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ORIGINAL RESEARCH

Extracellular Iron is a Modulator of the Differentiation of Osteoclast Lineage Cells

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Abstract Osteoclasts originate from the hematopoietic stem cell and share a differentiation pathway with the cells of the monocyte/macrophage lineages. Development and activation of osteoclasts, and as a consequence regulation of bone resorption, depend on two growth factors: macrophage colony-stimulating factor and receptor activator of NF-κB ligand. Furthermore, cell development and activity are modulated by a microenvironment composed of cytokines and growth factors and of the extracellular matrix. Membrane transporters are a means for cells to interact with their environment. Within this study, the expression of proteins regulating cellular iron homeostasis in osteoclast-like cells grown from bone marrow-derived progenitors was compared to the expression of this set of proteins by monocyte/macrophage lineage cells. In differentiating osteoclasts, levels of transcripts encoding *transferrin receptor 1* and *divalent metal transporter 1* (Slc11A2) were increased, while levels of transcripts encoding *ferroportin* (Slc40A1) and *natural resistance-associated macrophage protein 1* (Slc11A1) were decreased. Supplementation of the culture media with exogenous iron led to an increase in the proliferation of osteoclast progenitor cells and to the expression of a macrophage-like phenotype, while the development of osteoclasts was reduced. Upon transfer of mature OC onto a CaP substrate, iron depletion of the medium with the Fe^{3+}-chelator Deferoxamine Mesylate decreased CaP dissolution by ~30 %, which could be restored by addition of exogenous iron. During the 24 h of the assay, no effects were observed on total TRAP activity. The data demonstrate transcriptional regulation of the components of cellular iron transporters during OC development and suggests that iron homeostasis may contribute to fine-tuning of the RANKL-induced OC development.

Keywords Osteoclast · RANKL · DMT1 · Ferroportin · TfR1

Introduction

During repair, adaptation to mechanical needs, and homeostasis, bone continuously undergoes remodeling [6, 27]. An imbalance between osteoblastic formation and osteoclastic resorption leads to impairment of skeletal functions. An increase of resorption over formation underlies osteopenia and osteoporosis [30, 33], a failure in the development of osteoclasts or their dysfunction leads to osteopetrosis [8]. Osteoclasts, which are multinucleated giant cells, derive by fusion from mononuclear monocyte/macrophage lineage cells. Two growth factors, macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL), are required for the development of functional osteoclasts [7, 21]. The absence of either of these factors leads to an osteopetrotic phenotype caused by the failure of osteoclasts to develop. Besides these two essential growth factors, many

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components shape a specific microenvironment modulating the development and activation of osteoclast lineage cells [2, 3, 20]. It is, however, not only the presence of compounds within the microenvironment, but also the capacity of the cells to respond to them, that define cell fate at a given location and time.

Bone resorption by osteoclasts is a multistep process, beginning with attachment of the osteoclasts to the extracellular matrix of bone and formation of a sealing zone, continuing with the solubilization of the mineral matrix by extrusion of acid and ending with the digestion of the organic components of the ECM with lysosomal enzymes [4]. Among the diverse cellular interactions with the microenvironment, molecular transport significantly contributes to the osteoclasts' potential to respond to clues from the surroundings. Furthermore, the resorptive activity of osteoclast lineage cells is particularly dependent on functional transport mechanisms. During the resorption process, which requires acidification of the resorption lacuna, chloride transporter (CLC7) [22, 32], Cl^-/HCO_3^- antiporter [14], and H^+-transport through a specific H^+-ATPase [36] are essential. Inactivating mutations in each of the respective genes causes the development of inactive, resorption-incapable osteoclasts and of an osteopetrotic phenotype.

In the present study, the expression of molecules governing iron homeostasis was investigated during differentiation of osteoclast lineage cells. This comparison of monocytes/macrophages and osteoclasts revealed that iron transport molecules are differentially expressed during the development of these cell lineages, raising our interest in elucidating the role of iron in osteoclast development and function.

Materials and Methods

Differentiation of Osteoclasts In Vitro

Bone marrow cells were isolated from femora and tibiae of 6- to 8-week-old *C57Bl/6J* mice as described previously [3]. Epiphyses were removed, the bone marrow cavity was opened, and the cells were flushed out with αMEM (Invitrogen/Life Technologies, Dübendorf, CH)/10 % FBS (Sigma/Aldrich, Buchs, CH). Approximately 80×10^6 BMC were seeded in αMEM/10 % FBS, supplemented with M-CSF (30 ng/ml; Chiron, Emeryville, CA) into 75-cm^2 culture flasks and incubated at 37 °C in a humidified atmosphere with 5 % CO_2. After 24 h, the non-adherent, M-CSF-dependent osteoclast precursor cells (OPC) were counted and seeded into new culture dishes at a density of 3×10^5 cells/ml with 125 µl/cm^2 in αMEM/FBS/M-CSF supplemented with RANKL (recombinant human RANKL, 20 ng/ml, PeproTech, LuBioScience, Lucerne, CH). After 3 days, the medium was changed and osteoclasts developed within 5 days.

Differentiation of Osteoclasts and Macrophages

To investigate the changes in the expression of transcripts during the development of monocytes/macrophages and osteoclasts, OPC were grown in the absence and presence of RANKL, respectively. After 3 and 5 days in culture, total RNA was isolated from 3 individual cultures each, using RNeasy Mini Kits (Qiagen, Hombrechtikon, CH). To assess the effects of iron on cell development, the culture media were rendered iron-free by adding the Fe^{3+}-chelator Deferoxamine Mesylate (DFO; Calbiochem/Merck Millipore, Darmstadt, GER) at 10 µM, and the media were supplemented with different concentrations of iron sulfate (Fe^{2+}), iron chloride (Fe^{3+}), and Ferric Ammonium Citrate (Fe^{3+}; all from Sigma/Aldrich, Buchs, CH), respectively, ranging from 0 µM up to 40 µM.

Real-Time PCR

To confirm the development of osteoclasts and macrophages in dependence of the culture conditions, quantitative PCR for osteoclast and monocyte/macrophage products was performed using the following Assays-on-Demand (AoD; Life Technologies/Applied Biosystems, LuBioScience, Lucerne, CH): solute carrier family 9, subfamily B (cation/proton antiporter), member 2 (NHA2/Slc9b2; Mm01313329_m1), calcitonin receptor (Calcr; Mm00432271_m1), solute carrier family 4 (anion exchanger), member 2 (Slc4A2; Mm00436617_m1), chloride channel 7 (Clcn7; Mm00442400_m1), ATPase, H^+ transporting, lysosomal V1 subunit B1 (Atp6v1b; Mm00460309_m1), EGF-like module containing, mucin-like, hormone receptor-like sequence 1 (Emr1/F4/80; Mm00802529_m1), and colony-stimulating factor-1 receptor (Csf1r; Mm01266652_m1). To assess levels of transcripts encoding proteins involved in iron transport and storage, the AoD Mm00441941_m1 (transferrin receptor, TfR1), Mm00489837_m1 (Slc40a1, Ferroportin), Mm00435363_m1 (Slc11a2, DMT1, Nramp2), and Mm00443045_m1 (Slc11a1, Nramp1) were used. PCR was performed in an ABI7500 System (Life Technologies/Applied Biosystems, Dübendorf, CH) with a denaturing period of 20 s at 95 °C preceding 45 amplification cycles of 3 s at 95 °C and 30 s at 60 °C each.

Determination of Cell Proliferation

To assess the number of viable cells, the Cell Proliferation Kit II (Roche Diagnostics, Basle, CH) was used, following

the instructions of the manufacturer. Absorption measurements were carried out at 470 nm (reference wavelength 690 nm), using an Infinite 200Pro Spectrophotometer (Tecan Group Ltd., Männedorf, CH).

Quantification of Osteoclast Development

To visualize osteoclast development in culture, cells were stained for the osteoclast marker enzyme Tartrate Resistant Acid Phosphatase (TRAP). For this purpose, the cells were washed with phosphate-buffered saline (PBS; 137 mM NaCl/2.7 mM KCl/12 mM P_i, pH 7.4) and fixed for 10 min in 4 % paraformaldehyde at room temperature. The cells subsequently were stained using a TRAP staining kit (Sigma-Aldrich, Buchs, CH) as recommended by the manufacturer.

Alternatively, TRAP activity was determined in cell lysates. The cells were lysed in 0.1 % Triton X-100/1M NaCl and frozen at −20 °C. A 50 µl aliquot of the lysate was transferred into 96-well microtiter plates and mixed with an equal volume of p-nitrophenyl phosphate (4.61 mg/ml) in 40 mM Na-tartrate/50 mM Na-acetate, pH 4.8. After 60 min, the reaction was stopped with 50 µl 0.2 N NaOH and the absorbance was measured at 405 nm (reference wavelength 690 nm) using an Infinite 200Pro Spectrophotometer (Tecan Group Ltd., Männedorf, CH).

Osteoclast Activity Assay

To determine the capacity of osteoclasts to dissolve mineral, mature osteoclasts were seeded onto a layer of calcium phosphate (CaP). Similarly as described before [5], solutions of 0.12 M Na_2HPO_4 and 0.2 M $CaCl_2$ in 50 mM Tris/HCl pH 7.4 were preincubated overnight in a 5 % CO_2 incubator at 37 °C. Equal volumes were mixed and a CaP slurry precipitated. After washing twice with water, the slurry was resuspended in 1 ml of water/90 µl slurry. To the CaP suspension, $^{45}CaCl_2$ (PerkinElmer, Schwerzenbach, CH) was added to a specific activity of 1700 Bq/20 µg slurry. Of the spiked suspension, 200 µl was distributed into wells of 48-well plates, dried at room temperature for 3 days, and baked at 80 °C for 3 h. Before use, the CaP-coated plates were equilibrated with 30 % FBS in cell culture medium over night.

Osteoclasts were generated in cultures of OPC for 5 days with M-CSF (30 ng/ml and RANKL (20 ng/ml) in 5-cm-diameter UpCell dishes (Thermo Fisher Scientific Inc., Waltham MA, USA) as described above. Mature osteoclasts were detached from the dishes by incubating the plates at 4 °C. The cells from one UpCell dish were resuspended in 500 µl αMEM/FBS/M-CSF/RANKL and 50 µl was distributed into the CaP-coated 48-well plates.

After 24 h, the cell supernatants were collected and the solubilized ^{45}Ca was counted in a scintillation counter.

Results

Characterization of Differentiated Cells

For the analysis of the expression of transcripts encoding molecular transporters, M-CSF-dependent, non-adherent osteoclast progenitor cells were grown for 3 and 6 days with M-CSF/ ±RANKL. Cells grown in the presence of RANKL developed into osteoclasts, as shown by an increase in levels of transcripts encoding NHA2 (Fig. 1a), calcitonin receptor (Fig. 1b), the CL^-/HCO_3^- exchanger SLC4A2 (Fig. 1c), the chloride channel CLC7 (Fig. 1d), the osteoclast-specific V-type H^+-ATPase (Fig. 1e), and by the appearance of multinucleated, $TRAP^+$ cells. In the absence of RANKL, cells developed along the monocyte/macrophage lineage, as visualized by F4/80 (Fig. 1f) and c-fms (Fig. 1g) transcript levels.

Expression of Transcripts Encoding Cellular Iron Transport Molecules in Monocyte/Macrophage Lineage Cells

Transcripts encoding transferrin receptor 1 (TfR1) and ferroportin (Slc40A1) were highly regulated during the development of osteoclasts from monocyte/macrophage progenitor cells. Levels of transcripts encoding TfR1, which is mediating cellular uptake of iron, are increased in developing and mature osteoclasts (Fig. 2a), while ferroportin mRNA levels remained low in comparison to those in macrophages (Fig. 2b). As is the case for TfR1 and Slc40A1, transcripts encoding the divalent metal transporters Slc11A1 (NRAMP1) and Slc11A2 (DMT1) are reversely regulated in monocyte/macrophage cells and osteoclasts as well. Levels of transcripts encoding Slc11A1 are higher in monocyte/macrophage cells if compared to osteoclasts (Fig. 2c), while the reverse is the case for transcripts encoding Slc11A2 (Fig. 2d).

Effects of Extracellular Iron on the Differentiation of Monocyte/Macrophage Lineage Cells

In a next step, OPC were grown with M-CSF/RANKL and different concentrations of exogenous iron [$FeSO_4$ (iron sulfate), $FeCl_3$ (iron chloride), and FAC (ferric ammonium citrate)], ranging up to 40 µM, were added. After 3 and 5 days in culture, the number of viable cells was determined using an XTT-Assay and osteoclast development was assessed by TRAP activity in the cell lysate. Iron was

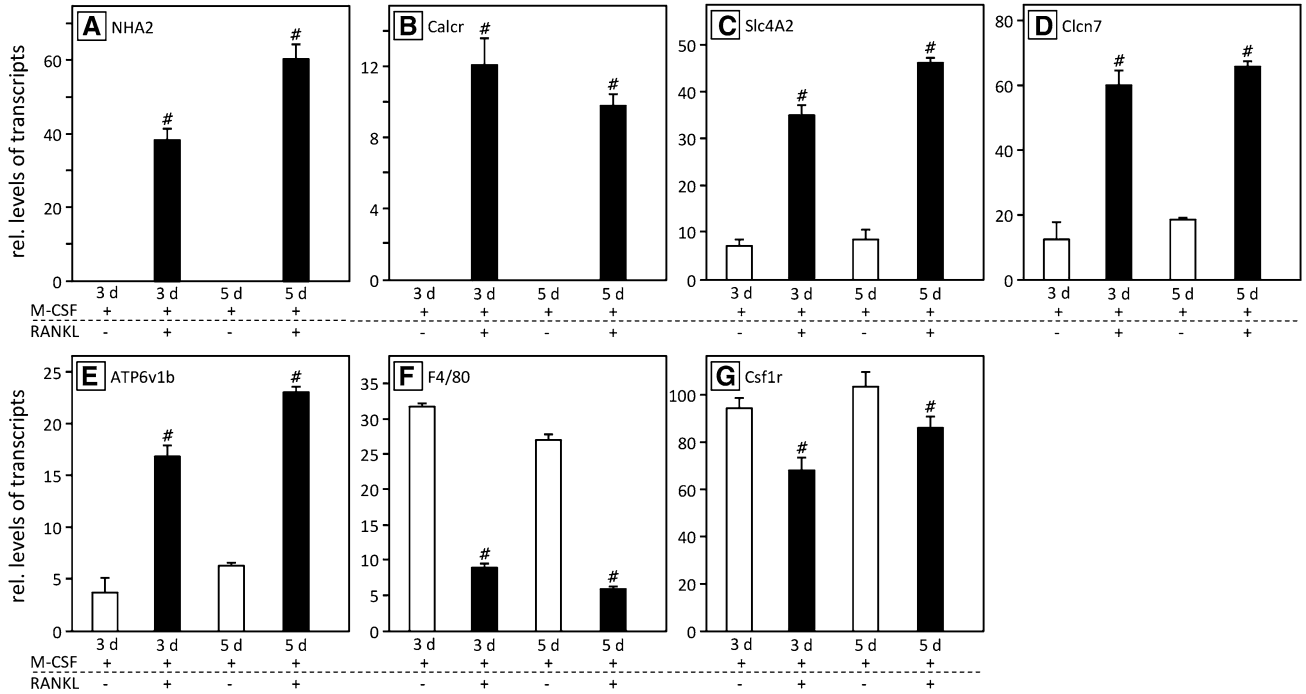

Fig. 1 Differentiation of monocyte lineage cells in vitro. To assess the development of osteoclasts in culture, the expression of mRNAs encoding markers for osteoclast lineage cells (NHA2 (**a**), Calcr (**b**)), of proteins required for resorption (Slc4A2 (**c**), Clcn7 (**d**), ATP6v1b (**e**)), of the pan-macrophage marker F4/80 (**f**), and of the M-CSF receptor (Csf1r (**g**)) was quantified by real-time PCR. Transcripts encoding osteoclast markers and functional proteins were detected mainly in cell cultures grown in medium supplemented with M-CSF and RANKL (*black bars*), F4/80 transcripts were expressed at high levels in cultures grown with M-CSF only (*white bars*), while Csf1r transcripts were found in all cell cultures. ($\#p < 0.05$). Values are given as mean ± SD, $n = 3$

found to lead to an increase in the number of viable cells dose dependently, the increase at 40 µM amounting to ~50 %, independently of the iron salt used in the study (Fig. 3a). Parallel to the increase in the number of viable cells, a decrease in TRAP activity in the cell lysate was observed, the decrease reaching approx. 70 % in cultures supplemented with 40 µM iron (Fig. 3b).

Modulation of Osteoclast Activity by Iron

Mineral dissolution and digestion of the organic matrix of bone are energy consuming steps. To assess the dependence of the osteoclasts' mineral-dissolving activity on the availability of iron, mature osteoclasts were seeded onto a layer of amorphous CaP which was spiked with ^{45}Ca. The cultures were rendered free of iron by supplementing the medium with DFO (10 µM), and the effects of exogenous iron were investigated by adding FAC in varying concentrations up to 40 µM. Deprivation of mature osteoclasts of iron by adding DFO to the medium reduced the cells' ability to dissolve CaP over 24 h by approx. 30 % as compared to control osteoclasts in normal culture medium (Fig. 4a). Upon addition of exogenous iron at concentrations of 10 µM and higher, osteoclast activity was restored to normal levels. During the 24 h period of the assay, iron deprivation and addition of exogenous iron to concentrations of up to 40 µM did not affect the number of TRAP positive cells, as assessed by determination of TRAP activity in cell lysates (Fig. 4b).

Effects of Iron Deprivation on the Expression of Transcripts Encoding Proteins Regulating Iron Homeostasis

DFO at 10 µM chelates the Fe^{3+} contained in the culture medium (10 % FBS containing approx. 3µM Fe^{3+}). As a consequence, only exogenous iron concentrations of ≥10 µM lead to a net availability of iron. When present continuously, iron dose dependently decreases the expression of transcripts encoding osteoclast products, while supporting macrophage differentiation, as visualized by levels of mRNAs encoding NHA2 and F4/80, respectively (Fig. 5a, b). Transcripts encoding TfR1 are highly upregulated under conditions of iron deprivation (Fig. 5d), while levels of transcripts encoding the iron storage protein ferritin (Tfh1) are increased in conditions of irons excess (Fig. 5e). Levels of transcripts encoding the iron transporter Slc40a1 (Fig. 5c) are high at iron concentrations of

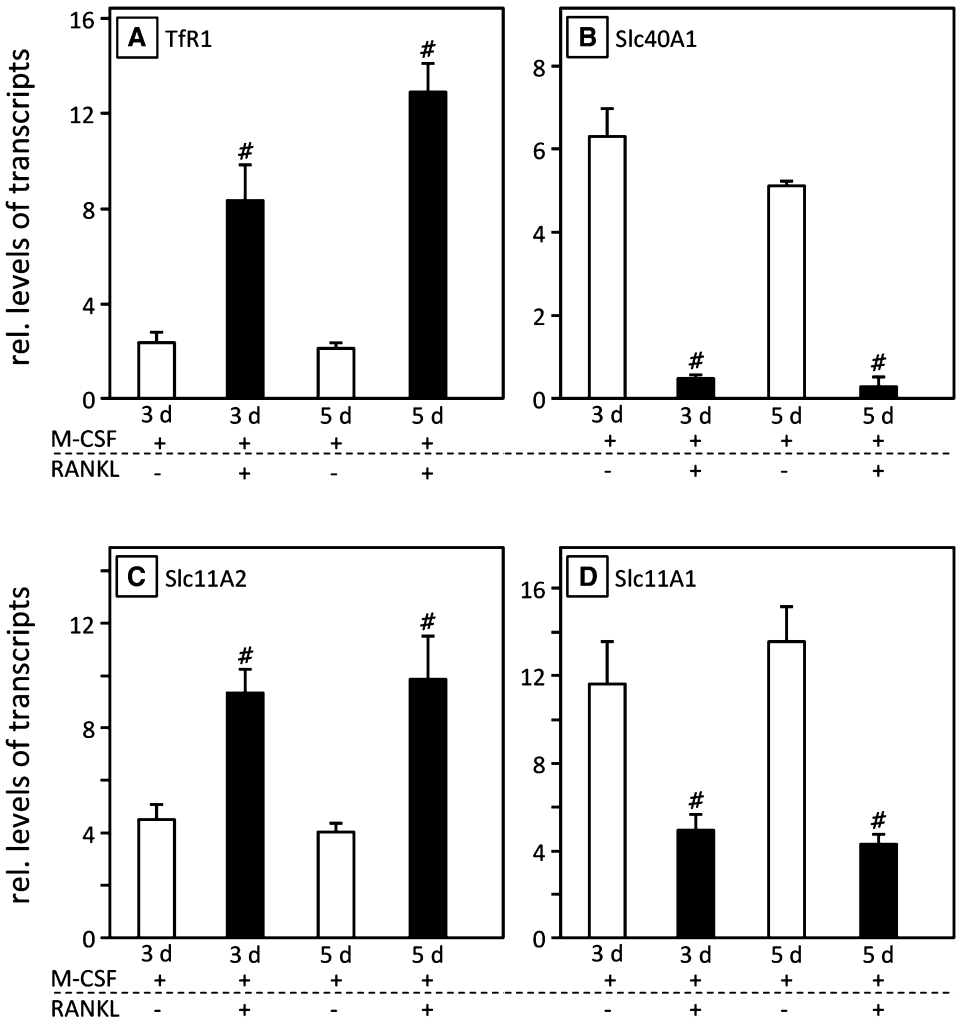

Fig. 2 Levels of transcripts encoding iron transport proteins in monocyte/macrophage lineage cells. Levels of transcripts encoding Transferrin Receptor (TfR1 (**a**)), Ferroportin (Slc40A1 (**b**)), Divalent Metal Transporter 1 (DMT1/Slc11A2 (**c**)), and Natural Resistance-associated Macrophage Protein One (NRAMP1/Slc40A1 (**d**)) were quantified by real-time PCR. Transcripts encoding TfR1 and Slc11A2 were increased in cultures of OPC grown with M-CSF and RANKL (*black bars*) after 3 days and after 5 days, while Slc40A1 and Slc11A1 transcript levels were increased in cultures grown with M-CSF alone (*white bars*) after 3 and 5 days. (#$p < 0.05$). Values are given as mean ± SD, $n = 3$

40 μM in osteoclasts and macrophages, as are transcripts encoding for Slc11A1 (Fig. 5f). In contrast, levels of transcripts encoding Slc11A2 are attenuated with increasing concentrations of exogenous iron (Fig. 5g).

Discussion

The expression of the molecular components of iron homeostasis in osteoclast lineage cells greatly depends on the cells' differentiation state. In particular, transcriptional regulation during osteoclastogenesis could be established for the iron transporters DMT1 (Slc11A2), NRAMP1 (Slc11A1), and Ferroportin (Slc40A1), and for the transferrin receptor (TfR1). Levels of transcripts encoding Slc11A1 and Slc40A1 were high in monocytes/macrophages as compared to differentiated osteoclasts, while levels of transcripts encoding Slc11A2 and TfR1 were highly expressed by mature osteoclasts. The data suggests that the biological functions of monocytes/macrophages require low iron uptake and high iron expulsion, while osteoclasts need to retain iron.

One of the major functions, besides host defense, of the cells of the monocyte/macrophage lineages is the recycling of iron from old and damaged red blood cells. Indeed, of the approx. 25 mg iron per day required by an adult human, close to 75 % are derived from hemoglobin in erythrocytes [41]. Monocyte/macrophage cells phagocytose the red blood cells and release iron through Slc40A1. In contrast, osteoclasts, to fulfill their main function of bone resorption, require high energy levels. Bone resorption is a two-step process: firstly, the hydroxyapatite is dissolved by generating acidic conditions within the resorption lacuna, and secondly, the organic matrix of bone is digested by osteoclast-derived lysosomal enzymes [4]. Both these processes, but in particular the generation of an acidic environment, which is driven by a proton-ATPase, consumes energy in the form of ATP [36]. ATP is generated in the respiratory chain of the mitochondria, and as a consequence, osteoclasts are particularly rich in these organelles.

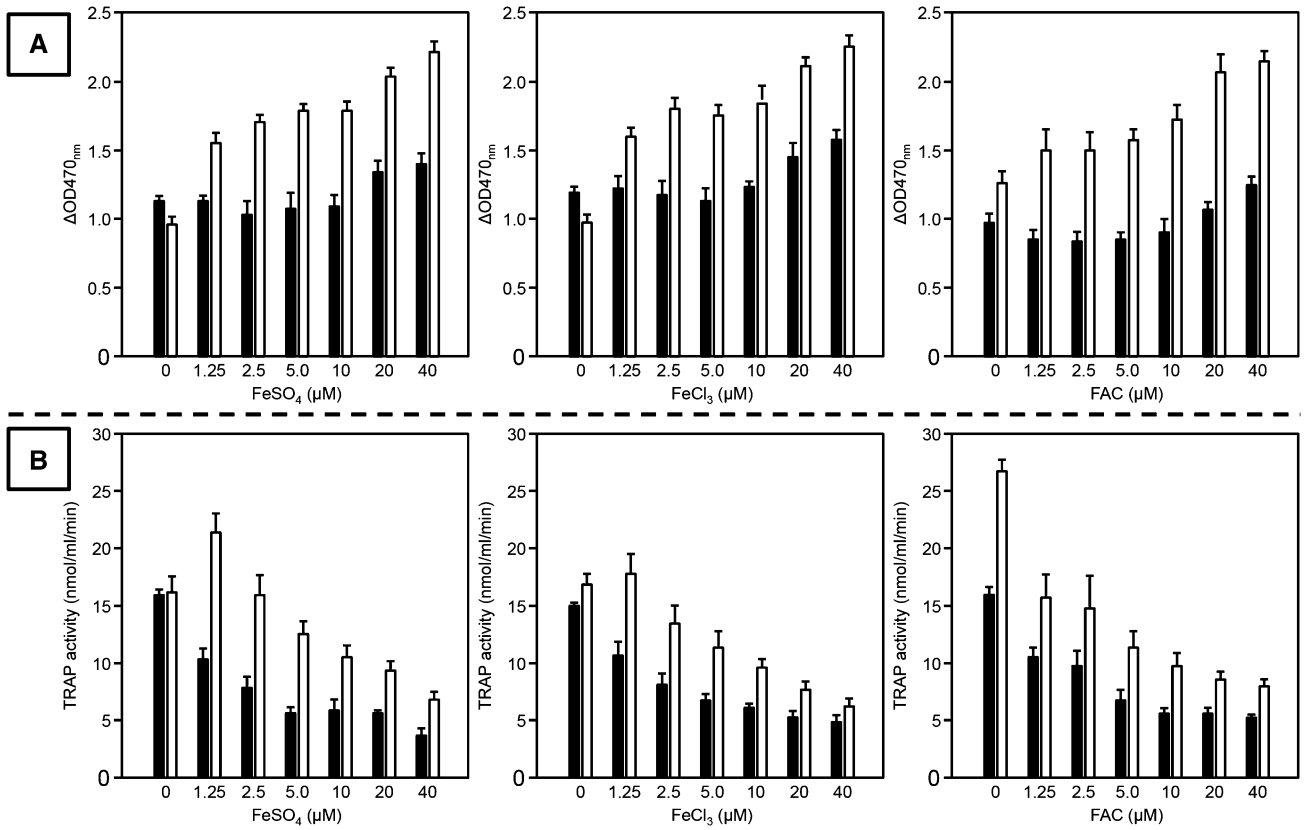

Fig. 3 Exogenous iron inhibits in vitro osteoclast formation. OPC were grown in the presence of exogenous iron at concentrations of up to 40 μM for 3 (*black bars*) and 5 days (*white bars*) in culture media supplemented with M-CSF (30 ng/ml) and RANKL (20 ng/ml). All three iron salts, FeSO4, FeCl3, FAC, used in the study stimulated the proliferation/survival the cells, as determined by XTT assays and measurements at 470 nm (**a**). Concomitantly with the increase in the number of viable cells, a decrease in total TRAP activity, as a measure of the development of osteoclast lineage cells, was observed in the cell lysates (**b**). Values are given as mean ± SD, $n = 6$

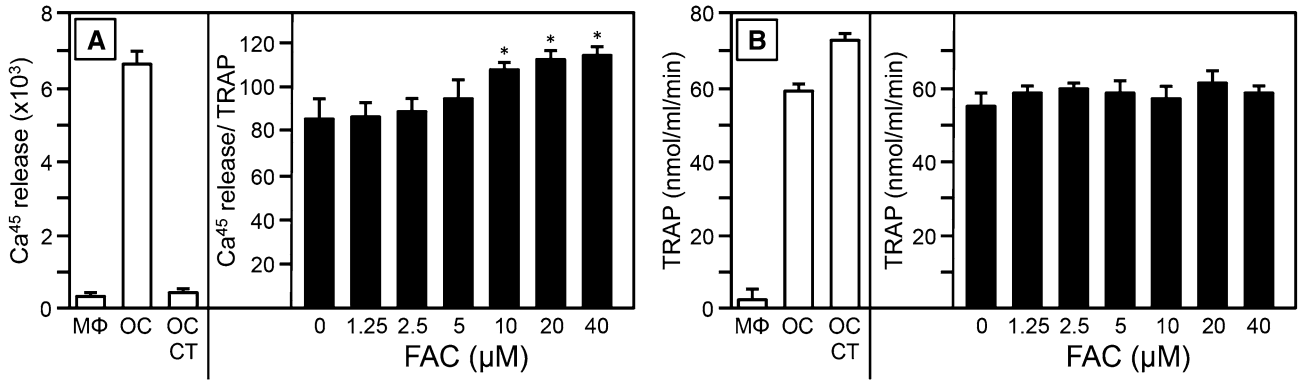

Fig. 4 The effect of exogenous iron on osteoclastic activity. Mature osteoclasts were seeded onto CaP layers containing Ca^{45} to assess the cells' ability to dissolve mineral, a crucial step in physiological bone resorption. The cells were cultured for 24 h in media supplemented with 10 μM FDO and up to 40 μM of exogenous iron (FAC). When FAC concentrations equaled or exceeded the concentration of FDO, the release of Ca^{45} was increased by approx. 30 % (*black bars*, **a**). M-CSF-dependent macrophages (MΦ) and OC in the presence of 10^{-9} M calcitonin (CT; *white bars*, **a**) caused a release of $Ca^{45} < 10$ % as compared to osteoclasts. At the end of the resorption assay, after 24 h on the CaP substrate, total TRAP activity in the cell cultures was not affected by the availability of exogenous iron (*black bars*, **b**). No TRAP activity was detected in cultures of MΦ, while enzyme activity was high in OC ± CT (*white bars*, **b**). Values are given as mean ± SD, $n = 6$; $^*p < 0.05$

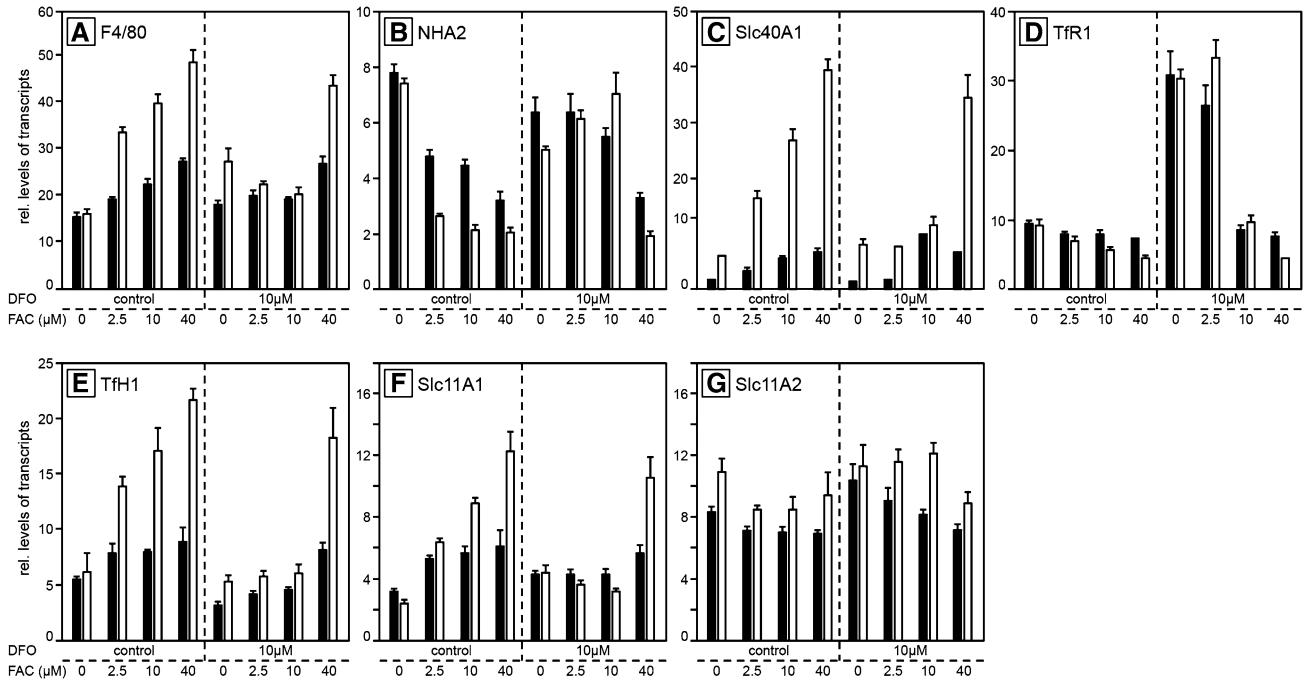

Fig. 5 Exposure to exogenous iron stimulates a macrophage-like phenotype. OPC were grown with M-CSF and RANKL, ± the iron chelator DFO for 3 days (*black bars*) and 5 days (*white bars*). In the absence of DFO, increasing concentrations of exogenous iron led to an increase in the levels of transcripts encoding monocyte/macrophage markers (F4/80 (**a**), Slc40A1 (**c**), TfH1 (**e**), Slc11A1 (**f**)), while attenuating the levels of transcripts encoding proteins that are highly expressed in osteoclasts (NHA2 (**b**), TfR1 (**d**), Slc11A2 (**g**)). Grown in culture media supplemented with DFO, the cultures assumed an osteoclast phenotype until exogenous iron levels exceeded 10 µM, the concentration of DFO, when the cells assumed a macrophage/monocyte phenotype. Values are given as mean ± SD, $n = 3$

Mitochondrial biogenesis has even been suggested as a fundamental pathway linked to osteoclast activation and bone metabolism [15, 31].

In contrast to the previous experiments by Ishii and colleagues [15], the experiments presented in this study suggest that iron exerts limited effects only on osteoclast development and activity in vitro. In the presence of DFO, a Fe^{3+} chelator virtually depleting the culture of iron, and osteoclast formation was affected only slightly and non-significantly. In the presence of excess iron, however, osteoclast development was attenuated and the proliferation of monocyte/macrophage lineage cells was increased. Resorptive activity of osteoclasts was affected by exogenous iron as well. Supplementing the culture medium with DFO (10 µM) caused a decrease of ^{45}Ca release of approx. 30 % over a period of 24 h. Addition of exogenous iron normalized the resorptive activity of osteoclasts and an excess of iron had no further effects.

These data suggest that sub-physiological levels of iron may cause a decrease in osteoclast activity, probably due to energy starving [18, 34]. High levels of iron may increase proliferation of progenitor cells due to its role as co-factor in numerous enzymes associated with proliferation, thereby decreasing osteoclastogenesis in vitro. Physiologically, however, the effects or iron may not be ending at the inhibition of bone resorption. Hemachromatosis, an iron storage disease, induces inflammatory processes and is accompanied by increased levels of the inflammatory cytokines TNFα and interleukin-1. The cytokine is a known stimulator of bone resorption, either by inducing the release of granulocyte–macrophage colony-stimulating factor (GM-CSF), thus increasing the size of the osteoclast progenitor pool, and by stimulating osteoclastogenesis directly by binding on TNF receptors on osteoclast progenitors [1, 12]. It has been suggested before that osteoclast progenitors in vivo exist in various pools outside of the marrow, in particular, as circulatory precursors in the blood [25, 26]. Upon homing to bone, the osteoprogenitors find an inflammatory osteoclastogenic microenvironment, in which they differentiate into mature osteoclasts becoming responsible for an increase in bone resorption [16, 19].

In the present study, the effects of the regulatory hepatic peptide hepcidin on in vitro osteoclastogenesis were not investigated. Hepcidin is the major regulatory factor of intra- and extracellular iron concentrations. By binding of hepcidin to Slc40A1 and stimulating internalization of the receptor, the cells' capacity to expulse iron is decreased. Hepcidin expression is strictly regulated at the transcriptional level [24]. The hepatic iron sensor TfR2 induces a signaling cascade requiring hemochromatosis protein

(HFE) and activating BMP signaling cascades via BMP6 [28]. Deficiency in TfR2 leads to deficiency in hepcidin, causing iron overload [39].

While with Slc11A2 of the enterocytes of the intestinal villi [9, 13] and the hepatic hormone hepcidin [10, 11] two major regulators of physiological iron levels are known, less knowledge is available concerning the physiological roles of iron. There are numerous iron storage diseases leading to the accumulation of iron deposits in organs and tissues [29]. Furthermore, it has been suggested that in postmenopausal women, iron accumulation may occur due to the cessation of menstruation [40]. Two major mechanisms may control the organism's reaction to excess iron: iron deposits may induce an inflammatory response [23] or may induce the generation of reactive oxygen species (ROS) [17]. As pointed out before, and as is known in many systemic inflammatory diseases, inflammatory processes are frequently accompanied by local and systemic bone loss. ROS may induce direct damage to proteins and nucleic acid, and by this process reinforce an inflammatory response.

Bone loss and osteoporosis have also been attributed to a deficiency in iron [35, 37]. This would be caused by the decreased availability of iron for enzyme systems that require iron and that are involved in the synthesis of collagen and in VitD activation and deactivation [34]. This effect in physiology, however, would be rather caused by an impairment of the function of the bone forming cells, the osteoblasts, rather than a modulation of osteoclast development and activation. Another interesting role of iron is its contribution to hypophosphatemia-induced rickets. This variety of the disease is due to phosphate wasting, which is caused either by a deregulation of osteocytic FGF23 or a constitutively active renal phosphate transporter. Iron deficiency induces osteocytes to release increased amounts of FGF23. Under physiological conditions, osteocytes control liberation and inactivation of FGF23, the latter process, however, being suppressed in conditions of iron deficiency and of chronic kidney disease (CKD), thus mimicking the phenotype of autosomal dominant hypophosphatemic rickets (ADHR) [38].

In conclusion, iron is a multifaceted metal most essential for physiology but with the potential to generate considerable damage. The control of iron contents is limited to uptake in the intestine through Slc11A2 and the hepcidin/Slc40A1 system, but a pathway to get rid of excess iron is missing. In the present report, it is demonstrated that the components of the iron homeostasis system are highly differentially expressed in cells of the monocyte/macrophage system and in osteoclasts, the levels being adapted to the specific needs of the respective cell type. With respect to the development and maintenance of the skeleton, the role of iron is somewhat controversial. While it is obviously essential for cell development and function, its role at the systemic level is less clear. Both, iron deficiency and iron excess have been correlated with bone loss and osteoporosis. While osteoporosis in situations with low iron levels may well be caused by specific cellular effects, such as energy metabolism, impairment of BMP signaling, or phosphate wasting, the effects of iron surplus are probably non-specific and are mediated through inflammatory processes. The universal role of iron and its ubiquitous presence may hinder the development of strategies that use iron as a therapeutic target.

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Compliance with Ethical Standards

Conflict of interest Wenjie Xie, Sebastian Lorenz, Silvia Dolder and Willy Hofstetter declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent All animal experiments were performed under the permit BE23/13 issued to WH by the Veterinary Office of the State of Bern.

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