraction of the incorporated stain [Gregory et al., 2004; Tataria et al., 2006]. A 96-well plate reader (BioTek ELx800, Fisher Scientific, Loughborough, UK) was used to measure absorbance of triplicate samples during the assay. The mineral content was normalised to the DNA content per well [Rago et al., 1990], and measurements of alkaline phosphatase activity were performed using SIGMAFAST pNPP reagents (Sigma) according to manufacturer's instructions. Data represent mean ± SEM (n= 3).

ESEM and XRD mineralization analysis:

For Environmental Scanning Electron Microscopy (ESEM), samples were fixed with ice-cold 4% paraformaldehyde for 15 min, and then washed in 0.1M cacodylate buffer containing 7% sucrose. Images were obtained using an environmental scanning electron microscope (Philips FEG ESEM) equipped with a Trecor detector set at an accelerating voltage of 10 kV under a vacuum of 4.0-5.8 torr and 6% humidity. X-ray diffraction measurements were carried out as previously described [Sottile et al., 2003].

Flow cytometry:

Cells harvested using trypsin/EDTA were pelleted by centrifugation 5 min at 200 g, and incubated with CD29 (Abcam), CD105 (AbSerotec), CD106 (AbSerotec), p75 (Abcam), CD90 (eBiosciences), CD44

(eBiosciences), SSEA4 (eBiosciences) conjugated antibodies at room temperature for 30 min. Samples were washed in PBS and stored on ice until FACS analysis on a Beckman-Coulter Altra flow cytometer.

Osteocalcin protein assay:

Confluent cells were treated for 21 days and osteocalcin concentrations present in the cultures were measured by enzyme-linked immunosorbent assay (ELISA) using the Invitrogen Osteocalcin Human Direct ELISA Kit according to the manufacturer's instructions. Data represent mean ± SEM (n= 4).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR):

RT-PCR was carried out as described elsewhere [Sottile et al., 2002], using the following primers (forward/reverse): clathrin (Clath): ttataatgggggaaaacagg / ttgtctttgttggcactga ; bone specific alkaline phosphatase (bALP): gcagccactgagcgttc / gtgggagtgcttgtatctc. The PCR reactions were heated at 95 ºC for 5min, before undergoing 30 cycles of [95 ºC for 30 s, 60 ºC for 45 s and 72 ºC for 1min].

Results:

Mesenchymal marker expression in multi- and nulli-potent cell types:

To compare the cellular characteristics of the HEK293, NTera and HeLa cells analysed with those of MSCs, we first evaluated the expression levels of a panel of surface markers CD29, CD105, CD106, p75, CD90, CD44 known to be expressed on mesenchymal progenitors [Mareschi et l., 2006; Jackson et al., 2007; Spiropoulos et al., 2010], as well as a surface marker associated with both MSCs and embryonal carcinoma cells SSEA4 [Gang et al., 2007; Andrews, 2002]. Analysis of cell suspensions by flow cytometry indicated that whereas control MSCs showed positive expression of mesenchymal markers ($CD29^{hi}$/$CD105^{hi}$/CD106/p75/$CD90^{hi}$/$CD44^{hi}$/$SSEA4^{hi}$) as expected (Fig.1), NTera cells and HeLa cells exhibited lower CD105 expression, undetectable CD106 expression and variable CD90 and CD44 expression. By contrast, HEK293 cells did not show detectable expression for CD105, CD106, CD90 and CD44, and low CD29 expression, revealing a profile significantly different from that of control MSCs. Only MSCs and NTera cells showed high SSEA4 expression.

Mineral deposition potential of osteogenic and non-osteogenic cells:

To assess the response to pro-osteogenic medium in HEK293, HeLa, and Ntera cell lines, we treated the cells with standard OM for up to 4 weeks and compared the outcome to MSC controls. This experiment showed a differential response to OM for the different cultures analysed. Bone nodules were visible by phase microscopy in the treated MSCs as expected, and the formation of mineral deposits could also be observed in HeLa and NTera cells. To confirm this observation, each cell type was evaluated by Alizarin Red S staining at various time-points to assess the extent of mineral deposition in each well (Fig.2). When exposed to OM for 2 to 3 weeks, the cell types analysed showed an ability to deposit mineral as highlighted by Alizarin Red staining, with the exception of HEK293 (Fig.2A-B). None of the cell types analysed showed any significant mineral deposition in the absence of OM treatment over the 28 days. To better compare the cell lines analysed, the amount of Alizarin Red staining was quantified at regular time-points over 4 weeks *in vitro* to establish the kinetic of mineral deposition (Fig.2C). In order to account for differences in growth characteristics, DNA content was used as normalisation parameter. HeLa cultures were the first to show visible mineral deposits as early as day 4-7, while both MSC and NTera cells showed noticeable staining at day 14. Expression of osteocalcin, an established osteogenic marker [Aubin et al., 1995], was measured in order to assess whether exposure to OS conditions triggered osteogenic differentiation the cultures undergoing mineral deposition. An Elisa assay carried out after 14 days (Fig.2D) showed that whereas no osteocalcin expression could be detected in HEK293 or NTera cells, HeLa cells were seen to express osteocalcin upon treatment, as seen in MSC controls.

Alkaline phosphatase expression in cell lines after treatment:

Changes in alkaline phosphatase activity, an assay widely used for osteogenic cultures [Aubin et al., 1995; Jaiswal et al., 1997], were analysed in the 4 cell lines considered to determine whether this parameter was affected by exposure to OM treatment. While MSCs showed extensive up-regulation of alkaline phosphatase activity upon OM exposure as expected, none of the 3 other cell types showed a similar response (Fig. 3A,B). Both NTera and HeLa cells showed high basal alkaline phosphatase activity in control conditions, which was not significantly affected by exposure to OM treatment. The alkaline phosphatase activity observed in HEK293 cells was found to be below detection levels in both control and OM conditions.

Since the alkaline phosphatase activity assay cannot discriminate between the various isoenzymes present in the cell, and to further characterise the nature of the cellular response observed under OM conditions, we analysed the presence of transcript for the bone/liver/kidney alkaline phosphatase ('bALP') in the cell models studied, in the presence or absence of OM. Expression of bALP, which typically increases in osteogenic cultures, was differentially regulated in the cell types studied. In MSCs, a significant upregulation of bALP mRNA was observed in OM conditions, whereas there was no detectable increase in bALP mRNA upon treatment of HeLa, NTera or HEK293 cells (Fig.3C). Whilst levels of bALP were below detection in HEK293 and HeLa cells, steady levels of bALP mRNA were detected in NTera cultures under both control and OM conditions.

Mineralization analysis:

To better characterise the material deposited in the cultures, environmental scanning electron microscopy ('ESEM') analysis was carried out for OM-treated cultures from the different cell lines (Fig. 4A-B). The spectral analyses obtained by ESEM measurements showed that the material collected in NTera and HeLa cells exposed to OM was composed of calcium phosphate mineral, as observed in MSCs cultures (Fig. 4B), while HEK293 cells did not show such deposits. Calcium phosphate ratios measured in MSCs and HeLa cultures (1.4) were higher than for NTera ccultures (1.2), lower than that of physiological calcified tissue (1.6). The nature of the mineral collected was further evaluated by X-ray diffraction analysis of the deposits harvested from OM-treated HeLa and NTera cultures (Fig. 4C). The results for both NTera and MSC showed strong diffraction patterns with peaks corresponding to hydroxyapatite [Markovic et al., 2004], whereas the HeLa-derived sample showed a weaker crystalline signal with limited detectable rings compatible with an apatite structure, suggesting a more amorphous nature.

Dexamethasone- and BMP-dependence:

To determine whether the induction of mineral deposition observed in HeLa and NTera was comparable to that observed in MSCs, we tested the effect of two regulators of osteogenic differentiation on the different cultures. Cells were first treated in the presence and absence of dexamethasone ('Dex'), a glucocorticoid known to increase mineralization in osteogenic cultures [Iba et al., 1995; Jorgensen et al., 2004] and routinely included in osteogenic treatments [Jaiswal et al., 1997; Pittenger et al., 1999; Sottile et al., 2002]. Alizarin red staining and subsequent quantitation revealed significant differences between the cultures (Fig.5A): while exposure to Dex was required for MSC mineralization *in vitro*, both NTera and HeLa cells

achieved significant mineralization when treated in the absence on Dex. In HeLa cell cultures, *in vitro* mineralization appeared significantly higher in the absence of Dex compare to full OS conditions.

A similar analysis was carried out in the presence or absence of dorsomorphin ('dorso'), a small molecule inhibitor of the BMP pathway [Yu et al., 2008] (Fig.5B). This assay showed that whereas OM-triggered mineralization in MSCs was negatively affected by inhibition of the BMP pathway, in line with the known pro-osteogenic effect of BMP signalling [Seib et al., 2009], both NTera and HeLa cells were able to efficiently deposit mineral despite BMP inhibition.

Discussion:

MSCs provide an efficient *in vitro* model for OM-induced mineralization, with a time-dependent increase in mineral deposition, upregulation of alkaline phosphatase activity and osteocalcin expression as decisive hallmarks of differentiation [Jaiswal et al., 1997; Halleux et al., 2001; Sottile et al., 2002]. The mineral deposition kinetics obtained for the MSC culture used here showed a sharp increase throughout the treatment, in line with previous reports [Okamoto et al., 2002; Sottile et al., 2003]. In addition, our results confirmed the dexamethasone-dependency of *in vitro* mineralization [Cheng et al., 1994; Jaiswal et al., 1997; Eijken et al., 2006], and the requirement for BMP signalling [Seib et al., 2009] in MSC cultures. MSCs formed calcium phosphate deposits throughout the ECM and the cell layer, as shown by the ESEM analysis, and produced an X-ray diffraction pattern characteristic of hydroxyapatite [Jaiswal et al., 1997; Sottile et al., 2003]. By contrast, our results establish HEK293 cells as a reliable negative control for osteogenesis research: HEK293 cells were shown to lack detectable expression of the mesenchymal markers CD29, CD105, CD106 and CD90, and we confirmed that this cell line is unable to mineralize in response to pro-osteogenic conditions *in vitro*. Our study further establishes that HEK293 cells do not show detectable alkaline phosphatase activity, even after exposure to OM conditions. In line with their lack of mineral deposition ability, HEK293 failed to display any basal or OM-induced osteocalcin expression, confirming their suitability as negative control for *in vitro* experiments. These results corroborate recent *in vivo* observations that HEK293 cells implanted in a diffusion chamber model lacked osteogenic potential even when the cells were exposed to OM conditions prior to injection [Tremoleda et al., 2008].

HeLa cells on the other hand emerged as an unsuitable negative control for osteogenic treatments. HeLa cells exhibited substantial mineral deposition *in vitro* in response to osteogenic medium. HeLa cells have previously been reported to have an indirect osteogenic effect when implanted in a mouse model of heterotopic bone formation [Ostrowski et al., 1975; Izbicka et al., 1997; Kochanowska et al., 2002b], however this was shown to result from paracrine BMP production which had an osteogenic effect on the surrounding host cells [Kochanowska et al., 2002a] and HeLa had not so far been reported to deposit mineral *in vitro*. The nature of the deposits collected in OM-treated HeLa cultures showed a calcium phosphate component, however the mineral did not seem to be as crystalline or ECM-bound as observed in MSC cultures. In addition, HeLa cells were shown to be strongly positive for alkaline phosphatase activity, in line with reports that they express several alkaline phosphatase isoforms [Boylan et al., 1996; Kniss et al., 2002]. This could explain the substantial mineral content observed under OM conditions, as high alkaline phosphatase activity has been reported to contribute to unspecific mineral deposition in other systems [Russell et al., 1986; Khouja et al., 1990, Hui et al. 1997]. The presence of osteocalcin was also detected in OM-treated cultures, which was unexpected. Although HeLa cells represent a widely used model as negative control for molecular and cellular analyses, the present observations indicate that these cells do not constitute an inert model for osteogenic treatment and mineralization studies.

Similarly, NTera-2 cells were also able to deposit phosphate calcium mineral in culture in response to OM conditions. These observations on an established human EC line are in line with previous reports of osteogenic characteristics in mouse EC-derived lines [Poliard et al., 1993; Shukunami et al., 1997]. The differentiation ability of existing human EC lines is heterogeneous [Pera et al., 1989; Duran et al., 2001]. NTera-2 cells originate from a human testis embryonal carcinoma and have been well characterised, including their propensity to form neuroectodermal lineages in response to retinoic acid [Andrews et al., 1984; Andrews, 2002]. Injections into mouse models indicate that human EC lines can form tumours containing a broad range of tissues, occasionally including cartilage [Pera et al., 1989; Andrews, 2002]. However, this differentiation has not been widely reported for NTera-2 [Duran et al., 2001], and so far reports describing their *in vitro* potential suggest a limited range of mesodermal cell types in NTera-2 cultures [Chadalavada et al., 2005; Pal and Ravindran, 2006; Simoes and Ramos, 2007]. Our study provides new evidence that NTera-2 cultures can deposit mineral *in vitro* after OM treatment. NTera cells are known to express the bALP [Draper et al., 2002], and as discussed in the case of HeLa cells, high alkaline phosphatase activity could contribute to this process. Although advanced analysis of the calcium phosphate deposits revealed a mineral structure compatible with that collected from osteogenic cultures, it is interesting to note that this phenomenon is not dependent on the presence of dexamethasone or BMP signalling, which are known to support mineralization in osteoprogenitor cultures [McCulloch et al., 1986; Cheng et al., 1994; Iba et al., 1995; Jaiswal et al., 1997; Eijken et al., 2006]. The absence of osteocalcin detection further brings into question the biological basis of this cellular response, and calls for further investigation to analyse the nature of this process.

β-glycerophosphate is widely used in osteogenic media as a source of organic phosphate known to support mineralization *in vitro* [Tenenbaum, 1981]. Although a high concentration is thought to play a role in non physiological responses in vitro [Khouja et al., 1990], the addition of 10mM β-glycerophosphate to the osteogenic supplements has remained routinely used by a wide range of groups performed differentiation assays with MSC cultures and other cellular models [McCulloch & Tenenbaum, 1986; Blumberg et al., 1997; Jaiswal et al., 1997; Bruder et al., 1998; Peter et al., 1998; Pittenger et al., 1999; Jaiswal et al., 2000; Halleux et al., 2001; Lin et al 2001, Sottile et al., 2002, Maeda et al., 2004; Rodriguez et al., 2004; Boland et al., 2004; Zayzafoon et al., 2004; Kasten et el., 2008; Park et al., 2009; Song et al., 2009; Briggs et al., 2009]. Previous reports have suggested that the combination of high β-glycerophosphate levels, as used here, and high alkaline phosphatase activity is conducive to the appearance of mineral in culture [Hui et al., 1997]. It is interesting to note that as previously reported for embryonic stem cell cultures [Gentleman et al., 2009], both NTera and HeLa cultures observed in this study to have high mineral levels in response to OM also exhibited high alkaline phosphatase activity. It will therefore be of interest to next evaluate the OM response achieved for these cultures in parallel with MSCs when using more physiological conditions, in order to further explore possible differences between these cultures. It is perhaps not uncharacteristic that HeLa and NTera cells, both cancer cell lines, could display some degree of mineral deposition. Indeed, the occurrence of mineral deposition outside of the normal osteogenic differentiation process has been observed in other contexts such as cancer. Cancer models and cell lines such as prostate or breast cancer cells are known to

have osteomimetic properties, including upregulation of osteoblast-associated genes [Koeneman et al., 1999; Lin et al., 2001; Amatschek et al., 2004; Liu et al., 2008]. Adenomas and adenocarcinomas sometime contain mineralized derivatives and calcium phosphate deposits with hydroxyapatite characteristics [Ilse et al., 1980; Andrews, 2002; Morgan et al., 2005], which would be compatible with our *in vitro* observation that non-osteogenic cells such as HeLa cells are capable of mineral deposition. However, the pathways driving this mineralization event *in vivo* are still unclear. *In vitro* models such as these could be useful for the characterization of cancer-related mineral deposition pathways, which could lead to the development of new prognostic tests.

These results point to the need for advanced tests for mineralization beyond histochemical staining, which has already been highlighted for osteogenic cultures [Bonewald et al., 2003; Declercq et al., 2005]. Observations on OM-treated HeLa cells and NTera cells suggest that more advanced physical techniques such as ESEM and XRD are also unable to dependably distinguish the osteogenic origin of the mineral deposited *in vitro*, underlining the value of diagnostics involving biological criteria. In this context, infrared spectroscopy may represent an interesting tool to further characterise differences the mineral found in different cultures [Boskey & Mendelsohn, 2005]. The use of high resolution transmission electron microscopy could also enable the evaluation of structural features of osteoblast-related mineralization such as extracellular matrix association [Boskey & Roy, 2008]. Our study suggests that another avenue of investigation that would supplement the existing data set could focus on testing the specific osteogenic regulators, such as dexamethasone or BMP pathway regulators, in cell cultures.

In conclusion, we have shown that both HeLa and NTera cultures can deposit mineral *in vitro*. HEK293 cells can be used as a reliable non-mineralizing control, with no detectable alkaline phosphatase activity or osteocalcin expression even in the presence of a high level of β-glycerophosphate as used here. Our results underline that reliance on mineral deposition as the main phenotypical trait on which to evaluate osteogenic differentiation is precarious. Responsiveness to Dex and BMP treatment provides a useful criterion to discriminate between MSC-like osteogenic response to osteogenic conditions, and unrelated cellular mineralization. The origin and physiological relevance of the mineral deposition observed in the non-osteogenic cell lines analysed here remain to be determined, and may contribute to the elucidation of calcification events associated with some cancers.

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Figure Legend:

Fig.1: Analysis of mesenchymal marker expression in human cell cultures by flow cytometry shows different expression levels for CD29, CD105, CD106, p75, CD90, CD44 and SSEA4 highlighting divergent surface marker expression between MSCs and the other cell types. ++: high expression level, +: clear expression level, +/-: dim expression level, -: no detectable expression.

Fig.2: Time-course of mineral deposition in response to OM treatment (added at day0) analysed by Alizarin red S staining in wells (A) and viewed under microscope at day28 (B). (C) Corresponding staining quantification values for the time-course normalised to DNA content. (D) ELISA assay measuring osteocalcin expression in the cell lines analysed at day21. Bar = 100μm.

Fig.3: Alkaline phosphatase (ALP) activity measured in cell lines treated with control and OM for 14 days. The enzymatic reaction was carried out in cultures (A) and the staining intensity was quantified (B). (C) Expression of bone/liver/kidney alkaline phosphatase mRNA (bALP) detected by RT-PCR, using Clathrin as housekeeping gene control.

Fig.4: Analysis of mineral deposits present in the different cultures. (A) ESEM analysis of cultures treated in CTRL or OM conditions (B) EDX spectrum analysis for cultures treated in OM for 21 days. (C) X-ray diffraction patterns of the mineral deposited after 4 weeks.

Fig.5: Effect of dexamethasone and BMP inhibition on mineral deposition in cell lines analysed at day 21. (A-B) Alizarin red S staining performed in cultures maintained in OM in the presence (‘OM +Dex’) or absence (‘OM -Dex’) of dexamethasone at day 21 (A) and corresponding quantitation (B). (C-D) Effect of the absence (‘OM’) or presence (‘OM+Dorso’) of dorsomorphin treatment on the mineral deposition analysed after 14 days of OM exposure, measured by Alizarin red staining (C) and corresponding quantitation (D). Data representative of 3 independent experiments.

References:

Amatschek, S., U. Koenig, H. Auer, P. Steinlein, M. Pacher, A. Gruenfelder, G. Dekan, S. Vogl, E. Kubista, K.H. Heider, C. Stratowa, M. Schreiber, W. Sommergruber (2004) Tissue-wide expression profiling using cDNA subtraction and microarrays to identify tumor-specific genes. Cancer Res *64(3)*: 844-856.

Andrews, P.W. (2002) From teratocarcinomas to embryonic stem cells. Philos Trans R Soc Lond B Biol Sci *357(1420)*: 405-417.

Andrews, P.W., I. Damjanov, D. Simon, G.S. Banting, C. Carlin, N.C. Dracopoli, J. Fogh (1984) Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2. Differentiation in vivo and in vitro. Lab Invest *50(2)*: 147-162.

Aubin, J.E., F. Liu, L. Malaval, A.K. Gupta (1995) Osteoblast and chondroblast differentiation. Bone *17(2 Suppl)*: 77S-83S.

Beresford, J.N., S.E. Graves, C.A. Smoothy (1993) Formation of mineralized nodules by bone derived cells in vitro: a model of bone formation? Am J Med Genet *45(2)*: 163-178.

Blumberg, P., R. Brenne, S. Budny, H. Kresse (1997) Increased turnover of small proteoglycans synthesized by human osteoblasts during cultivation with ascorbate and beta-glycerophosphate. Calcif Tissue Int *60(6)*:554-560.

Boland, G.M., G. Perkins, D.J. Hall, R.S. Tuan (2004) Wnt 3a promotes proliferation and suppresses osteogenic differentiation of adult human mesenchymal stem cells. J Cell Biochem *93(6)*:1210-1230.

Bonewald, L.F., S.E. Harris, J. Rosser, M.R. Dallas, S.L. Dallas, N.P. Camacho, B. Boyan, A. Boskey (2003) von Kossa staining alone is not sufficient to confirm that mineralization in vitro represents bone formation. Calcif Tissue Int *72(5)*: 537-547.

Boskey, A.L., R. Mendelsohn (2005) Infrared spectroscopic characterization of mineralized tissues. Vib Spectrosc 38(1-2):107-114.

Boskey, A.L., R. Roy (2008) Cell culture systems for studies of bone and tooth mineralization. Chem Rev *108(11)*:4716-4733.

Boylan, M.O., M. Athanassiou, B. Houle, Y. Wang, H. Zarbl (1996) Activation of tumor suppressor genes in nontumorigenic revertants of the HeLa cervical carcinoma cell line. Cell Growth Differ *7(6)*: 725-735.

Briggs, T., M.D. Treiser, P.F. Holmes, J. Kohn, P.V. Moghe, T.L. Arinzeh (2009) Osteogenic differentiation of human mesenchymal stem cells on poly(ethylene glycol)-variant biomaterials. J Biomed Mater Res A *91(4)*:975-984.

Bruder, S.P., N.S. Ricalton, R.E. Boynton, T.J. Connolly, N. Jaiswal, J. Zaia, F.P. Barry (1998) Mesenchymal stem cell surface antigen SB-10 corresponds to activated leukocyte cell adhesion molecule and is involved in osteogenic differentiation. J Bone Miner Res *13(4)*:655-663.

Buttery, L.D., S. Bourne, J.D. Xynos, H. Wood, F.J. Hughes, S.P. Hughes, V. Episkopou, J.M. Polak (2001) Differentiation of osteoblasts and in vitro bone formation from murine embryonic stem cells. Tissue Eng *7(1)*: 89-99.

Cancedda, R., M. Mastrogiacomo, G. Bianchi, A. Derubeis, A. Muraglia, R. Quarto (2003) Bone marrow stromal cells and their use in regenerating bone. Novartis Found Symp *249*: 133-143.

Chadalavada, R.S., J. Houldsworth, A.B. Olshen, G.J. Bosl, L. Studer, R.S. Chaganti (2005) Transcriptional program of bone morphogenetic protein-2-induced epithelial and smooth muscle differentiation of pluripotent human embryonal carcinoma cells. Funct Integr Genomics *5(2)*: 59-69.

Cheng, S.L., J.W. Yang, L. Rifas, S.F. Zhang, L.V. Avioli (1994) Differentiation of human bone marrow osteogenic stromal cells in vitro: induction of the osteoblast phenotype by dexamethasone. Endocrinology *134(1)*: 277-286.

Clover, J., M. Gowen (1994) Are MG-63 and HOS TE85 human osteosarcoma cell lines representative models of the osteoblastic phenotype? Bone *15(6)*: 585-591.

Declercq, H.A., R.M. Verbeeck, L.I. De Ridder, E.H. Schacht, M.J. Cornelissen (2005) Calcification as an indicator of osteoinductive capacity of biomaterials in osteoblastic cell cultures. Biomaterials *26(24)*: 4964-4974.

Draper, J.S., C. Pigott, J.A. Thomson, P.W. Andrews (2002) Surface antigens of human embryonic stem cells: changes upon differentiation in culture. J Anat *200(Pt 3)*: 249-258.

Duran, C., P.J. Talley, J. Walsh, C. Pigott, I.E. Morton, P.W. Andrews (2001) Hybrids of pluripotent and nullipotent human embryonal carcinoma cells: partial retention of a pluripotent phenotype. Int J Cancer *93(3)*: 324-332.

Eijken, M., M. Koedam, M. van Driel, C.J. Buurman, H.A. Pols, J.P. van Leeuwen (2006) The essential role of glucocorticoids for proper human osteoblast differentiation and matrix mineralization. Mol Cell Endocrinol *248(1-2)*: 87-93.

Gang, E.J., D. Bosnakovski, C.A. Figueiredo, J.W. Visser, R.C. Perlingeiro (2007) SSEA-4 identifies mesenchymal stem cells from bone marrow. Blood *109(4)*:1743-1751.

Gentleman, E., R.J. Swain, N.D. Evans, S. Boonrungsiman, G. Jell, M.D. Ball, T.A. Shean, M.L. Oyen, A. Porter, M.M. Stevens (2009) Comparative materials differences revealed in engineered bone as a function of cell-specific differentiation. Nat Mater. *8(9)*:763-770.

Gey, G.O., W.D. Coffman, M.T. Kubicek (1952) Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. Cancer Res *12*: 264-265.

Graham, F.L., J. Smiley, W.C. Russell, R. Nairn (1977) Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J Gen Virol *36(1)*: 59-74.

Gregory, C.A., W.G. Gunn, A. Peister, D.J. Prockop (2004) An Alizarin red-based assay of mineralization by adherent cells in culture: comparison with cetylpyridinium chloride extraction. Anal Biochem *329(1)*: 77-84.

Halleux, C., V. Sottile, J.A. Gasser, K. Seuwen (2001) Multi-lineage potential of human mesenchymal stem cells following clonal expansion. J Musculoskelet Neuronal Interact *2(1)*: 71-76.

Hui, M., S.Q. Li, D. Holmyard, P. Cheng (1997) Stable transfection of nonosteogenic cell lines with tissue nonspecific alkaline phosphatase enhances mineral deposition both in the presence and absence of beta-glycerophosphate: possible role for alkaline phosphatase in pathological mineralization. Calcif Tissue Int *60(5)*:467-472.

Iba, K., H. Chiba, N. Sawada, S. Hirota, S. Ishii, M. Mori (1995) Glucocorticoids induce mineralization coupled with bone protein expression without influence on growth of a human osteoblastic cell line. Cell Struct Funct *20(5)*: 319-330.

Ilse, G., N. Ryan, K. Kovacs, D. Ilse (1980) Calcium deposition in human pituitary adenomas studied by histology, electron microscopy, electron diffraction and X-ray spectrometry. Exp Pathol *18(7-8)*: 377-388.

Izbicka, E., C.R. Dunstan, D. Horn, M. Harris, S. Harris, R. Adams, G.R. Mundy (1997) Effects of human tumor cell lines on local new bone formation in vivo. Calcif Tissue Int *60(2)*: 210-215.

Jackson, L., D.R. Jones, P. Scotting, V. Sottile (2007) Adult mesenchymal stem cells: differentiation potential and therapeutic applications. J Postgrad Med *53(2)*: 121-127.

Jaiswal, N., S.E. Haynesworth, A.I. Caplan, S.P. Bruder (1997) Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem *64(2)*: 295-312.

Jaiswal, R.K., N. Jaiswal, S.P. Bruder, G. Mbalaviele, D.R. Marshak, M.F. Pittenger (2000) Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase. J Biol Chem *275(13)*:9645-9652.

Jorgensen, N.R., Z. Henriksen, O.H. Sorensen, R. Civitelli (2004) Dexamethasone, BMP-2, and 1,25-dihydroxyvitamin D enhance a more differentiated osteoblast phenotype: validation of an in vitro model for human bone marrow-derived primary osteoblasts. Steroids *69(4)*: 219-226.

Kasten, P., J. Vogel, I. Beyen, S. Weiss, P. Niemeyer, A. Leo, R. Lüginbuhl (2008) Effect of platelet-rich plasma on the in vitro proliferation and osteogenic differentiation of human mesenchymal stem cells on distinct calcium phosphate scaffolds: the specific surface area makes a difference. J Biomater Appl *23(2)*:169-188.

Khosla, S., J.J. Westendorf, M.J. Oursler (2008) Building bone to reverse osteoporosis and repair fractures. J Clin Invest *118(2)*: 421-428.

Khouja, H.I., A. Bevington, G.J. Kemp, R.G. Russell (1990) Calcium and orthophosphate deposits in vitro do not imply osteoblast-mediated mineralization: mineralization by betaglycerophosphate in the absence of osteoblasts. Bone *11(6)*:385-391.

Kimelman, N., G. Pelled, G.A. Helm, J. Huard, E.M. Schwarz, D. Gazit (2007) Review: gene- and stem cell-based therapeutics for bone regeneration and repair. Tissue Eng *13(6)*: 1135-1150.

Kniss, D.A., Y. Xie, Y. Li, S. Kumar, E.A. Linton, P. Cohen, P. Fan-Havard, C.W. Redman, I.L. Sargent (2002) ED(27) trophoblast-like cells isolated from first-trimester chorionic villi are genetically identical to HeLa cells yet exhibit a distinct phenotype. Placenta *23(1)*: 32-43.

Kochanowska, I.E., K. Wlodarski, A. Wojtowicz, A. Kinsner, K. Ostrowski (2002a) BMP-4 and BMP-6 involvement in the osteogenic properties of the HeLa cell line. Exp Biol Med *227(1)*: 57-62.

Kochanowska, I.E., K. Wlodarski, A. Wojtowicz, K. Niemira, K. Ostrowski (2002b) Osteogenic properties of various HeLa cell lines and the BMP family genes expression. Ann Transplant *7(4)*: 58-62.

Koeneman, K.S., F. Yeung, L.W. Chung (1999) Osteomimetic properties of prostate cancer cells: a hypothesis supporting the predilection of prostate cancer metastasis and growth in the bone environment. Prostate *39(4)*: 246-261.

Lin, D.L., C.P. Tarnowski, J. Zhang, J. Dai, E. Rohn, A.H. Patel, M.D. Morris, E.T. Keller (2001) Bone metastatic LNCaP-derivative C4-2B prostate cancer cell line mineralizes in vitro. Prostate *47(3)*:212-221.

Liu, F., N. Bloch, K.R. Bhushan, A.M. De Grand, E. Tanaka, S. Solazzo, P.M. Mertyna, N. Goldberg, J.V. Frangioni, R.E. Lenkinski (2008) Humoral bone morphogenetic protein 2 is sufficient for inducing breast cancer microcalcification. Mol Imaging *7(4)*: 175-186.

Maeda, S., M. Hayashi, S. Komiya, T. Imamura, K. Miyazono (2004) Endogenous TGF-beta signaling suppresses maturation of osteoblastic mesenchymal cells. EMBO J *23(3)*:552-563.

Mareschi, K., I. Ferrero, D. Rustichelli, S. Aschero, L. Gammaitoni, M. Aglietta, E. Madon, F. Fagioli (2006) Expansion of mesenchymal stem cells isolated from pediatric and adult donor bone marrow. J Cell Biochem *97(4)*:744-754.

Marie, P.J., O. Fromigue (2006) Osteogenic differentiation of human marrow-derived mesenchymal stem cells. Regen Med *1(4)*: 539-548.

Markovic M, F.B., Tung MS (2004) Preparation and comprehensive characterization of a calcium hydroxyapatite reference material. J Res Natl Inst Stand Technol *109*: 553-568.

McCulloch, C.A., H.C. Tenenbaum (1986) Dexamethasone induces proliferation and terminal differentiation of osteogenic cells in tissue culture. Anat Rec *215(4)*:397-402.

Morgan, M.P., M.M. Cooke, G.M. McCarthy (2005) Microcalcifications associated with breast cancer: an epiphenomenon or biologically significant feature of selected tumors? J Mammary Gland Biol Neoplasia *10(2)*: 181-187.

Okamoto, T., T. Aoyama, T. Nakayama, T. Nakamata, T. Hosaka, K. Nishijo, T. Nakamura, T. Kiyono, J. Toguchida (2002) Clonal heterogeneity in differentiation potential of immortalized human mesenchymal stem cells. Biochem Biophys Res Commun *295(2)*: 354-361.

Ostrowski, K., K. Wlodarski, D. Aden (1975) Heterotopic chondrogenesis and osteogenesis induced by transformed cells: use of nude mice as a model system. Somatic Cell Genet *1(4)*: 391-395.

Pal, R., G. Ravindran (2006) Assessment of pluripotency and multilineage differentiation potential of NTERA-2 cells as a model for studying human embryonic stem cells. Cell Prolif *39(6)*: 585-598.

Palmieri, A., F. Pezzetti, G. Brunelli, Z. Ilaria, F. Carinci (2009) A comparison between genetic portraits of normal osteoblasts and osteosarcoma cell lines. Indian J Dent Res *20(1)*: 52-59.

Park, K.W., H. Waki, W.K. Kim, B.S. Davies, S.G. Young, F. Parhami, P. Tontonoz (2009) The small molecule phenamil induces osteoblast differentiation and mineralization. Mol Cell Biol *29(14)*:3905-3914.

Pautke, C., M. Schieker, T. Tischer, A. Kolk, P. Neth, W. Mutschler, S. Milz (2004) Characterization of osteosarcoma cell lines MG-63, Saos-2 and U-2 OS in comparison to human osteoblasts. Anticancer Res *24(6)*: 3743-3748.

Pera, M.F., S. Cooper, J. Mills, J.M. Parrington (1989) Isolation and characterization of a multipotent clone of human embryonal carcinoma cells. Differentiation *42(1)*: 10-23.

Peter, S.J., C.R. Liang, D.J. Kim, M.S. Widmer, A.G. Mikos (1998) Osteoblastic phenotype of rat marrow stromal cells cultured in the presence of dexamethasone, beta-glycerolphosphate, and L-ascorbic acid. J Cell Biochem *71(1)*:55-62.

Pittenger, M.F., A.M. Mackay, S.C. Beck, R.K. Jaiswal, R. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, D.R. Marshak (1999) Multilineage potential of adult human mesenchymal stem cells. Science *284(5411)*: 143-147.

Poliard, A., D. Lamblin, P.J. Marie, M.H. Buc-Caron, O. Kellermann (1993) Commitment of the teratocarcinoma-derived mesodermal clone C1 towards terminal osteogenic differentiation. J Cell Sci *106 (Pt 2)*: 503-511.

Rago, R., J. Mitchen, G. Wilding (1990) DNA fluorometric assay in 96-well tissue culture plates using Hoechst 33258 after cell lysis by freezing in distilled water. Anal Biochem *191(1)*: 31-34.

Rodríguez, J.P., M. González, S. Ríos, V. Cambiazo (2004) Cytoskeletal organization of human mesenchymal stem cells (MSC) changes during their osteogenic differentiation. J Cell Biochem *93(4)*:721-731.

Russell, R.G., A.M. Caswell, P.R. Hearn, R.M. Sharrard (1986) Calcium in mineralized tissues and pathological calcification. Br Med Bull *42(4)*: 435-446.

Seib, F.P., M. Franke, D. Jing, C. Werner, M. Bornhauser (2009) Endogenous bone morphogenetic proteins in human bone marrow-derived multipotent mesenchymal stromal cells. Eur J Cell Biol *88(5)*: 257-271.

Shukunami, C., K. Ishizeki, T. Atsumi, Y. Ohta, F. Suzuki, Y. Hiraki (1997) Cellular hypertrophy and calcification of embryonal carcinoma-derived chondrogenic cell line ATDC5 in vitro. J Bone Miner Res *12(8)*: 1174-1188.

Simoes, P.D., T. Ramos (2007) Human pluripotent embryonal carcinoma NTERA2 cl.D1 cells maintain their typical morphology in an angiomyogenic medium. J Negat Results Biomed *6*: 5.

Song, I.H., A.I. Caplan, J.E. Dennis (2009) In vitro dexamethasone pretreatment enhances bone formation of human mesenchymal stem cells in vivo. J Orthop Res *27(7)*:916-921.

Sottile, V., C. Halleux, F. Bassilana, H. Keller, K. Seuwen (2002) Stem cell characteristics of human trabecular bone-derived cells. Bone *30(5)*: 699-704.

Sottile, V., A. Thomson, J. McWhir (2003) In vitro osteogenic differentiation of human ES cells. Cloning Stem Cells *5(2)*: 149-155.

Spiropoulos, A., M. Theodosaki, K. Stefanaki, G. Paterakis, M. Tzetis, K. Giannikou, E. Petrakou, M.N. Dimopoulou, I. Papassotiriou, E.S. Roma, E. Kanavakis, S. Graphakos, E. Goussetis (2010) Rapid clinical-scale propagation of mesenchymal stem cells using cultures initiated with immunoselected bone marrow CD105 cells. J Cell Mol Med. DOI: 10.1111/j.1582-4934.2010.01157.x

Tang, N., W.X. Song, J. Luo, R.C. Haydon, T.C. He (2008) Osteosarcoma development and stem cell differentiation. Clin Orthop Relat Res *466(9)*: 2114-2130.

Tataria, M., N. Quarto, M.T. Longaker, K.G. Sylvester (2006) Absence of the p53 tumor suppressor gene promotes osteogenesis in mesenchymal stem cells. J Pediatr Surg *41(4)*: 624-632.

Tenenbaum, H.C. (1981) Role of organic phosphate in mineralization of bone in vitro. J Dent Res *60*:1586-1589.

Thomas, P., T.G. Smart (2005) HEK293 cell line: a vehicle for the expression of recombinant proteins.J Pharmacol Toxicol Methods *51(3)*: 187-200.

Tremoleda, J.L., N.R. Forsyth, N.S. Khan, D. Wojtacha, I. Christodoulou, B.J. Tye, S.N. Racey, S. Collishaw, V. Sottile, A.J. Thomson, A.H. Simpson, B.S. Noble, J. McWhir (2008) Bone tissue formation from human embryonic stem cells in vivo. Cloning Stem Cells *10(1)*: 119-132.

Yu, P.B., C.C. Hong, C. Sachidanandan, J.L. Babitt, D.Y. Deng, S.A. Hoyng, H.Y. Lin, K.D. Bloch, R.T. Peterson (2008) Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. Nat Chem Biol *4(1)*: 33-41.

Zayzafoon, M., W.E. Gathings, J.M. McDonald (2004) Modeled microgravity inhibits osteogenic differentiation of human mesenchymal stem cells and increases adipogenesis. Endocrinology *145(5)*:2421-2432.

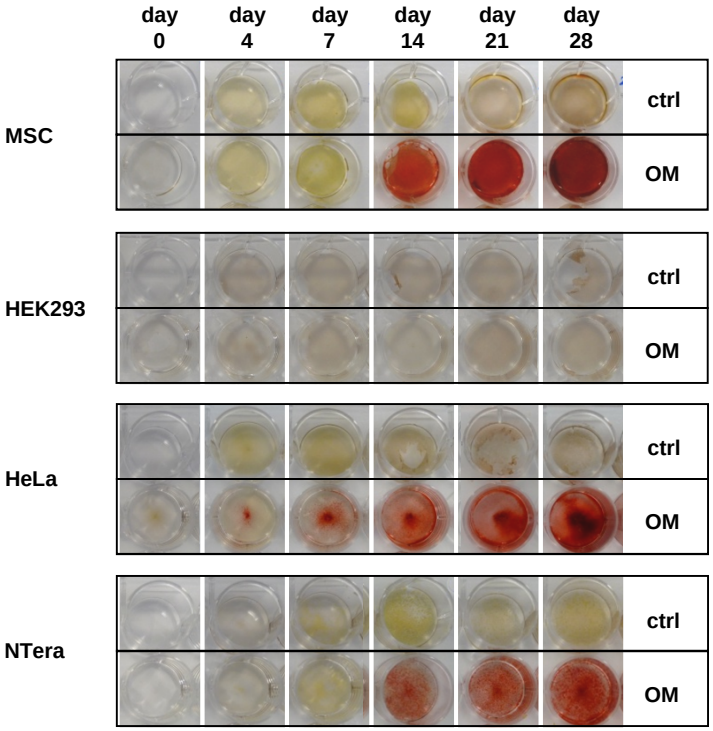
zur Nieden, N.I., G. Kempka, H.J. Ahr (2003) In vitro differentiation of embryonic stem cells into mineralized osteoblasts. Differentiation *71(1)*: 18-27.

Fig. 1

	CD29	CD105	CD106	p75	CD90	CD44	SSEA4
MSC	++	++	+	+	++	++	++
Ntera	++	-	-	-	++	+	++
HeLa	++	+	-	+	-	++	+
HEK293	+/-	-	-	+	-	+	+

Fig.2

A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MSC
HEK293
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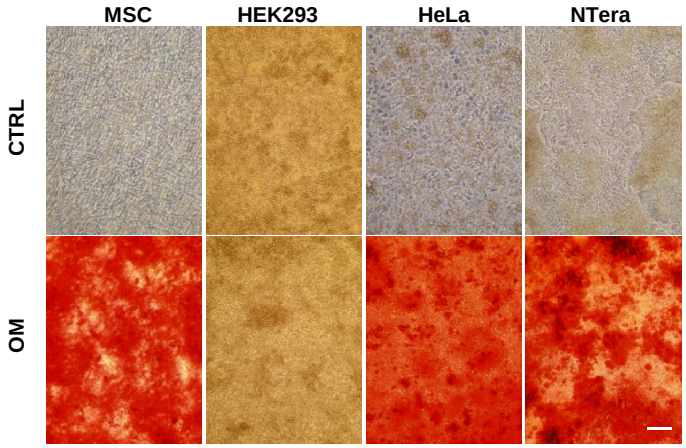
B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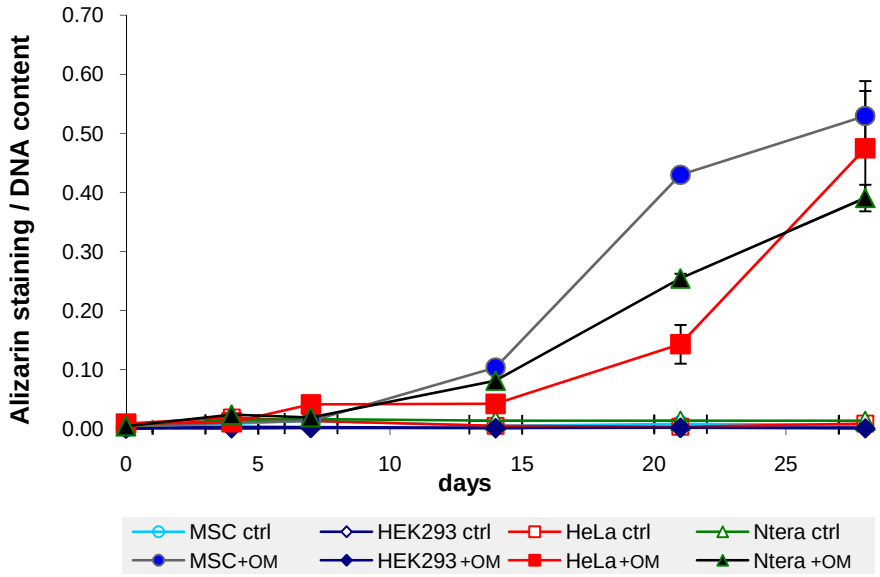
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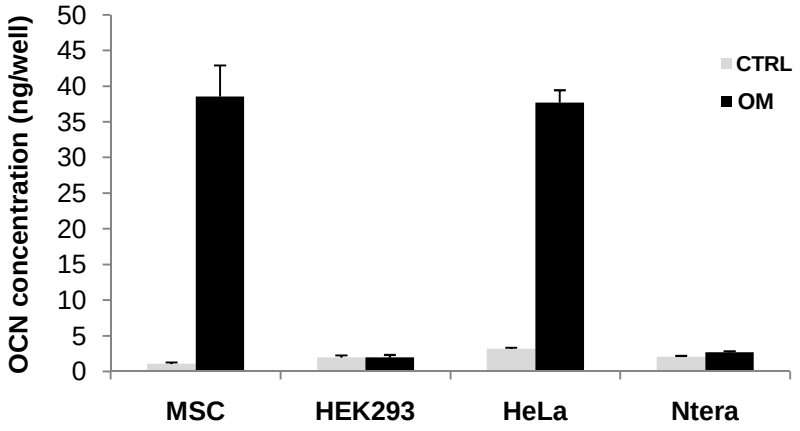
Ntera +OM

D


OCN concentration (ng/well)
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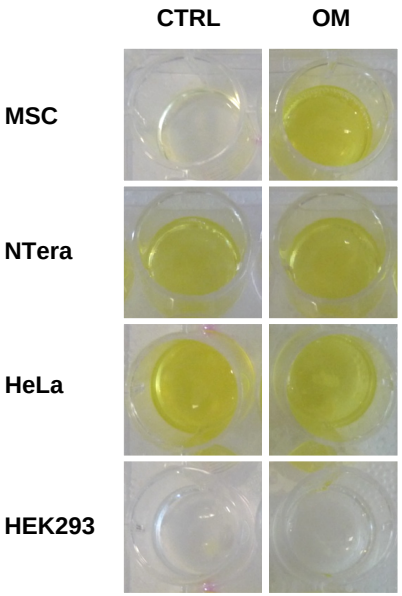
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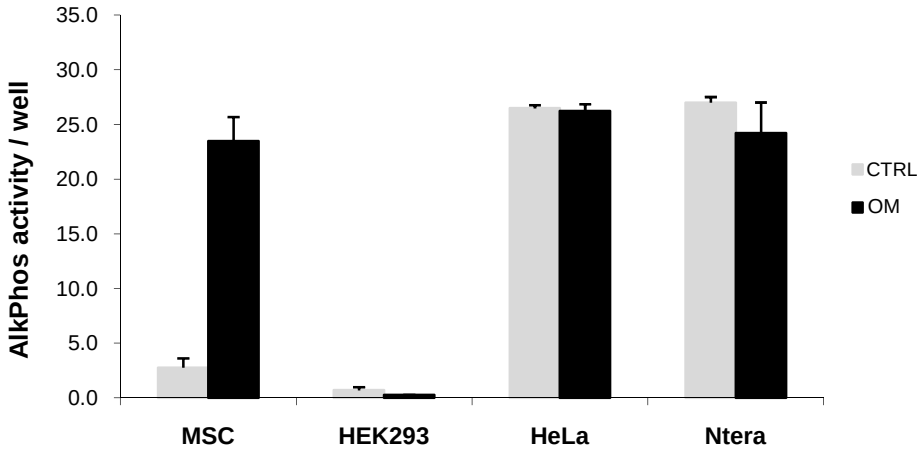
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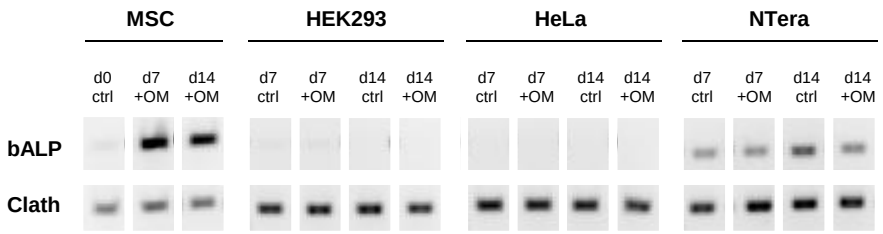
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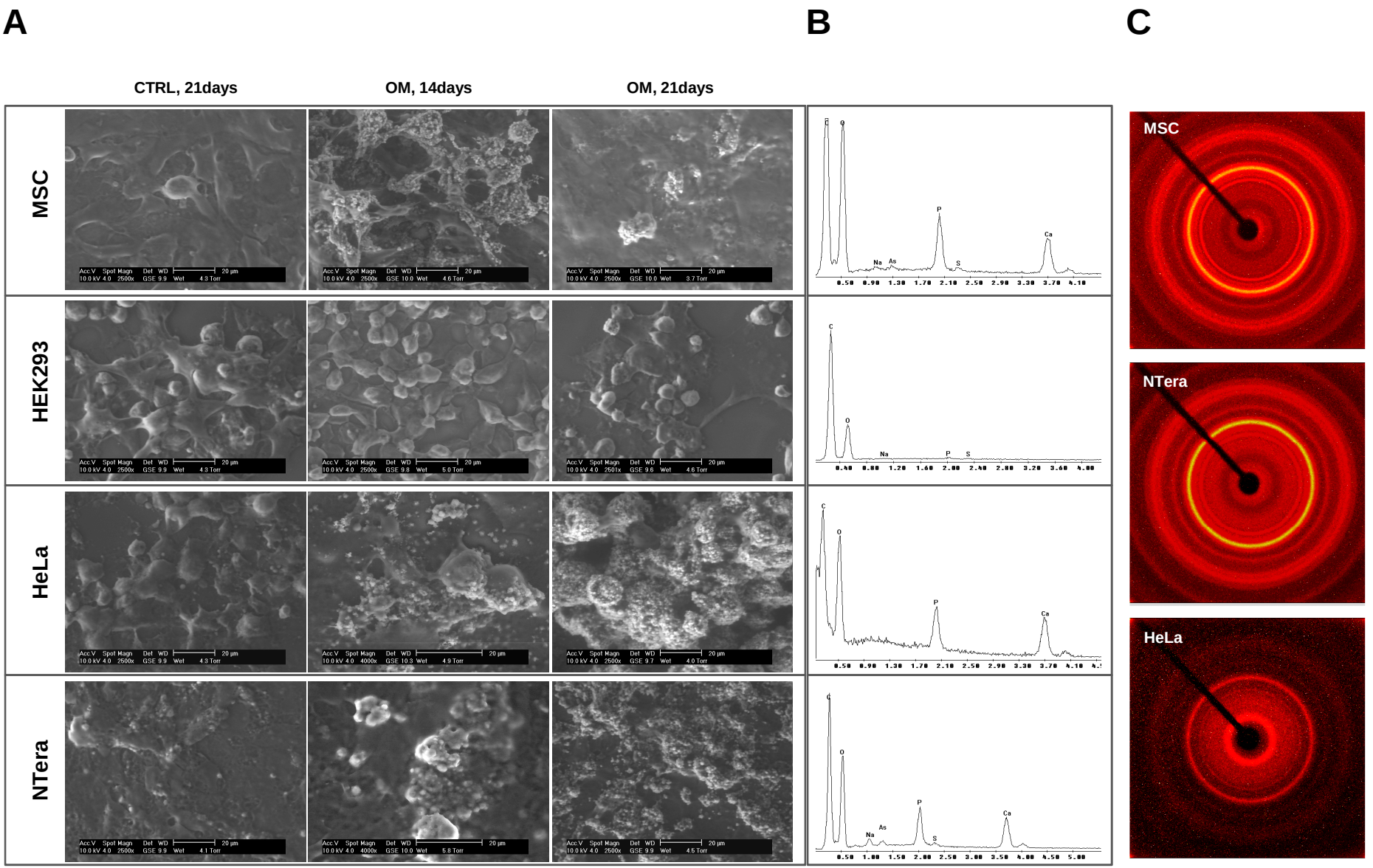
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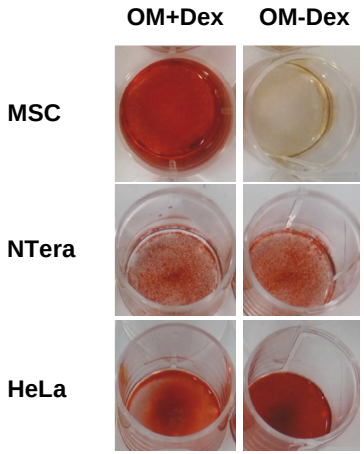
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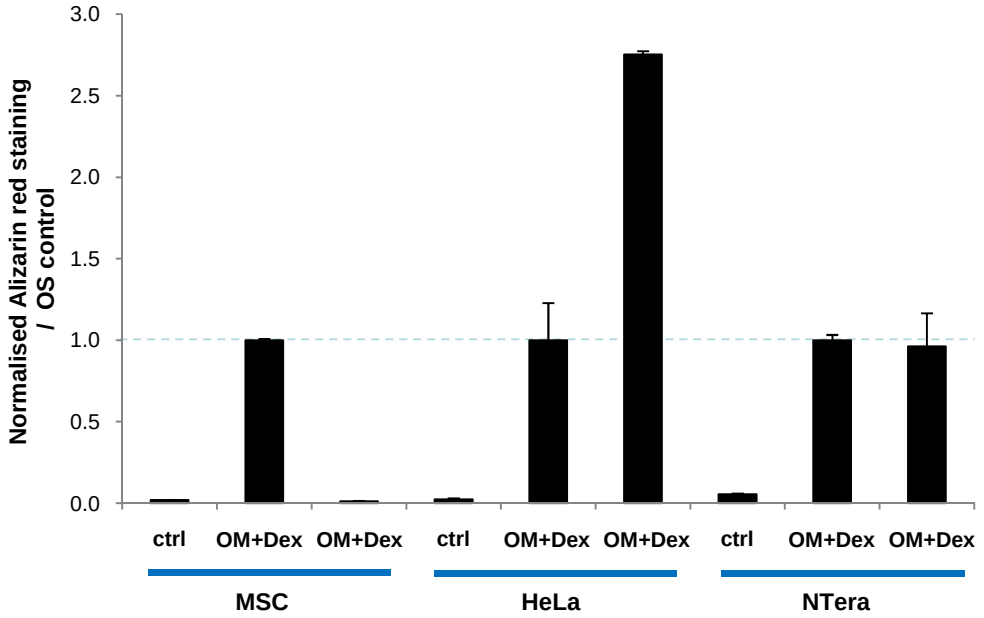
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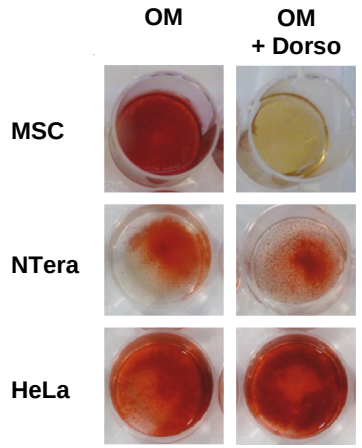
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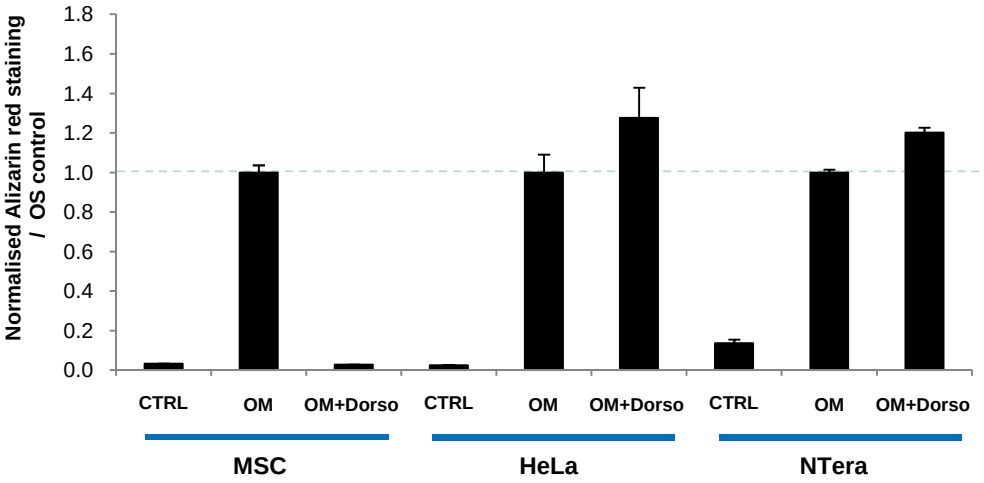
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