FORUM REVIEW ARTICLE

Role of Heme Oxygenase-1 in Postnatal Differentiation of Stem Cells: A Possible Cross-Talk with MicroRNAs

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Abstract

Significance: Heme oxygenase-1 (HO-1) converts heme to biliverdin, carbon monoxide, and ferrous ions, but its cellular functions are far beyond heme metabolism. HO-1 *via* heme removal and degradation products acts as a cytoprotective, anti-inflammatory, immunomodulatory, and proangiogenic protein, regulating also a cell cycle. Additionally, HO-1 can translocate to nucleus and regulate transcription factors, so it can also act independently of enzymatic function. **Recent Advances:** Recently, a body of evidence has emerged indicating a role for HO-1 in postnatal differentiation of stem and progenitor cells. Maturation of satellite cells, skeletal myoblasts, adipocytes, and osteoclasts is inhibited by HO-1, whereas neurogenic differentiation and formation of cardiomyocytes perhaps can be enhanced. Moreover, HO-1 influences a lineage commitment in pluripotent stem cells and maturation of hematopoietic cells. It may play a role in development of osteoblasts, but descriptions of its exact effects are inconsistent. **Critical Issues:** In this review we discuss a role of HO-1 in cell differentiation, and possible HO-1-dependent signal transduction pathways. Among the potential mediators, we focused on microRNA (miRNA). These small, noncoding RNAs are critical for cell differentiation. Recently we have found that HO-1 not only influences expression of specific miRNAs but also regulates miRNA processing enzymes. **Future Directions:** It seems that interplay between HO-1 and miRNAs may be important in regulating fates of stem and progenitor cells and needs further intensive studies. *Antioxid. Redox Signal.* 20, 1827–1850.

Introduction

Stem and progenitor cells

STEM CELLS (SCs) are defined by two major features. First, they can differentiate into diverse specialized cell types. Second, they are capable of self-renewal—after asymmetric division each SC can generate one daughter cell with a stemness characteristic, and one daughter cell that differentiates. In this manner SCs can maintain their own population. They can also undergo a symmetric division to self-renew and expand (193).

SCs can be classified according to their differentiation potency to (i) totipotent, (ii) pluripotent, (iii) multipotent, and (iv) unipotent (monopotent). Totipotent SCs can give rise to all embryonic as well as extraembryonic tissues (trophoblast

and placenta). This feature is evident only for zygote (which itself is not an SC, as does not self-renew), and cells within the first couple of divisions after fertilization (193).

Pluripotent embryonic stem cells (ESCs) appear at later stage of embryogenesis and their *in vitro* cultures are established from epiblast tissue of the inner cell mass of a blastocyst. They can develop into all cell types in embryo, regardless of the germ layer, and can form all cells of the organisms, except of the placenta. Among markers characteristic for pluripotent SCs are Oct4, Nanog, SSEA-1 (in mouse), or SSEA-4 (in human). In 2006 a breakthrough research demonstrated that pluripotent cells can be obtained from terminally differentiated somatic cells by an enforced expression of Oct4 and Sox2, accompanied by Klf4 and Myc or Nanog and Lin28. Reprogramming of human somatic cells was demonstrated a year

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later. Cells created this way are termed the induced pluripotent stem cells (iPS cells) and resemble ESCs, as they are able to differentiate in all somatic tissues as well as contribute to germline (18, 164, 193).

Multipotent SCs can differentiate into tissues of a single germ layer. Mesenchymal stem cells (MSCs) are regarded as multipotent, since they can differentiate into osteoblasts, chondrocytes, and adipocytes. Additionally, MSCs were postulated to give rise to functional endothelial cells or cardiomyocytes; however, this concept has not been conclusively proved so far (21, 22, 193). Despite suggestions that MSCs can be found in different adult tissues (*e.g.*, adipose-derived MSCs and skeletal-muscle-derived MSCs), it appears that they develop only in the bone marrow stromal compartment. Here, together with osteoblasts and adipocytes, MSCs create a niche for the other multipotent SCs—hematopoietic stem cells (HSCs). HSCs are capable of differentiation into erythroid, lymphoid, and myeloid lineages and give rise to all types of mature blood cells (21, 22, 193).

Monopotent (or unipotent) SCs are progenitor cells of a single tissue, and can differentiate into only one cell type. They still possess, however, a self-renewal ability. Muscle satellite cells (mSCs) are examples of adult SCs for skeletal muscle. They display a monopotent characteristic; although,

mSCs, when stimulated properly, can be reprogrammed to the other mesenchymal cell types—osteoblasts and adipocytes (10, 22, 173, 193).

microRNAs

microRNAs (miRNAs), the novel mediators governing SC commitment and differentiation, have been intensively studied over the last few years. They negatively regulate gene expression (19, 42, 45). Interestingly, genes involved in cell differentiation, which must be temporally downregulated, possess many miRNA binding sites (83).

miRNAs are short (19–25 nt) single-stranded RNAs. These regulatory molecules are encoded in genome and transcribed like other genes by the RNA polymerase II or III, what leads to the formation of long pri-miRNAs, with a hairpin structure and a fragment of double-stranded RNA (Fig. 1) (8, 198, 217). A significant proportion of miRNAs is not encoded by their own genes, but they are generated during the alternative splicing of mRNA genes (63). These so-called mirtrons are encoded in introns, and may regulate the expression of the gene from which they originate (32, 47, 218, 219).

Processing of pri-miRNAs is conducted first in the nucleus, where RNase type III (Drosha enzyme) together with heme

FIG. 1. Biogenesis of miRNA. Gene encoding exonic miRNA is transcribed by an RNA polymerase II or III to pri-miRNA, which is further processed to pre-miRNA by Drosha/DGCR8 complex. Other miRNAs (intronic miRNA) are spliced from the introns of mRNA-coding genes. Pre-miRNA is then translocated to the cytoplasm, where Dicer-TRBP converts it to miRNA duplex, devoid of hairpin structure. Due to helicase activity of Dicer, the double-stranded structure is unwinded and subsequently incorporated into RISC complex (including Argonaute protein), where it binds to target mRNA. After localization in P-bodies, depending on the complementarity between miRNA and targeted sequence, mRNA is either degraded or its translation is suppressed. miRNA, microRNA; TRBP, TAR-RNA binding protein.

protein DGCR8 form pre-miRNAs that are 60–100-nt-long hairpins (53, 238) (Fig. 1). Mirtrons however omit this stage, and after alternative splicing, together with pre-miRNA, they are transported to the cytoplasm by Exportin 5 and RanGTP (47, 63). Afterward, pre-miRNAs are processed by Dicer nuclease (which is an RNase III-type endonuclease), complexed with TAR-RNA binding protein (TRBP, which recruits and binds pre-miRNA) (198, 213). Following cleavage, a miRNA duplex of desired length of ~21 nt is formed, which is subsequently incorporated into RNA-induced silencing complex, consisting of Dicer, TRBP, and Argonaute protein (198, 213). The duplex is unwinded by a helicase domain of Dicer into passenger strand (typically degraded by RISC) and mature miRNA strand (198, 213). Then, the RISC complex delivers miRNA to the target mRNA. Binding occurs between seed sequence of miRNA (between nt 2 and 8) and usually 3'UTR of mRNA (213), although association with 5'UTR, promoter regions, and open reading frame is also possible (47). When the pairing is perfect, mRNA is degraded by endonuclease Argonaute2 in RNA processing bodies (P-bodies), containing deadenylases and decapping enzymes (198, 213, 217). However, in case of incomplete miRNA-mRNA binding, repression of translation is induced. Argonaute proteins upon binding to 3'UTR may compete with eIF4F for m7G sequence on mRNA, and therefore inhibit initiation of translation (198, 217). Post-initiation repression is also possible, when Argonaute proteins lead to disassembly of ribosome complex and

degradation of nascent protein (198, 217). Finally, degradation of mRNA occurs, initiated by polyA tail destabilization, resulting with translational repression (198, 217) (Fig. 1).

Heme oxygenase-1

Heme oxygenase-1 (HO-1, encoded by *HMOX1* gene) is regulated mainly at the transcriptional level (133, 178) (Fig. 2). However, as it was recently shown, expression of HO-1 is also regulated directly and indirectly by miRNAs (Fig. 2). Thus, miR-377 and miR-217 decrease HO-1 level in endothelial cells by interaction with 3'UTR of *HMOX1* mRNA (17). Additionally, miR-122 was shown to reduce HO-1 expression (172), whereas our recent work highlights a negative regulation of HO-1 by miR-200c in renal proximal tubular epithelial cells (195) and by miR-378 in lung cancer cells (189). The other important mediator is also miR-155 that targets Bach1 mRNA and thereby increases expression of HO-1 in endothelial cells and macrophages by enhancing Nrf2 activity (109, 171), as well as miR-196 and let-7, which have similar effects in hepatocytes (74, 75). miR-132 by silencing Nrf2 mRNA can decrease HO-1 expression (195). It is important to note that regulation of HO-1 by miRNAs can be cell-type specific, as, for example, neither miR-217 nor miR-377 is expressed in kidney proximal tubular epithelial cells (195).

It is not surprising that *HMOX1* expression can be regulated by miRNAs. However, our recent study revealed that

FIG. 2. Regulation of HO-1 expression. In a steady state, transcription of *HMOX1* gene is suppressed by Bach1 repressor that binds to ARE sequence and blocks binding of transcription factors. Stimulation of cell with various compounds leads to (i) dissociation of Bach1 repressor from ARE sequence; (ii) dissociation of Nrf2/Keap1 complex; (iii) association of Nrf2 to Maf proteins and binding to ARE sequence; (iv) activation of other transcription factors and their binding to other sequences within *HMOX1* promoter; (v) transcription of *HMOX1*. When a protein is produced, it can catalyze degradation of heme to CO, Fe²⁺, and biliverdin, the latter converted to bilirubin by biliverdin reductase. *HMOX1* protein can be processed by proteolysis to become an enzymatically inactive form that translocates to the nucleus where it affects activity of AP1 or NFκB transcription factors. Additionally, miR-377, miR-378, miR-200c, and miR-217 were shown to decrease HO-1 expression, whereas miR-155, miR-196 and let-7, as well as miR-132 regulate HO-1 expression indirectly, by affecting Bach1 and Nrf2, respectively. In human cells efficacy of transcription depends also on number of GT repeats in *HMOX1* promoter. ARE, antioxidant responsive element; HO-1, heme oxygenase-1.

both specific miRNA expression and global miRNA biogenesis can be regulated by HO-1 (103). This stresses additionally the significance of HO-1 as an important modulator of gene expression.

HO-1 is well known for its cytoprotective, proangiogenic, and anti-inflammatory properties, as well as for ability to regulate cell cycle. Although those effects were examined mostly in a cardiovascular bed (44, 84, 133), it has been shown that, apart from endothelium, smooth muscle cells, and immune cells, the HO-1 affects also other cell types, including stem and progenitor cells (62). At early stages of embryogenesis, it exerts proangiogenic, antioxidant, and anti-inflammatory activities in ESCs (104, 128, 214, 230, 251). Similar effects were observed in MSCs where HO-1 was induced, leading to improved transplantation outcome in the heart (82, 111, 186, 205, 216, 246), lungs (126), and kidneys (243). Moreover, HO-1 haploinsufficiency resulted in elevated mortality of HSCs (31) and was also important for cellular reprogramming, as its lack exacerbated oxidative-stress-induced cell death of murine iPS cells (128). HO-1 improves viability, proliferation, and production of proangiogenic factors in muscle progenitor cells (mSCs and myoblasts) (103, 112) and in so-called endothelial progenitor cells (EPCs; currently recognized as a heterogeneous population; see "Endothelial progenitor cells" sub-chapter) where, additionally, it facilitates migration (52). Eventually, also the MSC-, HSC-, and EPC-derived mature cells are affected by HO-1. Antiapoptotic, anti-inflammatory, and proliferative effects of HO-1 were reported in osteoclasts (179, 256), osteoblasts (34, 41, 43), myoblasts (80, 168, 182), leukocytes (234), and endothelial cells (133).

Interestingly, a growing body of evidence suggests a direct role of HO-1 in differentiation of stem and progenitor cells. In this review we attempt to summarize recent findings in this field, focusing on the molecular pathways that mediate the differentiation-related effects of HO-1, especially those involving miRNAs.

Embryogenesis and HO-1

Role of HO-1 in embryogenesis and differentiation of totipotent or pluripotent ESCs has not been studied extensively, but some indications begun to emerge suggesting that HO-1 may be important in embryonic and early fetal development.

First, although mice that lack HO-1 are viable, they are born from HO-1^{+/-} parents with frequency of 2–20% (depending on the genetic background, usually below 10%), less than predicted by Mendelian distribution (170, 251). In humans only two cases of complete HO-1 deficiency have been described (87, 236). These data suggest a mortality of murine and human embryos when HO-1 expression is missing. Indeed, HO-1, a cytoprotective (128) and immunomodulatory (214) enzyme, was postulated as a therapeutic target in immunological murine pregnancy complications (244), and its overexpression was shown to improve pregnancy outcome in mice (245). Interestingly, analysis of *HMOX1* promoter polymorphism in group of women affected by idiopathic recurrent miscarriage revealed the higher frequency of genotypes associated with increased HO-1 level, indicating that too high HO-1 expression or its too strong inducibility can also be deleterious (51).

Influence of HO-1 on fetal growth has been usually ascribed to its role in placental vascularization (104, 230, 251). In fact, lack of HO-1, inhibition of its activity or stimulation of HO-1 in

placenta by means of adenoviral vectors, resulted respectively in the reduced or improved growth of fetus in *in vivo* murine and rat models (104, 251). HO-1 was also necessary for proper trophoblast invasion in an *in vitro* model of growth of human cytotrophoblast cells on matrigel (142). Accordingly, in mice (226, 251), rats (104, 227), and humans (141), HO-1 can be detected both in embryonic and extra-embryonic tissues, starting from early stages of pregnancy. Its generation in trophoblast peaks when placenta is being formed, and then declines toward the end of pregnancy, remaining however still higher than in embryo (226, 227). Increased expression during embryogenesis, followed by its gradual downregulation when pregnancy progresses, may suggest that HO-1 plays a role in early developmental processes.

Placental or trophoblastic HO-1 may affect embryogenesis *via* regulation of insulin-like growth factor (IGF) binding protein-1, what was shown in an *in vivo* experiment performed in rats (104). IGF was shown to enhance the blastocyst formation, increase the number of blastomeres in cultured murine embryos, and facilitate the establishment of stem cell lines (131). Recently Lin *et al.* proved that HO-1 can directly affect differentiation of pluripotent SCs (128). They exploited a model of embryonic fibroblasts, isolated from HO-1^{+/+}, HO-1^{+/-}, and HO-1^{-/-} mice, and then reprogrammed to iPS cells through enforced expression of c-Myc, Oct3/4, Klf4, and Sox2. At the beginning, the cells did not differ, displaying similar level of alkaline phosphatase, Oct4, and SSEA-1. Prolonged cell culture showed however, that lack of HO-1 augments the spontaneous differentiation, as evidenced by reduced expression of pluripotency markers in HO-1^{-/-} cells. These results were further confirmed, when genetic or pharmacological silencing of HO-1 was performed in HO-1^{+/+} iPS cell cultures (128). Inhibition of spontaneous differentiation can be possibly mediated by HO-1-derived carbon monoxide, as treatment with CO could restore Oct4 expression in HO-1-deficient cells (128). Indeed, CO has already been demonstrated to be important for embryogenesis (250). The role of reactive oxygen species (ROS) has to be considered in those settings, with low levels preserving the self-renewal of SCs while higher ROS levels initiating differentiation (199). One can also suggest that the Erk1/2 kinases, upregulated in HO-1^{-/-} iPS cells (128), may play a role. This signaling pathway is involved in differentiation of ESCs (110, 197), and its inhibition prevents neural or mesodermal maturation while promotes self-renewal of ESCs (153).

The other possible mediators through which HO-1 could affect the maturation of ESCs are miRNAs. Recently miRNAs have been demonstrated to regulate the pluripotency maintenance or lineage commitment in ESCs (19). Since a link between HO-1 and miRNAs in ESCs has not been studied yet, we can only extrapolate the results obtained in other cell types. For instance, in the myoblast cell line, HO-1 is a potent negative regulator of DGCR8 expression, leading to a decrease in a total miRNA pool (103). Importantly, deletion of DGCR8 (224), similarly as deletion of Dicer (152), resulted in disturbed differentiation of ESCs. Also, HO-1 overproduction in myoblasts inhibited expression of Lin28 and Let7 (103), two factors necessary for maintenance of stemness and for promoting the cell cycle exit (136, 145). Eventually, HO-1 in myoblasts reduced expression of miR-34a (103)—which belongs to a cluster that negatively regulates Oct4, Sox2, Nanog, and Klf4 (235)—and inhibited production of miR-290, miR-

293, and miR-295 (103) - the cluster of miRNAs that restrains the early differentiation in murine ESCs (127). In contrast, miR-145, which also inhibits the expression of pluripotency markers (235), was upregulated in response to HO-1 in murine myoblast cell line (103). Thus, one can hypothesize that, apart from relatively well-documented role of HO-1 in formation of placenta, the proper activity of this enzyme may be necessary for early embryogenesis and regulation of lineage commitment of pluripotent cells. This supposition, however, has to be verified in a future work.

Hematopoiesis and HO-1

Unraveling the biology of hematopoietic stem and progenitor cells (HSPCs) began in 1960s (187, 209). Since then, extensive research on HSPCs allowed to understand better the main aspects of their activity, leading to first successful clinical application of SCs (208, 228). Recently, a growing body of evidence indicates that some mechanisms of self-renewal and differentiation found in HSPCs are shared by other tissue-specific SCs (25). Therefore, uncovering the role of HO-1 in hematopoiesis may also suggest how this enzyme is involved in SC-driven processes in other tissues.

It is estimated that 10^6 new blood cells have to be produced every second to provide their steady-state levels in human peripheral blood (25). Maintenance of this dynamic homeo-

stasis is possible due to the activity of bone marrow HSPCs. Differentiation and expansion of these cells follow defined hierarchical scheme and are strictly controlled. HO-1 was reported to play a role in regulation of this process, including stages of early stem and progenitor cell differentiation as well as maturation of effector cells.

HSCs with their multipotent character and self-renewal capabilities stand at the top of hematopoietic hierarchy (Fig. 3). Despite the very low number and a quiescent character of these cells, their function is critical to maintain hematological homeostasis (25). It is understandable that during evolution HSCs have been equipped in many mechanisms that protect them from stress conditions during postnatal differentiation. Cao *et al.* evidenced that HO-1 is one of these crucial protective factors (31). Paradoxically, HO-1^{+/-} heterozygous mice showed more rapid recovery in the model of 5-fluorouracil myelotoxic injury or better reconstitution at initial time points after bone marrow transplantation. However, when the HO-1^{+/-} animals were exposed to chronic stress or HO-1^{+/-} HSPCs were serially transplanted, significant decrease of hematopoietic recovery and exhaustion of HSPC reserve was finally observed (31). These results suggest an important role of HO-1 in regulating the balance between self-renewal and differentiation of HSPCs under environmental stress. It was proposed that reported HO-1-dependent

FIG. 3. Modulation of hematopoiesis by HO-1. HO-1 deficiency accelerates differentiation but inhibits self-renewal of HSCs under stress conditions. Downregulation of Bach1 suppresses the MPP/CMP transition, but confirmation of whether this effect is HO-1 dependent requires further studies (dotted line). HO-1 activity in central macrophages of erythroblastic islands results in elevated CO concentration and regulates the erythroblast maturation acting through modulation of TNF α and α -subunit of α 4 β 1 integrin. Decreased expression of HO-1 in megakaryocytes leads to increased generation of ROS what enhances production of platelets. By regulating the heme level in B-cell progenitors, HO-1 affects B-cell differentiation in a Bach2-dependent manner. CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor; HSC, hematopoietic stem cell; MEP, megakaryocyte-erythroid progenitor; MPP, multipotent progenitor; ROS, reactive oxygen species; TNF α , tumor necrosis factor α .

inhibition of stress-induced HSPC proliferation is reliant on CO. Reduced HO-1 expression and lowered CO concentration led to a decreased activation of p38-MAPK pathway and, consequently, to low levels of p21 in cycling cells (31).

Similarly, decrease in a long-term blood chimerism was observed after bone marrow transplantation from *Nrf2*^{-/-} mice (146). Although *Nrf2* is one of the main transcription factors driving expression of HO-1, the HO-1 mRNA levels in HSPCs from *Nrf2*^{-/-} mice were not altered, suggesting that mechanism of this effect is independent of HO-1 activity (146). However, the effect of *Nrf2* may be similar, as recent study demonstrated that HSC and progenitor cell compartment was expanded in *Nrf2*-deficient mice at the expense of HSC quiescence and self-renewal (215).

In next stage of hematopoiesis, HSCs differentiate to multipotent progenitors (MPPs) and subsequently to common myeloid progenitors (CMPs) or common lymphoid progenitors (CLPs) (Fig. 3). The transition to CLPs might be affected by HO-1 as indicated by studies done in *Bach1*^{-/-}*HO-1*^{-/-} mice (190). Such mice exhibited higher numbers of HSC and MPP fractions in bone marrow, but lower numbers of CMPs and, consequently, CMP-derived progeny, including dendritic-cell-restricted progenitors, common dendritic cell progenitors, monocytes, macrophages, and dendritic cells. It suggests that *Bach1*/*HO-1* pathway may be important in committing HSCs to CMPs (190). Nevertheless, the discussed event in *Bach1*^{-/-}*HO-1*^{-/-} mice is, at least to some extent, HO-1 independent, as *Bach1*^{-/-}*HO-1*^{+/+} mice show similar decrease in macrophage and dendritic cells as *Bach1*^{-/-}*HO-1*^{-/-} phenotype. Therefore, the exact role of HO-1 in HSC/CMP transition has to be clarified in a model of *Bach*^{+/+}*HO-1*^{-/-} mice. Other pathways dependent on *Bach-1*/*Nrf2* may play a role (215).

Several reports evidenced the involvement of HO-1 in differentiation of lineage committed progenitors (Fig. 3). The first studies regarding its role in maturation of hematopoietic progenitors concerned erythropoiesis (2, 77). Erythroblasts differentiate in the bone marrow and spleen within the defined niches called erythroblastic islands (20, 36). These structures were characterized as a central macrophage with many surrounding and interacting erythroid cells at different stages of maturation. Interestingly, the lack of HO-1 expression in erythroblasts is well described in both K562 erythroid cell line (7) and primary human bone marrow-derived erythroid progenitors (7). Oppositely, HO-1 is constitutively expressed in macrophages; therefore, it has been suggested that HO-1 activity in these cells is crucial to regulate erythropoiesis (211). Indeed, exogenous CO accelerated the differentiation of K562 cells *in vitro* (211). Moreover, in the *in vivo* model of *HO-1*^{+/+} bone marrow transplantation, the differentiation of erythroblasts was impaired (30). This effect was mostly visible in the spleen as a prevailing site of erythropoiesis under stress conditions and to lesser extent in the bone marrow. Interestingly, higher percentage of tumor necrosis factor α (TNF α)-expressing macrophages was found in the spleen of *HO-1*^{+/+} mice. As TNF α inhibits erythropoiesis, this may explain the erythropoietic defect in *HO-1*^{+/+} individuals (30). Further, HO-1-deficient proerythroblasts displayed a decreased surface expression of α -subunit of α 4 β 1 integrin (CD49d) that is crucial for adhesion to central macrophages, what might contribute to alterations of erythropoiesis.

HO-1 may also regulate thrombopoiesis (Fig. 3) (150, 159). Treatment of Meg-01 human megakaryoblastic cell line or

primary human cord blood-derived megakaryocytes with prostaglandin-J₂ (PGJ₂) led to platelets release and concomitantly upregulated HO-1 expression. However, when HO-1 activity was inhibited by tin protoporphyrin IX (SnPPPIX) before PGJ₂ stimulation, the release of platelets was further increased. Additionally, PGJ₂-induced platelet production was accompanied by elevation of oxidative stress. Hence, it is likely that the well-known antioxidant properties of HO-1 may inhibit the platelet production (159). Similar effect may be exerted by *Nrf2* that shares DNA-binding specificities with NF-E2 p45, an essential regulator of megakaryopoiesis. Accordingly, it has been demonstrated that p45 through competing with *Nrf2* promotes increased oxidative status, what enhances platelet gene expression and megakaryocytic maturation (150).

Contribution of HO-1 to development of murine lymphoid lineages has been suggested in case of B-cell maturation (Fig. 3). Accordingly, HO-1 expression increases with stages of B-cell differentiation, being the lowest in pre-B, moderate in immature-B, higher in mature-B, and the highest in plasma cells (225). As proposed, HO-1 regulates intracellular heme levels that affect the humoral immunity in *Bach1*- and *Bach2*-dependent mechanism. In turn, *Bach1* and *Bach2* modulate expression of HO-1, indicating a presence of negative feedback loop (159).

Despite increasing number of studies, many aspects of how HO-1 influences hematopoiesis need to be further elucidated. A growing body of evidence underlines the crucial role of stromal cells within HSC niche in controlling the HSC self-renewal and quiescence (223). It remains unclear whether HO-1 modulates HSC potential by regulating the niche components. Moreover, the nuclear form of HO-1 that lacks an enzymatic activity has been recently described (130). That raises a question whether the involvement of HO-1 in hematopoiesis described until now is solely dependent on its enzymatic activity or to some extent also on its not well-characterized non-enzymatic functions.

Importantly, recent results strongly support the hypothesis that miRNAs are crucial factors shaping the HSPC development (160). Taking into consideration that HO-1 may globally affect the miRNA production (103), including particular miRNAs known to regulate hematopoiesis, it is reasonable to hypothesize that a role of HO-1 in HSPC differentiation is at least partially miRNA dependent. One of the potential candidates is miR-146a, whose forced overexpression caused disturbed kinetics of differentiation and decreased engraftment and survival of HSCs (196). The levels of miRNA-146a seem to be strictly connected with HO-1 expression, as overexpression of the enzyme led to increased levels of miR146a in murine myoblast cell line (C2C12 cells) (103), while T regulatory cells from spleens of *HO-1*^{-/-} mice showed decreased 146a expression (unpublished data). Moreover, the miRNAs 15a (100, 249), 142-3p (23), 155 (161), and 196b (169), whose knockdown or overexpression deregulates hematopoiesis, may be also regulated by HO-1 (103). Therefore, the potential influence of HO-1 and miRNA cross-talk on hematopoietic differentiation is particularly interesting and needs to be further studied.

HO-1 Role in Cancer SCs

Increasing understanding of SC properties led to discovery that certain fraction of cancer cells display similar characteristics to the tissue SC populations (174). Many reports evidenced that solid tumors and leukemias contain a small fraction

of quiescent cancer cells that show enhanced self-renewal capacity and long-term tumor-initiating potential. These cells were called cancer SCs and are thought to be the most therapy-resistant fraction of tumor cells (49). As HO-1 was shown to regulate stress response and differentiation of adult SCs, it has been hypothesized that HO-1 activity may play an important role in cancer SC biology.

There are several reports showing that high HO-1 expression selects the most resistant tumor cells (15). Subclones of human lung adenocarcinoma cell line (CL3) with highest HO-1 expression were highly insensitive to toxic effects of arsenite (117). Further, HO-1 was shown to be a crucial factor responsible for chemotherapy resistance of human cholangiocarcinoma cells (101). Other studies demonstrated that HO-1 protects chronic myeloid leukemia against cell death induced by BCR/ABL inhibitors (139, 140). The resistance of multiple myeloma to bortezomib and lenalidomide is also linked to high expression of HO-1 (15). Consistently, silencing of HO-1 increased the sensitivity of the acute myeloid leukemia (AML) cells to cytarabine and daunorubicin chemotherapeutics (69). Apart from HO-1-dependent chemotherapy and drug resistance, high HO-1 expression may provide protection of cancer cells against photodynamic therapy (158).

The majority of studies that prove the cytoprotective role of HO-1 in cancer cells claimed that mechanism of HO-1-dependent resistance is connected with regulation of ROS (15, 69, 98, 101, 117, 188). Additional pathway has been proposed in case of AML cells; HO-1 counteracted induction of apoptosis caused by NF κ B inhibition (176) and provided resistance to TNF α -induced cell death (177). Importantly, many cytoprotective effects ascribed to Nrf-2 were shown to be dependent on HO-1 activation (96, 134).

There is a body of evidence that HO-1 enhances survival and drug resistance in neoplasms. Drug resistance and tumor reoccurrence after therapy may result from presence of cancer SCs. Therefore, it is justified to postulate that HO-1 is important in this rare cell fraction. The first observation that supports this hypothesis came from work by Herrmann *et al.* (70), who studied primary AML cells isolated from patients. They sorted AML cells with CD34⁺CD38⁻ and CD34⁺CD38⁺ phenotypes, which previously were shown to be enriched in leukemia stem and progenitor cells (206), and confirmed high HO-1 expression within these subpopulations (70). The water-soluble inhibitors of HO-1, SMA-zinc protoporphyrin IX (ZnPPiX) and PEG-ZnPPiX (154), inhibited *in vitro* growth of CD34⁺CD38⁻ and CD34⁺CD38⁺ AML cells in a dose-dependent manner (70). Accordingly, incubation of AML cells with SMA-ZnPPiX reduced the occurrence of leukemia *in vivo* after xenotransplantation to immunocompromised mice (70), what confirmed the possibility to use HO-1 inhibitors to block leukemia-initiating cells.

Over last decade, the involvement of miRNAs in cancer pathophysiology has been investigated profoundly (210). Apart from functioning as an oncogene or a tumor suppressor, some miRNAs were revealed to be associated with self-renewing and therapy-resistant population of cancer cells (234), including the leukemic SCs (40). For example, miR-196b has been suggested to take part not only in self-renewal of hematopoietic progenitors, but also in leukemic SCs. It was less abundant in more differentiated cells, while its overexpression increased the replating potential of leukemic progenitors and partially blocked their differentiation (169). On

the other hand, in AML patients, miR-204 was downregulated (58). Interestingly, upregulation of miR-196b and downregulation of miR-204 were observed in C2C12 cells in response to HO-1 overexpression (103). Such cells injected to immunodeficient mice behaved like tumor cells, did not differentiate, and formed lung metastasis (102).

Among miRNAs that are connected with HO-1 expression, miR-29a, miR-34a, miR-126, miR-145, miR-146a, miR-155, and miR-378 were found to be deregulated in various cancer SC populations (40, 50). Their functions and mechanisms that regulate their expression can, however, differ in SCs derived from distinct tumors.

Summing up, it appears that HO-1 activity in cancer SCs could result in tumor resistance to therapy. Therefore, the HO-1 inhibitors have grown into potential anticancer drugs. However, more studies on HO-1 role in strictly defined cancer SCs are needed. The possible influence of HO-1 on self-renewal and differentiation of cancer SCs seems to be particularly interesting.

Osteoclastogenesis and HO-1

Bone is a dynamic structure, whose constant remodeling in postnatal life is a consequence of balance between bone production by osteoblasts and bone resorption by osteoclasts, the specialized cells formed by fusion of monocyte-macrophage precursors. Osteoclastogenesis is tightly regulated by several cytokines (Fig. 4). Macrophage colony-stimulating factor promotes differentiation of HSCs into preosteoclasts, and their fusion into multinucleated, mature osteoclasts. Receptor activator for NF κ B ligand (RANKL), which is a member of TNF α family, by binding to its receptor (receptor activator for NF κ B [RANK]) and afterward to TNF α receptor associated factor 6, activates several signaling pathways during differentiation, including Erk1/2, p38, JNK, and PI3K/Akt kinases. This leads to activation of numerous transcription factors, such as NF κ B and c-Fos, which induce expression of another transcription factor NFATc1 (NFAT2). It in turn acts as a co-factor of AP1 transcription factor and activates expression of target genes, specific for osteoclasts: tartrate resistant acid phosphatase (TRAP), cathepsin K, or calcitonin receptor. The major inhibitor of this process is osteoprotegerin, a soluble ligand for RANK, which inhibits RANKL-dependent signaling (24, 207, 252). Osteoclastogenesis can also be augmented by proinflammatory cytokines, for example, TNF α (24, 207, 252). Recently, the high mobility group box 1 (HMGB1) nuclear transcription factor, which can be released by damaged cells, was shown to upregulate maturation of osteoclasts by binding to advanced glycation endproduct receptors (254).

Several lines of evidence indicate that HO-1 plays a role in osteoclast maturation during postnatal life (Fig. 4). In contrast to preosteoclasts, the mature osteoclasts do not respond to hemin stimulation and do not upregulate HO-1 level, suggesting that HO-1 affects rather osteoclast differentiation than function. Accordingly, HO-1 expression is decreased in mature osteoclasts, and possibly such downregulation is necessary for proper osteoclastogenesis (256). This supposition has been confirmed recently in two independent studies, which showed that HO-1 is inhibited during osteoclast differentiation induced by stimulation of RANK with RANKL (68, 179). HO-1 inhibition in differentiating osteoclasts may depend on Bach1 transcriptional repressor, as this effect was less pronounced in the Bach1^{-/-} cells (68). Additionally, the RANKL-dependent

FIG. 4. HO-1 as an inhibitor of osteoclastogenesis. HO-1 is downregulated during differentiation of osteoclasts by RANKL-dependent signaling. Elevated HO-1 inhibits osteoclastogenesis probably *via* inhibiting c-Fos and NFATc1 transcriptional activities. Thereby it decreases expression of markers of terminally differentiated osteoclasts (e.g., TRAP) and prevents bone resorption. HO-1 abolishes also secretion of HMGB1 and downregulates signaling from TNF α , which are known to induce osteoclast maturation. Similarly, inhibition of osteoclastogenesis is also exerted by bilirubin and CO. Additionally, HO-1 may decrease activity of p38 and Erk1/2 kinases or DGCR8, and downregulates ROS (involved in upregulation of RANKL/RANK signaling pathway), all of them known as regulators of osteoclast maturation, whereas it may upregulate expression of miR-146a that is an inhibitor of osteoclastogenesis. Black—effects exerted by HO-1; gray—known pathways of osteoclastogenesis; spots—hypothetical pathways regulated by HO-1 in differentiating osteoclasts. (→)—induction; (—)—inhibition; (+)—in cooperation with. HMGB1, high mobility group box 1; RANK, receptor activator for NFκB; RANKL, receptor activator for NFκB ligand; TRAP, tartrate resistant acid phosphatase.

HO-1 downregulation can be mediated by declined p38 signaling (179). It appears that suppression of HO-1 enables the release of pro-osteoclastogenic HMGB1 (179). Moreover, ROS were demonstrated to be critical in RANKL-dependent signaling, as their presence is necessary for proper activation of downstream signaling pathways (Akt, p38, JNK, and ERK1/2 kinases) (67, 114), while HO-1 is known to affect differentiation of other cell types *via* inhibition of O $_2^{\bullet-}$ (14). Thus, it seems that inhibition of HO-1 may result in elevation of ROS, required for the RANKL/RANK signaling (179).

Induction of HO-1 with hemin inhibits osteoclastogenesis in *in vitro* culture of primary murine preosteoclasts or RAW-D osteoclast precursors (179, 256) and, as a result, *in vitro* bone resorption (256). These effects were reversed by siRNA-mediated HO-1 silencing or upregulated after HO-1 gene transfer (179), and to some extent, they could be mimicked by HO-1 byproducts—bilirubin and CO (179, 256). Since proliferation of osteoclast precursor cells was increased and apoptosis was decreased, hemin-induced differences in the number of mature osteoclasts as well as reduced transcription of TRAP and calcitonin were attributed to impaired differentiation (179, 256).

The detailed mechanisms by which HO-1 regulates osteoclastogenesis have not been elucidated so far. It has been shown that induction of HO-1 expression with hemin reduced

the expression of RANK (256) and release of HMGB1 (179) during *in vitro* differentiation of primary preosteoclasts. Similarly, in the Bach1-deficient primary preosteoclast cells, inhibition of differentiation was accompanied by attenuated RANKL/RANK signaling and decreased activity of c-Fos and NFATc1 (68). HO-1 may, to some extent, mediate these effects, as it is upregulated in Bach1 $^{-/-}$ preosteoclasts, and its silencing partially restores osteoclastogenesis (68).

Increase in HO-1 expression may also be responsible for inhibition of osteoclast differentiation of murine primary bone marrow-derived macrophages cultured *in vitro* and treated with inducers of Nrf2: tert-butylhydroquinone (237), delta-methrin (180), and kahweol (56). Upregulated HO-1 expression was parallel to decreased HMGB1 release (56, 237), inhibition of Erk1/2 activity (56, 180), and attenuation of NFATc1-dependent transcription (56, 237). One can hypothesize that these pathways are involved in regulation of osteoclastogenesis by HO-1.

Inhibitory effect of HO-1 on osteoclast maturation and bone resorption was confirmed *in vivo*. HO-1 induced by cobalt protoporphyrin IX (CoPP) abolished inflammatory osteoclastogenesis mediated by TNF α (256), and TNF α -induced bone loss was decreased in Bach1-deficient mice (68). On the other hand, Nrf2 $^{-/-}$ animals were reported to develop severe

joint injury and spontaneous bone fracture, what might be due to increased formation of osteoclasts (231). Moreover, reduced expression of HO-1 was demonstrated in patients with rheumatoid arthritis, which is an inflammatory disease characterized by increased osteoclast activity and bone destruction (54, 256). It appears that high HO-1 activity leading to elevated bilirubin level can protect bone from erosion (256).

Like many other processes, osteoclastogenesis is regulated by miRNAs (Fig. 4). Despite few studies that report the role of miR-223, miR-155, or miR-21 in murine *in vitro* culture of preosteoclast cell line and primary cells (85, 233), this issue has not been investigated sufficiently yet. Some observations suggest, however, that miRNAs might mediate effects of HO-1 in osteoclasts. First, decreased expression of Dicer or DGCR8 proteins suppresses biogenesis of miRNAs in osteoclasts and slows osteoclastogenesis (148, 200). Similar influence on DGCR8 expression and miRNA production can result from HO-1 activation in murine myoblast C2C12 cell line (103). Next, in C2C12 cells, HO-1 was shown to upregulate miR-146a (103), known to abolish osteoclast differentiation *in vitro* via inhibition of NFATc1 transcription factor (155). The crosstalk between miRNAs and HO-1 in osteoclastogenesis is, however, hypothetical and needs examination.

In conclusion, HO-1 inhibits osteoclastogenesis and expression of proteins characteristic for terminally differentiated osteoblasts *via* decrease in RANKL/RANKL signaling, decrease in HMGB1 secretion, and maybe through miRNA-dependent pathways (Fig. 4).

Osteoblastogenesis and HO-1

New bone tissue is formed by osteoblasts, the cells of mesenchymal origin. Following lineage commitment, osteoprogenitors proliferate intensively and differentiate into mature osteoblasts, generating osteogenic extracellular matrix and conducting bone mineralization. When trapped and isolated in the bone matrix, osteoblasts give rise to mitotically inactive osteocytes, which can further activate their precursors in a paracrine manner (81, 119).

Commitment of MSCs into osteoblastic lineage during postnatal life is induced by cooperation of Wnt/ β -catenin, bone morphogenic protein 2 (BMP2), and runt-related transcription factor 2 (Runx2) (Fig. 5). Runx2 is a main transcription factor controlling osteoblastogenesis during skeletal development and postnatal life, and its deficiency precludes formation of mineralized skeleton. It is both sufficient and obligatory to promote osteoblastic differentiation of MSCs.

FIG. 5. HO-1 as a stimulator (A) and inhibitor (B) of osteoblastogenesis. HO-1 activates PI3K/Akt and pAMPK signaling pathway and reduces ROS level, inducing differentiation and viability of osteoblasts. Additionally, HO-1 may induce osteoblastogenesis *via* activation of Wnt/ β -catenin signaling. It could also interfere with differentiation process by potentially decreasing miRNAs involved in regulation of transcriptional cascade of Runx2 (miR-133, miR-211, miR-182, and miR-29a). By modulating the level of miR-210 and miR-206, HO-1 could induce BMP2 signaling and expression of Cx43. On the other hand, HO-1 decreases expression of Runx2 and markers of differentiated osteoblasts, what is mediated by CO and bilirubin, and could be also achieved by decreased activity of c/EBP δ . HO-1 may possibly induce miR-135, a repressor of Runx2, and reduce miR-29b, what induces differentiation by affecting Wnt/ β -catenin and BMP2 signaling. Nrf2, an HO-1 inducer, abolishes Runx2 activity. Black—effects exerted by HO-1; gray—known pathways of osteoblastogenesis; spots—hypothetical mechanisms regulated by HO-1 in differentiating osteoblasts. (→)—induction; (—|)—inhibition; (+)—in cooperation with. BMP2, bone morphogenic protein 2.

Runx2 binding sequences are located in the promoters of several genes characteristic for osteoblasts, such as collagen 1 α , osteopontin, bone sialoprotein, osteocalcin, osteonectin, or alkaline phosphatase. Expression of Runx2 is precisely controlled, and decreases during terminal stages of differentiation, what is necessary for proper bone development. It is potentiated by BMP2 or Wnt/ β -catenin signaling pathways, while inhibited by Sox9 or Nkx3.2, the inducers of chondrogenesis. Transcriptional activity of Runx2 is controlled by the plethora of coactivators. Namely, CCAAT/enhancer binding protein α and β (c/EBP α and c/EBP β) upregulate, together with Runx2, the expression of bone-specific growth factors or their receptors. Activating transcription factor 4, whose expression is required at later steps of osteoblast maturation, interacts with Runx2 or FoxO1 to induce transcription of genes typical for terminally differentiated osteoblasts. Interestingly, Runx2 is activated by FoxO1 in differentiating MSCs, whereas in mature osteoblasts or committed osteoblastic progenitors such an interaction can be opposite. This may depend on production of ROS, which are known to reduce bone mass and osteoblast differentiation through activation of FoxO proteins. Finally, Runx2 increases expression of osterix, another transcription factor that promotes osteoblastogenesis. Osterix elevates expression of major osteoblastogenic genes and, together with its cofactor NFATc1, activates the Wnt/ β -catenin signaling pathway, which can be additionally stimulated by BMP2 and transforming growth factor β (6, 81, 105, 119, 125, 137, 229).

Role of HO-1 in postnatal osteoblastogenesis has been studied in different models, but still there is no consensus whether it induces, inhibits, or has no effect on this process (Fig. 5). Some reports showed a slight increase in HO-1 expression during *in vitro* osteoblastic differentiation of human MSCs (14), and an enhanced bone mineralization after *in vitro* stimulation of mesenchymal osteoblast precursor cells (14, 221) or primary osteoblasts isolated from osteoarthritic patients (41) with CoPPIX. Markers of osteogenesis (BMP2, Runx2, and osteonectin) were, however, unchanged after pharmacological stimulation of HO-1 expression or inhibition of its activity (14, 41). Yet, their upregulation or reduction was observed upon CoPPIX or tin mesoporphyrin IX (SnMPPIX) treatment, respectively, when cells were incubated additionally with high concentration of glucose (14) or with interleukin-1 β (IL-1 β) (41). Similar effect was observed when HO-1-overexpressing primary osteoblasts were stimulated with IL-1 β (41). HO-1 was also suggested to be a mediator of *in vitro* osteoblastogenesis of primary rat or human MSCs induced by curcumin (64) and osteoprotegerin (221), or as a factor protecting MSCs from nicotine-dependent inhibition of osteogenic differentiation (92).

Induction of HO-1 expression with CoPPIX, especially under hyperglycemic conditions, leads to activation of AMPK (14, 221) and Akt (221) kinases in human primary MSCs cultured *in vitro* (Fig. 5), what can be reversed by SnMPPIX (221). Both these signaling pathways upregulate osteoblastic differentiation (94), and can be proposed as mediators of osteoblastogenic activity of HO-1. Another possible mechanism of augmented maturation of human osteoblasts is HO-1-dependent decrease in production of O₂^{•-} (6, 14). Forced expression of HO-1 by means of lentiviral vectors induces also Wnt10 *in vivo* in a murine adipose tissue (29). Wnt10 was shown to increase osteoblast differentiation (105) and may be responsible for some effects of HO-1 activation, similarly to

other factors that may promote osteoblastogenesis instead of adipogenesis (1, 60, 202, 203).

HO-1 was reported to support differentiation of human dental pulp cells into odontoblasts (99), which share many similarities with osteoblasts. They also originate from mesenchymal progenitors, rely on Runx2 as a regulator of differentiation, and express the same components of extracellular matrix (28). Pharmacological stimulation of HO-1 with CoPPIX upregulated the odontoblast differentiation markers, whereas its pharmacological or genetic inhibition, as well as scavenging the CO or ferrous iron, exerted opposite effects (99). Accordingly, pharmacological inhibition of HO-1 activity with ZnPPIX or SnPPIX blocked *in vitro* osteogenesis induced by pachymic acid (118), substance P (102), mechanical stress (116), or simvastatin (147), and reduced the expression of odontoblast differentiation markers, such as alkaline phosphatase, osteonectin, osteocalcin, osteopontin, and bone sialoprotein (102, 116, 118, 147).

On the other hand, Nrf2 was shown to repress transcriptional activity of Runx2 and inhibit osteoblastogenesis (71). This is in line with inhibitory role of HO-1 in osteoblast *in vitro* differentiation observed for primary osteoblastic cells isolated from rat calvaria, where genetic overexpression or pharmacological activation of HO-1 reduced mineralization and decreased expression of alkaline phosphatase, osteocalcin, and Runx-2, with only a slight decrease in cell viability (132). In accordance, treatment with bilirubin or CO-releasing molecule reduced osteoblast differentiation rate, the effect reversed by ZnPPIX (132). Additionally, HO-1/ferritin axis was reported to inhibit smooth muscle cell calcification and osteoblastic differentiation (242). As HO-1-derived CO potently decreases activity of c/EBP δ in murine myoblast C2C12 cell line (103), one can suppose that influence of HO-1 on osteoblastogenesis may be partially mediated by cEBP-dependent pathways. Finally, some experiments showed that neither overexpression of HO-1 (253) nor its deficiency in the primary MSCs isolated from HO-1^{-/-} mice (243) modulated the rate of osteoblastic maturation *in vitro*.

Recent studies have underlined a role of miRNAs in formation of osteoblasts from both MSCs and C2C12 cell line (76). In the latter cells, we have demonstrated that miRNA expression profile is significantly influenced by high level of HO-1 activity (103). Thus, one can expect that similar changes might be induced during osteoblastogenesis, if expression of HO-1 is simultaneously increased. Overexpression of HO-1 in C2C12 cells caused a decrease in expression of miR-133a/b, miR-182, miR-206, miR-211, and miR-29a (which have been demonstrated to inhibit osteoblast maturation of C2C12 cells, murine MSCs, and primary murine osteoblasts isolated from rodent calvaria *in vitro*), while upregulated miR-210 (known to induce *in vitro* differentiation of murine stromal cells into osteoblasts) (76, 97, 103). Attenuating the osteoblastic maturation by miRNAs results from directly targeting the mediators critical for osteoblastogenesis: Runx2 (by miR-133 and miR-211), Cx43 (by miR-206), osteonectin (by miR-29a), and FoxO1 (by miR-182) (76, 78, 86, 97, 123). Conversely, miR-210 targets the activin receptor, thereby blocking the signaling from inhibitors of BMP pathways (149). Thus, it appears that alternations in expression of miRNAs may promote the osteoblastic differentiation, and might be potential executors of HO-1-related effects (Fig. 5).

However, induction of HO-1 in C2C12 cells also elevates the expression of miR-135, considered as a factor inhibiting

osteoblastic maturation of C2C12 cells *via* targeting Runx2 (123). Similarly, HO-1 seems to downregulate miR-29b (103), which was shown to decrease the expression of various inhibitors of differentiation in osteoblasts from rat calvaria, such as activin receptor or β -catenin interacting protein 1 (122). In this manner, HO-1 might exert also the inhibitory effect on osteoblastogenesis (Fig. 5).

Confusing results that demonstrate both stimulatory and inhibitory impact of HO-1 on maturation of osteoblasts may be due to differences in cell models used (MSCs or dental pulp cells *vs.* primary osteoblasts). It should also be noticed that although pro-osteogenic activity of HO-1 has been reported in a few independent studies, HO-1 alone was insufficient to induce differentiation (14, 41, 102). Instead, it rather potentiated or decreased the effects of other factors (14, 41, 102). However, such a separation of modulatory function of HO-1 and its direct influence on osteoblastogenesis requires further experimental verification.

Adipogenesis and HO-1

Formation of adipose tissue in an adult organism includes two important stages—commitment of MSCs to adipogenic lineage followed by terminal differentiation of preadipocytes to mature adipocytes. After a lineage commitment, cultured preadipocytes have to be growth arrested, and then begin to proliferate synchronously, undergoing multiple rounds of mitosis, finished by terminal differentiation, manifested by a dramatic cell-shape change, accumulation of triglycerides, and *de novo* lipid synthesis (135, 204).

Several factors are engaged in regulation of the commitment process (Fig. 6). BMP2 and BMP4 have an activating role as they induce expression of cytoskeletal proteins necessary for changing the cell shape from fibroblast like to the round one. Wnt/ β -catenin pathway triggers the transcription of c-myc and cyclin-D1, important for mitotic clonal expansion process. Its role, however, is not so unequivocal. High level of Wnt10b, which decreases upon the later stages of differentiation, leads to inhibition of adipogenesis through blocking the expression of major adipogenic transcription factors—peroxisomal proliferator activated receptor γ (PPAR γ) and c/EBP α . In contrast, Wnt5b upregulates PPAR γ and enhances adipogenesis. Finally, Hedgehog and its ligand Sonic (Shh) interfere with the adipogenic commitment, although the precise mechanism of this effect remains unknown (135, 204, 220).

The committed preadipocytes are growth arrested before their clonal expansion is induced by activation of IGF and cyclic AMP (cAMP) signaling pathways (Fig. 6). First, the cAMP response element binding protein (CREBP) is activated to induce the expression of c/EBP β . After phosphorylation and dimerisation, the c/EBP β protein acquires the DNA-binding properties and, together with c/EBP δ , activates expression of master regulators of adipogenesis, c/EBP α and PPAR γ transcription factors, binding to the c/EBP regulatory elements found in their promoters. The c/EBP α displays antimitotic activities, whereas PPAR γ forms a heterodimer with retinoid-X-receptor- α to activate PPAR-response element in target genes, whose expression is typical for mature adipocytes. Both transcription factors upregulate each other and activate expression of large group of adipogenic proteins, including lipoprotein lipase, intracellular lipid binding protein, or sterol-regulatory-element-binding protein 1, which, in turn, induce production of lipogenic enzymes (135, 204).

FIG. 6. HO-1 as an inhibitor of adipogenesis. HO-1 decreases expression of c/EBP α , PPAR γ , and enzymes characteristic for mature adipocytes by interaction with Wnt/ β -catenin and Hh/Shh pathways. Nrf2, an HO-1 inducer, suppresses adipogenic differentiation too. HO-1 may reduce expression of miR-200c and miR-378, resulting in a decline of differentiation mediated *via* Wnt or c/EBP α / β , respectively. Possibly, HO-1 can also inhibit adipogenesis by decrease in ROS production, DGCR8 expression, and CO-mediated downregulation of c/EBP δ / β activities. Black—effects exerted by HO-1; gray—known pathways of adipogenesis; spots—hypothetical mechanisms regulated by HO-1 in differentiating adipocytes. (→)—induction; (—|)—inhibition; (+)—in cooperation with. Hh, Hedgehog; PPAR γ , peroxisomal proliferator activated receptor γ ; Shh, Sonic Hedgehog.

Osteoblasts and adipocytes are believed to originate from common precursors—MSCs. The shift in a differentiation potential of MSCs from osteoblastic to adipogenic lineage is due to a cooperation of various factors, which can support bone formation concomitantly with suppressing adipogenesis, and *vice versa* (1, 60, 202, 203). In such a situation, HO-1 might have an opposite effect on osteoblast and adipocyte differentiation during postnatal life. And indeed, it was reported that when HO-1 in MSCs induces osteoblastogenesis, it represses adipocyte development (14, 221).

Accordingly, *in vitro* studies have revealed that human (14, 91, 221) and murine (121) bone-marrow-derived MSCs cultured under adipogenic conditions differentiated into mature adipocytes more reluctantly when additionally stimulated with CoPPiX. This effect was reversed if HO-1 expression was inhibited by siRNA (221). Moreover, *in vivo* stimulation of

obese mice and rats with CoPPIX (27, 156) or stably genetic overexpression of HO-1 in mice fed a high fat diet (29) decreased the size and number of adipocytes. Again, the opposite effect was observed after SnMPIX stimulation (29).

Expression of HO-1 is lower in obese diabetic individuals as demonstrated in Zucker fatty rats (91), obese mice (121), and Zucker diabetic fatty rats (156). It can be also decreased in diabetic patients (157). HO-1 overexpression (29) or treatment with CoPPIX (91, 121, 156, 191) reduced subcutaneous and visceral *in vivo* fat deposits as well as decreased the elevated synthesis of lipids, what was reversed by pharmacological inhibition of HO-1 (121, 191). These effects may result from antioxidant properties of HO-1 (91, 121), as adipogenic activity of elevated H_2O_2 that activates c/EBP β , c/EBP α , and PPAR γ and promotes clonal expansion was recently proved in 3T3-L1 preadipocytes (113, 241) and fibroblasts (240). In muscle-derived SCs, ROS were necessary for induction of adipogenesis in hyperglycemia through activation of PKC (4). What is more, the antioxidative quercetin was able to reverse H_2O_2 -induced adipogenesis, concomitantly with an increase in HO-1 expression (240). Accordingly, activation of Nrf2 reduced adipogenic differentiation through decreasing the ROS levels and suppressing the c/EBP β , c/EBP α , and PPAR γ (184). Since Nrf2-dependant effects are not always mediated by HO-1, and among Nrf2 mediators there is also HIF-1 α (3, 247), the effect of Nrf2/HO-1/ROS axis on these transcription factors must be further experimentally proved. In addition, it must be kept in mind that mitochondrial ROS can also inhibit adipogenesis as they control expression of the adipogenic repressor CHOP-10/GADD153 (33).

HO-1 expression is downregulated during adipogenesis in parallel with increased expression of PPAR γ (14, 221, 222), fatty acid synthase (FAS), and c/EBP α (222) in human MSCs differentiating *in vitro*. Moreover, enforced overexpression of HO-1 *in vivo* led to inhibition of PPAR γ and c/EBP α in murine adipose tissue, the effect reversed by HO-1 activity inhibitor (29). The hindering role of HO-1 in adipogenesis has also been supported indirectly by the observation that epoxyeicosatrienoic acids (EETs), inhibitors of adipocyte differentiation, upregulate HO-1 in adipogenic cultures of human bone marrow-derived MSCs (93, 192, 222), probably due to the drop in expression of Bach1 (192, 222). Induction of HO-1 was accompanied by reduction of lipid content in mature adipocytes as well as by downregulation of FAS, c/EBP α , and PPAR γ in differentiating cells (93, 222). Decrease in lipid deposits was reversed by pharmacological inhibition of HO-1, proving that suppression of adipogenesis by EETs was HO-1 dependent (93).

Effects exerted by EETs were attributed to augmented phosphorylation of Akt (93). Since CoPPIX, the HO-1 activator, increased the activity of Akt in adipocytes of obese mice (27), it was postulated that HO-1 affects development of adipose tissue *in vivo* in rats through modulation of Akt-dependent pathways (192). However, as Akt kinase is rather regarded as a positive stimulator of adipogenesis (39, 55, 167), the mechanism of antiadipogenic effects of HO-1 involves probably also other pathways and needs further elucidation.

Another suggested mechanism might involve the AMPK activity (27, 192, 221). Phosphorylation of this kinase was elevated in rat adipose tissue upon CoPPIX treatment and suppressed when SnMPIX was applied *in vivo* (192). However, recently the pAMPK was demonstrated to induce osteo-

blastogenesis on the expanse of adipogenesis (94), the effect opposite to that exerted by HO-1 on adipocyte differentiation. On the other hand, HO-1 could inhibit differentiation of adipocytes *via* regulation of Wnt/ β -catenin pathway (Fig. 6). Its activation upregulates β -catenin, shh, and Wnt10b and downregulates Wnt5b in murine adipose tissue, with specificity confirmed through applying the HO-1 inhibitor (29). Worth mentioning, the HO-1-derived CO is able to decrease expression of c/EBP β and c/EBP δ transcriptional activities, in murine macrophages and C2C12 cells, respectively (103, 201), what could potentially inhibit transcription of adipogenic genes and mediate the antiadipogenic properties of HO-1. Finally, it should be kept in mind that apart from the reports that prove inhibition of adipogenic differentiation caused by HO-1 activity, there are a few studies that demonstrate that adenoviral HO-1 overexpression in MSCs (253) or HO-1 deficiency in MSCs isolated from HO-1^{-/-} mice (243) does not exert any effect on adipogenesis.

As it has been recently reported, HO-1 strongly regulates expression of miRNAs in C2C12 cell line and primary satellite cells (103). Since C2C12 cells are of mesenchymal origin, capable of adipogenic differentiation under proper conditions (5, 73, 79, 115), one can suspect that HO-1-dependent changes in miRNA levels may also be relevant to adipogenesis. Regulation of adipocyte maturation by miRNAs has been widely studied and modulation of such crucial pathways as PPAR γ , c/EBP α , and Wnt signaling has been reported (143, 175). Among miRNAs, the miR-200 family is regarded as an inducer of adipogenesis (Fig. 6). Thus, expression of miR-200a/b/c increases during *in vitro* adipogenic differentiation of murine stromal derived cells, leading to inhibition of Wnt signaling pathway (89). Interestingly, production of miR-200c was decreased in response to HO-1 overexpression in C2C12 cells (103) and, reversely, miR-200c decreased HO-1 in proximal tubular epithelial cells (195). Similar downregulation was found in case of miR-378 (103); another miRNA whose increased production, characteristic for adipogenesis, is responsible for elevated lipid synthesis in 3T3-L1 preadipocytes, *via* changes in c/EBP α and c/EBP β transcriptional activities (59). Therefore, HO-1 can be suggested to influence adipogenesis also through regulation of miR-200c and miR-378. Further, it was shown that knockdown of Dicer or Drosha, resulting in decreased miRNA biogenesis, inhibits *in vitro* adipogenic differentiation of human bone marrow-derived stromal cells (165). Although the effect of DGCR8 knockdown has not been examined yet, one may suppose that disturbed miRNA processing resulting from HO-1-dependent inhibition of DGCR8 (103) may be also one of possible pathways that affect maturation of adipocytes.

To sum up, HO-1 seems to decrease adipogenesis by regulating the c/EBP α , PPAR γ , or Wnt/ β -catenin activities, and possibly by miRNA-dependent mechanisms (Fig. 6).

Myogenesis and HO-1

mSCs represent a specific type of adult SCs, with small amount of cytoplasmic organelles and dense heterochromatin. They are considered as monopotent progenitor cells committed to skeletal muscle myocytes, which are indispensable for muscle regeneration during postnatal life (173). In an adult organism, under normal conditions, mSCs remain quiescent on a periphery of muscle fibers, but upon muscle damage they rapidly leave their niche to become activated

and they proliferate myoblasts, capable of differentiating and fusing into multinucleated myocytes. Within few days they form a large number of new muscle fibers or increase the size of already existing ones (12, 26, 35). Although mSCs have been supposed to differentiate also into adipocyte or osteocyte lineages (11), recent reports have not confirmed their multipotent SC characteristic (173). However, they indeed undergo the asymmetric divisions, when one daughter cell initiates differentiation while the other one self-renews and can robustly replenish quiescent cell reservoir, what is distinctive for SCs (46, 106).

Quiescent satellite cells are defined by the presence of Pax7 transcription factor, whose expression is critical for mSC development and, in consequence, for muscle growth or regeneration (173). Pax7 maintains mSCs in an undifferentiated state and its expression decreases upon differentiation of myoblasts. However, during their activation, Pax7 induces a group of key transcription factors (MyoD, myogenin, Myf5, and Myf6), so-called myogenic regulatory factors (MRFs).

Myf5 and MyoD are upregulated during activation and proliferation of mSCs. MyoD causes predominantly a cell cycle arrest and promotes a proliferation to differentiation (26, 35, 166). Since forced overexpression of MyoD in non-myogenic cells is able to convert them into fusing myoblasts (38), it is perceived as a master regulator of myogenic differentiation. Once expressed, MyoD initiates a cascade of myogenic commitment, driving the production of all MRFs. Together with myocyte enhancer factor 2 (MEF2), MyoD directly induces the expression of myogenin and Myf6 at terminal stages of differentiation. This is essential to stimulate fusion of cells into multinucleated myotubes, expressing the markers of mature muscles, such as myosin heavy chain (MHC), creatine phosphokinase (CPK), or desmin (26, 35, 107, 166).

Recently, MRFs together with MEF2 have been demonstrated to control transcription of so-called myomirs, namely,

miR-1, miR-133a, miR-133b, and miR-206, a group of miRNAs specific for skeletal or cardiac muscle. Their temporal expression in differentiating myoblasts fine tunes the output of MRFs and is necessary for proper muscle development (194, 212). Both miR-1 and miR-206 inhibit proliferation and promote differentiation of myoblasts *via* blocking the DNA polymerase II and downregulating the MRF inhibitors (37, 95). Their upregulation coincides with increased level of markers of differentiation (MyoD, myogenin, MHC, and CPK) and fusion of myoblasts (95). Although MRFs activate also expression of miR-133a and miR-133b, whose expression is elevated during myoblast differentiation in a similar manner as miR-1 and miR-206 (194, 212), there are inconsistent results regarding their role in this process. In some studies they were described as inhibitors of myoblast proliferation, although not as potent as miR-1 or miR-206 (95). In others they were reported to stimulate proliferation and inhibit differentiation, possibly through direct targeting the MyoD and MEF2 (37).

We have shown that HO-1 is a potent regulator of differentiation of muscle progenitors into myocytes and myotubes. This was surprising as in an earlier article lentiviral-mediated HO-1 overexpression was reported to have no effect on *in vivo* myoblast maturation of primary porcine myogenic cells (112). Possibly a short period of differentiation, analysis of marker connected with terminal differentiation only, and low transduction efficacy of HO-1 construct (112) could hinder the detection of potential influence. We found that either in immortalized C2C12 murine myoblast cell line retrovirally transduced for stable HO-1 overexpression or in primary mSCs isolated from mice of different HO-1 genotype (HO-1^{+/+}, HO-1^{+/-}, or HO-1^{-/-}), HO-1 potently inhibits a myogenic program (Fig. 7). Specifically, HO-1 led to increased proliferation of myoblasts, whereas myotube formation as well as expression of MyoD, myogenin, and MHC were strongly reduced. All these effects were reversible by SnPPiX

FIG. 7. HO-1 as an inhibitor of myogenesis. HO-1 decreases myogenic differentiation by reducing MRF *via* c/EBPδ or SDF-1α. HO-1 and SDF-1α abolish also expression of myomirs (miR-1, miR-133a, miR-133b, and miR-206) and miR-378, whereas induces miR-146a. HO-1 inhibits DGCR8 and lowers total miRNA biogenesis, but does not affect expression of MRFs or myomirs *via* this pathway. Black—effect exerted by HO-1; gray—known pathways of myogenesis; spots—mechanisms regulated by HO-1 in differentiating myoblasts. (→)—induction; (—|)—inhibition; (+)—in cooperation with. MRFs, myogenic regulatory factors.

or HO-1-specific siRNA. Moreover, HO-1-overexpressing C2C12 cells transplanted into *caput gastrocnemius* muscle of NOD-SCID mice formed tumors infiltrating healthy tissue and metastasizing to the lungs, whereas their control counterparts formed normal myofibers. Further analysis revealed that inhibition of differentiation is not connected to the reduced oxidative stress, but it depends on HO-1-derived CO, which inhibits nuclear translocation of c/EBP δ and decreases its binding to MYOD promoter, thereby blocking the MyoD transcription (103).

An additional pathway that mediates the inhibitory effect of HO-1 involves possibly the SDF-1 α , whose production was strongly upregulated in C2C12 cells in response to HO-1 activation (103). This chemokine is known to decrease formation of myotubes by myoblasts, the effect mediated by activation of atypical PKC ζ (163) and by regulation of MyoD expression (255). We found that stimulation of C2C12 cells with exogenous SDF-1 α mimicked the influence of HO-1 overexpression either on myotube formation or on generation of MRFs (103). Moreover, HO-1 activity decreased production of miR-1, miR-133a, miR-133b, and miR-206 in C2C12 cells through a MyoD-independent pathway. The importance of this mechanism has been illustrated by experiments where enforced overexpression of miR-133b or miR-206 in HO-1-overexpressing cells partially restored the differentiating potential of myoblasts. Worth mentioning, stimulation with SDF-1 α protein imitated the effects of HO-1 on myomir expression (103).

Apart from classical myomirs, HO-1 affects also the other miRNAs in differentiating muscle progenitors. Thus, production of miR-146a, which was proven to delay differentiation of myoblasts (108), was increased in C2C12 cells that overexpress HO-1 (103). On the other hand, miR-378, which negatively regulates expression of myogenic repressor of MyoD transcription (MyoR) (57), was decreased in response to HO-1 activity (103).

Interestingly, HO-1 reduces the expression of DGCR8, leading to inhibition of miRNA biogenesis in C2C12 cells and resulting in significantly lower total pools of pre-miRNA and mature miRNA (103) (Fig. 7). Enforced overexpression of DGCR8 in the HO-1-overexpressing C2C12 cells was enough to rescue the proper level of miRNA production, but did not influence differentiation of myoblasts or expression of MRFs. Accordingly, it has been reported that elimination of Dicer, another enzyme involved in miRNA processing, resulted in perturbed myogenesis, without affecting the MRFs (162). Nevertheless, despite the fact that HO-1 suppresses myogenic differentiation of myoblasts, its overall effect on skeletal muscle regeneration may also depend on the other activities, especially on enhancing the vascularization, as we have shown recently (80). Since a crosstalk between angiogenesis and myogenesis is crucial for regeneration processes (151), and HO-1 exerts proangiogenic properties (133), a decline in differentiation of myoblasts can be overwhelmed by increased exogenous signals from HO-1-potentiated blood vessel formation (80).

To conclude, HO-1 inhibits differentiation of myogenic progenitors by decreasing production of myomirs and almost complete blocking the c/EBP δ -dependent expression of MyoD. CO and SDF-1 α are mediators of the observed effects, whereas total miRNA biogenesis is affected due to inhibition of DGCR8 (Fig. 7).

Miscellaneous

Involvement of HO-1 in other postnatal developmental processes has also been postulated, although not extensively characterized.

Chondrogenesis

Although HO-1 has not been examined in the context of chondrogenic differentiation of MSCs, the role of Nrf2 has been clearly demonstrated. During embryonic development, expression of Nrf2 is restricted to the proliferating and prehypertrophic chondrocytes, in contrast to the terminally differentiated cells, secreting collagen X and osteopontin (72). When prechondrogenic cell line was engineered to overexpress Nrf2, a decrease in differentiation potential was observed, evident by a declined expression of terminal chondrogenic markers (72). The underlying mechanism has not been identified, but one can suspect a role for HO-1, despite it is not the only mediator of Nrf2 action. Especially, that HO-1 is known to influence the chondrocyte viability (144) or secretion of growth factors (65), and has already been reported to counteract chondrocyte senescence induced by IL-1 β (41, 43).

Endothelial progenitor cells

HO-1 plays an important role in functioning of EPCs, which originate from bone-marrow-derived SCs and are suggested to postnatally develop into mature endothelium (52). Nevertheless, recently the origin and nature of EPCs has become a matter of intense debate. Their differentiation capacity is being questioned and more data indicate for rather paracrine effect of those cells, named currently proangiogenic cells (239). Nevertheless, it has been convincingly demonstrated that HO-1 improved re-endothelialization both after pharmacological (16, 232) or genetic (129) induction, the process in which EPCs could play a role. HO-1 induction by probucol and succinobucol increased both the bone-marrow-located and the released EPCs (232), which formed also more colonies, while differentiation of EPCs from knockout mice was impaired (232). Likewise, lack of HO-1 leads to reduced formation of capillaries both *in vitro* and *in vivo*. These HO-1-related properties were, however, attributed to increased migration, viability, and proliferation of EPCs, as well as elevated proangiogenic cytokine secretion (52) (Grochot-Przeczek *et al.*, unpublished data). However, an impaired differentiation of EPCs deficient in HO-1 has been observed in the *in vitro* assays (Deshane *et al.*, unpublished data) (232).

So far, the role of HO-1 in regulation of EPC differentiation through effect on angiomirs (the miRNAs involved in blood vessel formation) has not been investigated. Nevertheless, the fact that HO-1 decreases the expression of miR-378 (189) can be considered in that context. Further studies are necessary, however, to elucidate the potential interactions, which might be further complicated by the problematic characteristic of cells defined as EPCs (239).

Myofibroblasts

Decrease in Nrf2 was associated with enhanced myofibroblast phenotype in idiopathic pulmonary fibrosis, while Nrf2 activation increased myofibroblastic dedifferentiation (9). Also, HO-1 was shown to reduce the rate of fibrosis in hepatic myofibroblasts (120). Likewise, the Nrf2/HO-1 axis

appears to play a protective role against renal fibrosis by inhibiting the epithelial-mesenchymal transition into myofibroblasts (90, 185). For example, activation of Nrf2 or HO-1 counteracted the profibrotic effects of ochratoxin A in renal proximal tubular cells. This effect was related to changes in miRNA levels (195).

Neurogenesis

Despite the fact that neuron formation is most prominent during prenatal life, recent studies have demonstrated its occurrence in several parts of fully developed mammalian brain. Neurogenesis in adulthood is accomplished, in a very limited range, by neural progenitor or SCs (48). It was suggested that ROS can directly influence the neuronal SC commitment promoting the switch from neurogenic to gliogenic lineage (181).

Nrf2 has already been demonstrated to augment the differentiation of neuroblastoma cell line into a neural lineage, what was revealed by increase in number of mature neuronal cells that express terminal markers, such as microtubule-associated protein 2 (248). This effect was probably mediated by ROS (181). Indeed, treatment with antioxidants reduced generation of ROS, decreased nuclear translocation of Nrf2, and halted a shift in differentiation of neural SCs toward gliogenic lineage on expense of neurogenesis (181). Interestingly, HO-1 was downregulated in MSCs during neural differentiation (13). Also in cultured astroglial cells, the relatively high expression of HO-1 gradually decreased in parallel with their terminal maturation (124). These observations may suggest a role for HO-1 in regulation of neurogenesis. Although its functions in nervous system have been extensively examined, the beneficial effects of HO-1 activity were mainly attributed to the enhanced cell survival, proliferation, and migration, or to inhibited inflammatory response (183). However, understanding a potential direct role of HO-1 in neural differentiation needs further research.

Cardiogenesis

Until recently, like a brain, the mammalian heart was regarded as a fully differentiated organ, with no regenerative capacity. It has been shown however, that cardiomyocytes can slowly divide and, even more important, the heart contains a resident progenitor cell population, what supports a possibility of myocardial renewal in adults (138).

Taking into account an important role of HO-1 in skeletal myogenesis (103), similar effects could be also suspected in cardiac myogenesis. This issue remains, however, mostly unaddressed. So far, it was found that HO-1-derived CO does enhance cardiac regeneration by facilitating the mobilization of cardiac progenitor cells, and improving their migration, proliferation, or viability (111). Similarly, induction of HO-1 in the transplanted MSCs augmented their regenerative potential in the infarcted or ischemic myocardium. These benefits were attributed to better cell viability or improved balance between production of proangiogenic growth factors and proinflammatory cytokines (82, 186, 205, 216, 246), without assessing the rate of MSC differentiation into functional cardiomyocytes.

Noteworthy, recent study has demonstrated that lentiviral overexpression of HO-1 in MSCs transplanted into the infarcted myocardium significantly increased the expression of cardiomyogenic transcription factors, suggesting that HO-1

can promote the maturation of cardiomyocytes (88). Accordingly, improved differentiation of cardiac SCs was observed after stimulation of Nrf2 expression (61, 66). However, further experiments have to be conducted to elucidate a precise role of HO-1 in cardiomyocyte development and to distinguish it from the effects on cell viability or proliferation.

Conclusions and Future Perspectives

Over the last decade the novel role of HO-1 as a regulator of differentiation was demonstrated in diverse tissues (Fig. 8). It became clear that HO-1 influences major developmental processes, like hematopoietis, osteogenesis, myogenesis, or adipogenesis, and is perhaps important for maintaining pluripotency. Additionally, HO-1 can be involved in regulating the differentiation of chondrocytes, myofibroblasts, neurons, and cardiomyocytes.

The variety of tissues, where HO-1-induced effects have been observed, support a hypothesis that its role in differentiation of stem and progenitor cells may be meaningful in embryonic development and in regeneration of adult organs. However, vast majority of experiments have been conducted *in vitro* so far, with pharmacological regulators of HO-1 activity. This can always be associated with a risk of unspecific results. Thus, such studies should be verified by use of genetic silencing of HO-1, experiments on primary cells isolated from mice of different HO-1 genotype, and finally *in vivo*, in proper animal models.

The mechanism that mediates the observed effects is tissue specific and has been elucidated only in some cell types. They can be associated with influencing the oxidative status and

FIG. 8. Summary of HO-1-related miRNAs in differentiation of various cell types. miRNAs that mediate observed effects (black) or suggested to play a role in HO-1-regulated differentiation (gray) are shown.

ROS generation or, instead, they can be mediated by products of HO-1 enzymatic activity, with CO emerging as the most meaningful effector. Importantly, recent studies indicate that HO-1 may act through modification of miRNA processing efficacy and through regulation of specific miRNA expression. This opens a new possibility to understand the role of HO-1 in cell biology and elucidate why effects of HO-1 are strictly dependent on the cell type.

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Abbreviations Used

AML = acute myeloid leukemia
ARE = antioxidant responsive element
ATF4 = activating transcription factor 4
BMP2 = bone morphogenic protein 2
c/EBP $\alpha/\beta/\delta$ = CCAAT/enhancer binding protein $\alpha/\beta/\delta$
cAMP = cyclic AMP
CLP = common lymphoid progenitor
CML = chronic myeloid leukemia
CMP = common myeloid progenitor
CoPPiX = cobalt protoporphyrin IX
CPK = creatine phosphokinase
CREBP = cAMP response element binding protein
EETs = epoxyeicosatrienoic acids
EPC = endothelial progenitor cell
ESC = embryonic stem cell
FAS = fatty acid synthase
GMP = granulocyte–macrophage progenitor
Hh = Hedgehog
HMGB1 = high mobility group box 1
HO-1 = hemeoxygenase-1
HSC = hematopoietic stem cell
HSPC = hematopoietic stem and progenitor cell
IGF = insulin-like growth factor
IL-1 β = interleukin-1 β
iPS cells = induced pluripotent stem cells
M-CSF = macrophage colony-stimulated factor
MEF2 = myocyte enhancer factor 2
MEP = megakaryocyte–erythroid progenitor
MHC = myosin heavy chain
miRNA = microRNA
MPP = multipotent progenitor
MRF = myogenic regulatory factor
MSC = mesenchymal stem cell
mSC = muscle satellite cell
PGI $_2$ = prostaglandin- I_2
PPAR γ = peroxisomal proliferator activated receptor γ
RAGE = receptors for advanced glycation endproducts
RANK = receptor activator for NF κ B
RANKL = receptor activator for NF κ B ligand
ROS = reactive oxygen species
Runx2 = runt-related transcription factor 2
SC = stem cell
Shh = Sonic Hedgehog
SnPPiX/SnMPIX = tin protoporphyrin IX/tin mesoporphyrin IX
SREBP-1 = sterol-regulatory-element-binding protein 1
tBHQ = tert-butylhydroquinone
TNF α = tumor necrosis factor α
TRAF6 = TNF α receptor associated factor 6
TRAP = tartrate resistant acid phosphatase
TRBP = TAR-RNA binding protein
ZnPPiX = zinc protoporphyrin IX