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New Insights into Intracellular Locations and Functions of Heme Oxygenase-1

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Abstract

Significance: Heme oxygenase-1 (HMOX1) plays a critical role in the protection of cells, and the inducible enzyme is implicated in a spectrum of human diseases. The increasing prevalence of cardiovascular and metabolic morbidities, for which current treatment approaches are not optimal, emphasizes the necessity to better understand key players such as HMOX1 that may be therapeutic targets. ***Recent Advances:*** HMOX1 is a dynamic protein that can undergo post-translational and structural modifications which modulate HMOX1 function. Moreover, trafficking from the endoplasmic reticulum to other cellular compartments, including the nucleus, highlights that HMOX1 may play roles other than the catabolism of heme. ***Critical Issues:*** The ability of HMOX1 to be induced by a variety of stressors, in an equally wide variety of tissues and cell types, represents an obstacle for the therapeutic exploitation of the enzyme. Any capacity to modulate HMOX1 in cardiovascular and metabolic diseases should be tempered with an appreciation that HMOX1 may have an impact on cancer. Moreover, the potential for heme catabolism end products, such as carbon monoxide, to amplify the HMOX1 stress response should be considered. ***Future Directions:*** A more complete understanding of HMOX1 modifications and the properties that they impart is necessary. Delineating these parameters will provide a clearer picture of the opportunities to modulate HMOX1 in human disease. *Antioxid. Redox Signal.* 20: 1723–1742.

Introduction

HEME OXYGENASES (HMOX) are rate-limiting enzymes that degrade heme (iron protoporphyrin IX) to carbon monoxide (CO), ferrous iron (Fe^{2+}), and biliverdin IXα. Biliverdin IXα is, subsequently, converted to bilirubin IXα by biliverdin reductase (BVR). HMOX enzymatic activity consumes three moles of molecular oxygen (O_2) per mole heme oxidized with electrons originating from NADPH and supplied by cytochrome P450 reductase (CPR) (164). The catabolism of heme is schematically represented in Figure 1. Notably, HMOX use heme as both a substrate and a prosthetic group (195). As HMOX degrade heme, the major source of iron in our body, they play a key role in whole body iron recycling/homeostasis. In addition, HMOX are implicated in vascular biology and cellular protection against stress (155). More recently, HMOX has been reported to activate the transcriptional machinery that drives the induction of antioxidant genes (27, 101), likely in part, independent of its enzymatic activity (27, 65). The convergence of these different properties stresses the importance of HMOX as a key agent that protects the cell.

The HMOX family is represented by two distinct enzymes: heme oxygenase-1 (HMOX1) and heme oxygenase-2 (HMOX2). Human HMOX1 and HMOX2 are paralogs, sharing ~42% similarity in their amino-acid sequences (29). Both proteins possess a common 24-amino-acid sequence known as the ''heme-binding pocket'' or ''HMOX signature'' that facilitates the catabolism of heme (110). While both proteins utilize the same substrate and cofactor, they are different in their physiological properties and regulation. For example, HMOX1 is induced in response to a variety of external stimuli, while HMOX2 is ubiquitously expressed.

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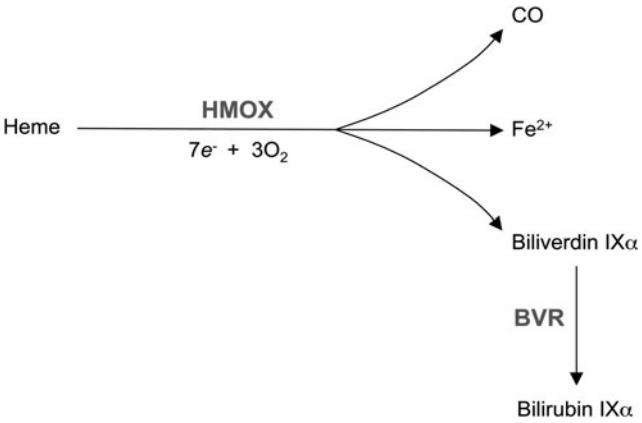

FIG. 1. Pathway of heme catabolism. HMOX enzymes catalyze the initial step in heme catabolism. HMOX oxidizes heme (Fe protoporphyrin IX) to biliverdin IXα. This reaction consumes three molecules of molecular oxygen (O_2) and seven electrons donated from NADPH by CPR, and it produces ferrous iron (Fe^{2+}), CO, and biliverdin IXα as the products. Biliverdin IXα is then reduced to bilirubin IXα by BVR. BVR, biliverdin reductase; CO, carbon monoxide; CPR, cytochrome P450 reductase; HMOX, heme oxygenase.

HMOX1 is a 32 kDa protein that is anchored to the endoplasmic reticulum (ER) by a single hydrophobic transmembrane segment (TMS) in the *C*-terminus (146, 196). HMOX1 colocalizes with CPR, and their interaction is required for maximal HMOX enzymatic activity (69, 103). In addition, HMOX1 has been demonstrated to localize to other organelles, including caveolae, mitochondria, and the nucleus (53, 83, 152), raising the possibility that HMOX1 may play a role in addition to heme degradation.

HMOX1 is typically expressed in mononuclear phagocytes of the spleen, liver, and bone marrow (164), although its expression and activity has been detected across almost all tissues assessed to date. HMOX1 is strongly induced by a number of chemical and physical stresses, including heat shock, heme and hemin, cytokines, lipopolysaccharide (LPS), growth factors, oxidative stress and hydrogen peroxide (H_2O_2), hypoxia, CO, and Fe starvation [reviewed in ref. (135)].

HMOX2 has a molecular mass of 36 kDa and is expressed ubiquitously, with particularly high levels in the brain (156). Unlike HMOX1, HMOX2 contains heme regulatory motifs that act as a thiol/disulfide redox switch regulating the K_d for heme (193). To date, only corticoids are known to induce HMOX2 (110). HMOX2 is implicated in oxygen sensing and the regulation of the vascular tone of at least some vascular beds (183). CO acts as a vasodilator in peripheral vessels, whereas CO derived from HMOX2 acts as a vasoconstrictor in the cerebral circulation by preventing cystathionine β-synthase and forming the vasodilator hydrogen sulfide (117).

This review will focus on HMOX1, elaborating on the protein chemistry, subcellular localization, and therapeutic utility of this enzyme in cardiovascular diseases and diabetes mellitus.

Post-Translational and Structural Modifications of HMOX1

Little is known about the potential regulation of HMOX1 by post-translational modifications, although there is increasing appreciation that structural modifications, for example, truncation, underpin non-canonical functions of HMOX1 (27, 101). An *in silico* analysis of the human HMOX1 protein predicts a number of potential sites for post-translational modifications. These and those determined by the mining of mass spectrometry data and/or experimentally confirmed by *in vitro* or *in vivo* experiments are listed in Figure 2. Care needs to be taken, however, when interpreting data solely based on *in silico* analyses until these observations are confirmed. In the next section, we discuss the current knowledge of post-translational and structural modifications of HMOX1.

Phosphorylation

Phosphorylation, the addition of a phosphate group onto highly conserved, specific tyrosine, serine, or threonine residues, is a well-recognized post-translational modification. HMOX1 contains a strong consensus sequence for serine/threonine phosphorylation by the protein kinase, Akt. Akt phosphorylates recombinant human HMOX1 at serine 188 as determined by studies in the human embryonic kidney cell line, HEK293T (137). Phosphorylation at S188 leads to a modest increase in HMOX activity when compared with the non-phosphorylated HMOX1 protein. Fluorescence resonance energy transfer experiments demonstrated that a serine to asparagine point mutation at residue 188 in HMOX1 resulted in a lower K_d for the interaction between CPR and BVR than that observed for wild-type HMOX1. This suggests that the negative charge produced by phosphorylation at S188 increases the affinity of HMOX1 for these proteins. Therefore, any increase in Akt activity, as is observed in response to a range of stimuli (135), could conceivably lead to an increase in HMOX activity.

More recently, HMOX1 phosphorylation at serine/threonine residues was detected in human brain samples (8, 19). It was reported that basal HMOX activity was significantly inhibited by brief treatment of neuron/glia cell cultures with inhibitors of the MEK and ERK signaling pathways (19). While there are several potential ERK phosphorylation sites on HMOX1, many of these are not conserved among divergent species (Fig. 2). Furthermore, there is no evidence that ERK directly phosphorylates HMOX1. Interestingly, and similar to BVR, HMOX1 has a conserved docking site/motif for ERK FXF (DEF motif) that is important for BVR to form a complex with MEK/ERK (95). On activation, MEK is released from ERK and the BVR/ERK complex enters the nucleus. Thus, it appears possible that the DEF motif in HMOX1 may act in a manner similar to that reported for BVR and be used as one mechanism to shuttle HMOX1 into the nucleus.

Palmitoylation

Palmitate is a 16-carbon saturated fatty acid that can be covalently attached to a number of eukaryotic proteins. There is no clear consensus sequence motif for palmitoylation, with the modification occurring at any one or more cysteine residues through a thioester linkage. The thioester bond is cleaved readily, enabling palmitoylation to play a significant role in cell signaling, subcellular trafficking, and protein–protein interactions. With regard to palmitoylation of HMOX1, only the murine and chicken proteins contain a cysteine residue (Fig. 2), suggesting that HMOX1 in these

FIG. 2. Potential post-translational modifications of HMOX1. Human, mouse, rat, and chicken HMOX1 protein sequences were aligned using COBALT:Multiple Alignment Tool (http://ncbi.nlm.nih.gov/tools/cobalt). Modifications reported in the literature and/or elucidated from mass spectrometry data mining are indicated as follows: green shading = acetylation, red box = ubiquitination, orange shading = palmitoylation, blue shading = phosphorylation, and gray shading = orthologous residues. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

species could be palmitoylated. Indeed, murine HMOX1 was found to be palmitoylated in the murine B16 melanoma cell line (107). It is not clear under what circumstances murine HMOX1 may be palmitoylated *in vivo*, and there is no indication as to whether palmitoylation affects HMOX activity. Surprisingly, despite a lack of cysteine residues, human HMOX1 has been reported to be palmitoylated in platelets (39), although to date, the effect of such palmitoylation on HMOX1 function remains unknown.

Acetylation

Acetylation of lysine residues is a reversible modification that plays an essential role in regulating gene expression. Lysine acetylation has also been shown to be important for cell cycle, nuclear transport, and chromatin remodeling. When assessing the residues that are important for the interaction between rat HMOX1 and CPR, nine (*i.e.*, K18, K22, K39, K48, K69, K149, K153, K179, and K196) out of a total of 15 lysine residues were identified by mass spectrometry (MALDI-TOF) as acetylated (61). Interestingly, K149 and K153 were protected from acetylation in the presence of CPR. However, in the absence of CPR, rat HMOX1 was acetylated at all nine lysine residues, and this led to a reduction in HMOX activity. These data suggest that at least in rats, CPR may modulate HMOX activity *via* inhibition of K149 and K153 acetylation. HMOX1 was also acetylated at K39 in human cancer cell lines (24) and at K18 in human liver tissue (199). The significance of acetylation of human HMOX1 at these residues remains unclear. In the instance of the K18 or K39, acetylation may confer nuclear localization to HMOX1, which may be associated with changes in gene transcription (27, 101). Indeed, acetylation of other transcription factors has been shown to promote gene transcription; for example, acetylation of Nrf2 at K588 and K591 facilitates binding to the *HMOX1* promoter and induces gene transcription *in vitro* (81).

Ubiquitination

Degradation of proteins by the ubiquitin-proteasome pathway enables cells to respond to a changing environment. Signal-dependent ubiquitination frequently results in the complete degradation of the targeted protein. HMOX1 protein turnover occurs *via* the ubiquitin proteasome system in the ER-associated membrane and has been shown for vascular smooth muscle cells (SMCs), HEK293 cells (100), and rat adrenal pheochromocytoma PC12 cells (189). In HEK293T cells, ER-resident E3 ubiquitin ligase is responsible for HMOX1 degradation *via* an interaction with the TMS region (100). However, it remains unclear how truncated HMOX1 protein in which the TMS sequence has been lost is turned over. Interestingly, treatment of the human colon adenocarcinoma cell line, HCT116, with the proteasome inhibitor bortezomib resulted in K39, K69, K86, K148, K153, and K243 residues of HMOX1 becoming ubiquitinated; while treatment with the proteasome inhibitor epoxomicin led to ubiquitination of K179 and K256 (85). These data suggest that not all ubiquitination events are regulated identically.

Dimerization and oligomerization

A lack of cysteine residues in HMOX1 led to the assumption that HMOX1 acts as a monomer. However, Hwang *et al.* demonstrated that in the ER of HEK293 cells, HMOX1 gives

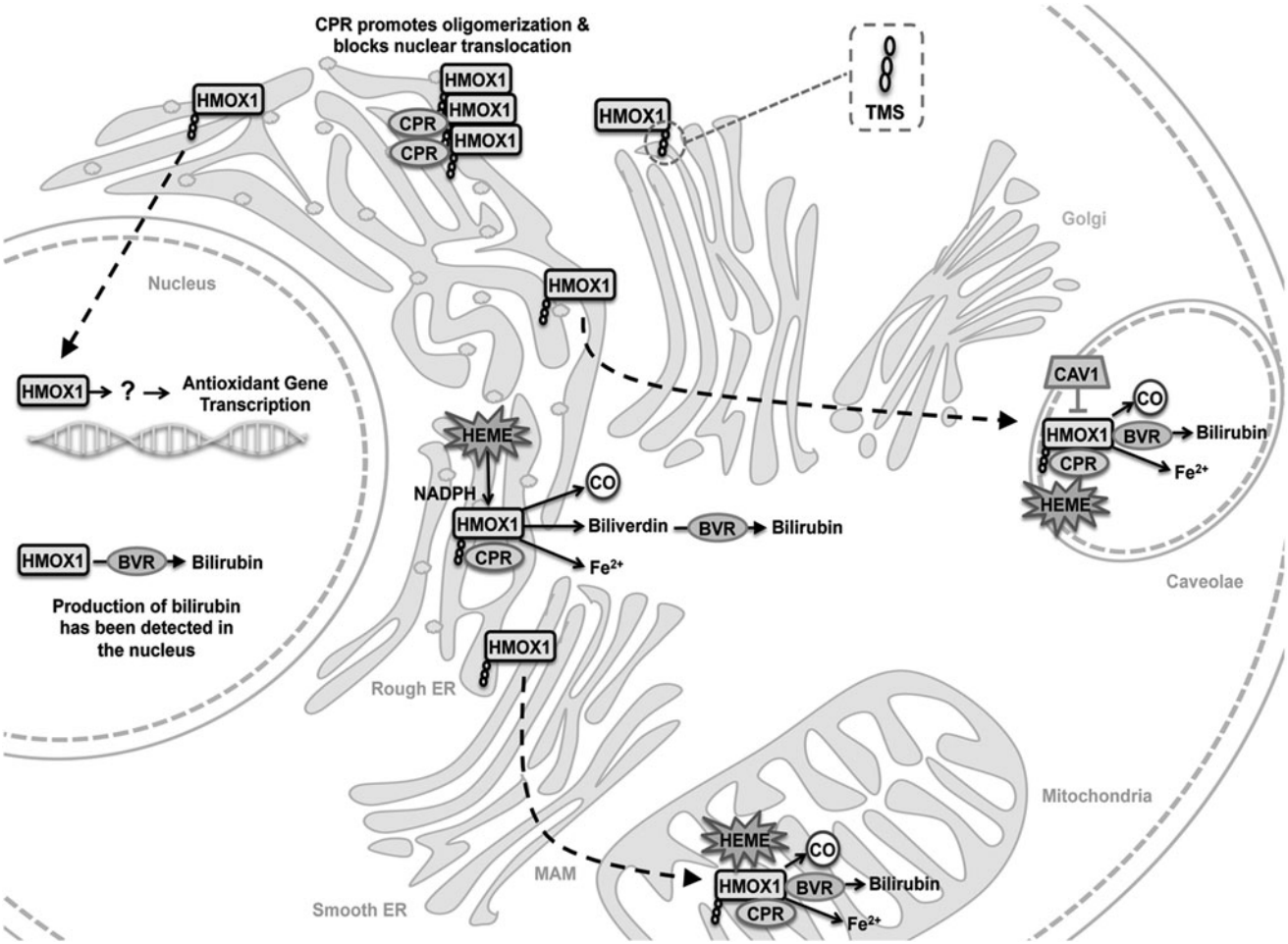

FIG. 3. Subcellular localization of HMOX1. HMOX1 localizes to different subcellular compartments. HMOX1 is tethered to the ER membrane by a trans-membrane sequence (TMS). CPR colocalizes with HMOX1 on the ER to facilitate heme degradation to CO, Fe^{2+}, and biliverdin. Biliverdin is then converted to bilirubin by BVR. HMOX1 may traverse the ER and MAM compartments to the mitochondria. In the mitochondria, HMOX1 is anchored to the inner mitochondrial membrane, where it may detoxify mitochondrial heme. HMOX1 may be transported to caveolae and the plasma membrane through the ER and Golgi apparatus, where it similarly detoxifies heme. The activity of HMOX1 in caveolae may be modulated by CAV1 that binds to and decreases HMOX activity. CPR can promote oligomerization of HMOX1 to increase its stability and enzymatic activity and to prevent nuclear translocation. Cleavage of the TMS enables truncated HMOX1 to enter the nucleus, where it can induce the transcription of antioxidant response genes. CAV1, caveolin-1; ER, endoplasmic reticulum; MAM, mitochondrial membrane associated; TMS, transmembrane segment.

rise to dimers and oligomers, the formation of which were essential for HMOX activity (70). Using mutant HMOX1 constructs in fluorescence resonance energy transfer and co-immunoprecipitation experiments, the TMS region was shown to be the interface for protein–protein interaction. HMOX1 dimers have also been observed in preparations of lipid vesicles (112). The TMS region is also responsible for binding CPR, which maximizes the catalytic activity of HMOX1 (69). Moreover, in HEK293 cells, CPR promotes HMOX1 oligomerization, which can prevent hypoxia-induced translocation of HMOX1 to the nucleus (103).

Truncation

Microsomal full-length HMOX1 (32 kDa) is trypsinized easily, resulting in a water-soluble truncated protein that typically lacks a 23–55-amino-acid hydrophobic *C*-terminal TMS (194). Purified recombinant full-length HMOX1 can also be cleaved by thrombin (69). In these and other studies, protein cleavage has given rise to at least three types of truncated HMOX1, including 27, 28, and 30 kDa isoforms. The 23-amino-acid truncated HMOX1 ($HMOX1_{\Delta 23}$) has been crystallized, and this led to the identification of histidine 25 within the proximal alpha helix as the ligand for heme Fe (144). Truncated forms of HMOX1 have been reported to localize to the nucleus (see next), with cysteine proteases having been implicated in proteolytic cleavage (101). While the TMS of HMOX1 enhances its interaction with CPR and BVR (see earlier), there is convincing evidence that HMOX1 lacking the *C*-terminus retains catalytic activity. Thus, earlier studies established unambiguously that isolated purified human HMOX1 with approximately 67 of its *C*-terminus amino-acid residues deleted retains ~50% of the enzymatic activity of full-length HMOX1 when purified CPR is supplied as the source of electrons [see, *e.g.*, refs. (143, 144, 181)]. In addition to CPR, ascorbate is an established alternative source of

electrons for truncated HMOX1, yielding biliverdin IXα as the stereospecific product (168, 177).

Subcellular Localization of HMOX1

Since its discovery on microsomes (163), the location of HMOX1 has been assigned traditionally to the ER. More recently, however, HMOX1 has been reported to be present in other cellular compartments, including caveolae, mitochondria, and the nucleus (Fig. 3). In the ER, caveolae and mitochondria HMOX1 appear to be anchored by the TMS and to co-localize with CPR and BVR, suggesting heme degradation as its primary role. However, we are unaware of evidence for the presence of CPR in the nucleus, where truncated HMOX1 likely regulates gene transcription. In this section, we will review the localization of full-length and truncated HMOX1 within different cellular compartments.

Endoplasmic reticulum

The ER is the site of protein, lipid, and carbohydrate synthesis, and for the packaging of these molecules into vesicles for delivery-to-end organelles. The ER is also important for the regulation of cellular calcium, glycosylation, insertion of integral membrane proteins, disulfide bond formation and protein folding, and drug metabolism. Disturbances in redox regulation, glucose deprivation, cellular calcium, and viral infections can lead to ER stress and the unfolded protein response. Interestingly, HMOX1 and CO play integral roles in many disease processes in which ER stress is implicated. These include cardiovascular and metabolic diseases (84, 104). As previously discussed, HMOX1 is anchored to the ER *via* a hydrophobic *C*-terminus TMS (146). It has also been ascertained that the orientation of ER-bound HMOX1 is toward the cytosol (56). Within the ER or in response to stress such as hypoxia, the TMS may be cleaved, resulting in truncated HMOX1 that may then translocate to the cytoplasm and the nucleus (70, 101).

Caveolae

At the plasma membrane, there are specialized microdomains that are enriched in cholesterol and glycosphingolipids, and these are known as lipid rafts and caveloae. Cell signaling proteins localize to these lipid-rich areas for vesicular transport and rapid induction of signaling cascades in response to external stimuli. Caveolins (CAVs) are membrane proteins that are predominantly found in caveolae. CAVs form scaffolds on which signaling molecules can assemble, thereby facilitating rapid cell signaling responses.

One of the first reports of non-ER compartmentalization of HMOX1 was in the caveolae of rat pulmonary artery endothelial cells (ECs) (83). When treated with LPS, heme, or hypoxia, a proportion (25–40%) of HMOX1 protein was found to localize to a detergent resistant fraction that contained CAV-1. Co-immunoprecipitation studies confirmed that HMOX1 directly interacted with CAV1 to reduce HMOX activity (83), as was previously seen with endothelial nitric oxide synthase (48). When cells were depleted of cholesterol, HMOX1 disappeared from the detergent-resistant fraction. Moreover, as CAV1 was decreased, HMOX activity increased. Similarly, LPS treatment of isolated murine peritoneal macrophages resulted in HMOX1 translocation to caveolae, while exogenous CO enhanced the binding of CAV1 to toll-like receptor 4 and inhibited pro-inflammatory signaling (179). Cadmium has also been shown to cause HMOX1 and CAV1 association in mouse mesangial cells (78), and it is now appreciated that CAV1 is involved in the regulation of HMOX activity (159).

Mitochondria

Mitochondria are the energy powerhouses of the cell, producing energy in the form of ATP. In addition, mitochondria play key roles in signaling, apoptosis, cell survival, cellular growth, and heme synthesis. As such, "mitochondrial dysfunction" is implicated in many aspects of cardiovascular diseases, diabetes complications, and aging.

Mitochondrial HMOX activity was initially reported in the southern multimammate mouse (*Mastomys coucha*) (152), although that study did not discriminate between HMOX1 and HMOX2 activity. More recently, treatment of rat pulmonary EC with heme or LPS has been reported to result in a proportion of HMOX1 protein becoming associated with cell fractions containing cytochrome *c* (83). In fact, HMOX1 appears to be constitutively expressed in mitochondria of rat liver, where it colocalizes with BVR in the inner mitochondrial membrane (28). This suggests that mitochondrial HMOX1 may play a role in the detoxification of mitochondrial heme (see next).

Nucleus

Critical functions that occur within the nucleus include regulation of gene transcription and cell cycle progression. HMOX1 has been detected in the nucleus of various cell types, including brown adipose tissue (53), prostate cancer cells (136), astroglial cells (97), EC (83), squamous cell carcinoma, (51), Hepa, NIH3T3 cell lines (101), dendritic cells (52), and cerebral cortex tissue (117). Our laboratory has shown that the yeast homolog *Hmx1* may translocate to the nucleus and regulate gene transcription, although the possibility of ER-associated *Hmx1* contamination has not yet been ruled out (27). However, we have shown that hemin treatment leads to nuclear translocation in two mammalian cell types, namely human Jurkat T-cell lymphoma cells (Fig. 4) and rat aortic SMC (unpublished data).

Heme and hypoxia have also been shown to cause translocation of truncated HMOX1 to the nuclei (101). In that study, it was demonstrated that loss of the TMS was required for nuclear translocation. As noted earlier, truncation decreases (but does not eliminate) CPR-dependent activity of HMOX1. Indeed, overexpression of human $HMOX1_{\Delta 24}$ in HEK293T cells was reported to significantly lower HMOX activity compared with cells transfected with full-length HMOX1 (1.2-fold increase in HMOX activity for $HMOX1_{\Delta 24}$ truncated protein compared with 6.0-fold increase for full-length HMOX1) (70). As mentioned earlier, this could be due to truncated HMOX1 being located primarily in the nucleus where CPR was absent (101, 103, 182).

Proposed Modes of Action of HMOX1 at Different Cellular Locations

The primary role of HMOX1 across all species is undoubtedly the degradation of heme, which has led to the retention of

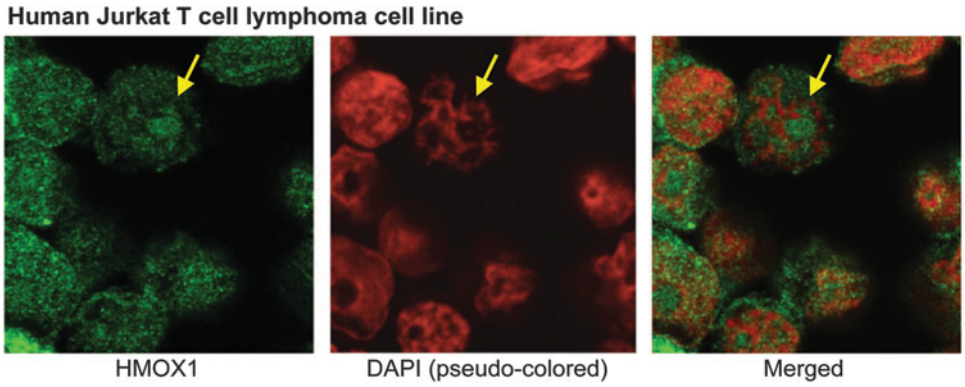

FIG. 4. Nuclear localization of HMOX1. Human Jurkat T-cell lymphoma cells were treated with 30 μM hemin for 24 h; the nuclei were isolated by fractionation and stained for HMOX1 (SPA-895, Stressgen and AlexaFluor 488; Life Technologies) and DAPI. Fluorescent photomicrographs were taken using a Leica SPEII confocal microscope. HMOX1 staining is shown in green. DAPI images were pseudo colored from blue to red, and HMOX1 and pseudo-colored DAPI images were merged using Leica LAS AF Lite software. Yellow *arrows* indicate nuclei. Magnification = 100×. DAPI, 4′,6-diamidino-2-phenylindole. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

the HMOX genes throughout evolution. However, variations in the HMOX1 protein, post-translational modifications, and subcellular localization indicate an expanded repertoire of function (Fig. 3). Recent studies also indicate that HMOX1 shuttles between subcellular compartments, as summarized in Table 1. Therefore, it may be appropriate to consider HMOX1 as a "dynamic" protein.

The association of HMOX1 with the ER and its orientation toward the cytosol (56) is not random. Rather, it is an efficient way to deal with the redox-active Fe^{2+} generated as a result of heme degradation. Free iron derived from heme commonly induces expression of the heavy chain of ferritin (43) and the Fe^{2+} exporter ferroportin (33). Together, ferritin and ferroportin efficiently sequester and remove redox-active Fe^{2+}, thereby minimizing Fenton chemistry-induced oxidative damage. Moreover, colocalization of BVR and CPR with HMOX1 ensures efficient conversion of biliverdin to bilirubin. In caveolae, the proximity of HMOX1, BVR, and CPR (83) as well as ferroportin would be expected to increase the efficiency of heme degradation and Fe export.

It has been shown that in isolated murine peritoneal macrophages, the LPS-induced translocation of HMOX1 from the ER to the caveolae proceeds by a p38 MAPK-dependent mechanism (179). In addition, this translocation was blocked

Table 1. Translocation of Heme Oxygenase-1 to Non-Endoplasmic Reticulum Compartments

Treatment	*Cell type*	*Compartments*	*References*
15-deoxy-$\Delta^{12,14}$-prostaglandin J2	Murine cortical neurons	Cytoplasm, microsomes	(86)
Cigarette smoke extract	A549 aveolar cell line	Mitochondria, cytoplasm	(147)
	Beas-2b bronchial epithelial cell line	Mitochondria, cytoplasm	(147)
Co-PP IX	Rat renal cells	Mitochondria	(172)
Heme	NIH3T3 fibroblast cell line	Nucleus	(101)
Hemin	Head and neck squamous cell carcinomas	Nucleus	(51)
	A549 alveolar cell line	Mitochondria, cytoplasm	(147)
	Beas-2b bronchial epithelial cell line	Mitochondria, cytoplasm	(147)
	PC3 prostate cancer cell line	Nucleus, cytoplasm	(136)
	LnCAP prostate cancer cell line	Nucleus, cytoplasm	(136)
	Rat liver	Mitochondria, microsomes	(28)
Hypoxia	NIH3T3 fibroblast cell line	Nuclear	(101)
	HEK293T embryonic kidney cell line	Nuclear	(103)
	Rat pulmonary artery endothelial cells	Caveolae	(83)
Indomethacin	Rat gastric mucosa	Mitochondria	(10)
LPS	Murine bone marrow derived macrophages	Cytoplasm	(34)
	Murine peritoneal macrophages	Caveolae	(179)
	A549 alveolar cell line	Mitochondria, cytoplasm	(147)
	Beas-2b bronchial epithelial cells	Mitochondria, cytoplasm	(147)
	Rat liver	Mitochondria, microsomes	(28)
Palmitoylation	Murine B16 melanoma cell line	Mitochondria associated membrane	(107)

LPS, lipopolysaccharide.

by brefeldin-A that disrupts Golgi complexes, suggesting that Golgi processing is also involved (Fig. 3). As mentioned earlier, CAV1 is enriched in caveolae, where it has been shown to bind to and inhibit the activity of HMOX (78, 83, 159). Indeed, deletion of CAV1 increases, while CAV1 overexpression inhibits LPS-induced HMOX activity in EC (83). CAV1 interferes with the binding of hemin to HMOX1 (159). Thus, CAV1 may be considered a cellular regulator of HMOX activity.

Electron microscopy studies indicate that mitochondrial HMOX1 is localized to the inner mitochondrial membrane (28), although HMOX1 lacks a typical mitochondria-targeting sequence as assessed by *in silico* analysis. Mitochondria are linked to the ER *via* mitochondrial-associated membranes (MAM) (54) that represent a likely route for HMOX1 trafficking between the two organelles. In the mouse melanoma cell line B16, inhibition of palmitoylation has been reported to result in translocation of HMOX1 from the MAM to the ER (107). Whether palmitoylation is a requisite for HMOX1 shuttling, however, is questionable, given that this modification does not occur in all species. Nevertheless, these data suggest that HMOX1 may shuttle between these organelles.

As mentioned earlier, mitochondrial HMOX1 colocalizes with BVR and CPR (28). Thus, one function of mitochondrial HMOX1 appears to be the regulation of mitochondrial heme content, in concert with heme synthesis that also occurs in mitochondria (129). Mitochondrial HMOX1 also plays a role in apoptosis. Treatment of rat renal cells with the HMOX1 inducer cobalt protoporphyrin IX results in translocation of HMOX1 to mitochondria and attenuation of the release of cytochrome *c* (172). HMOX1 induction was further associated with increased transcription and phosphorylation of the antiapoptotic protein Bcl2. Hemin and cigarette smoke extract also increase mitochondrial HMOX1 expression in A549 alveolar and Beas-2b bronchial epithelial cell lines (147) that was associated with preservation of ATP production.

The mode of action of nuclear HMOX1 appears to depart somewhat from the membrane-associated forms of HMOX1 discussed so far. Known inducers of HMOX1, such as hypoxia and hemin, are associated with nuclear translocation of HMOX1 (Table 1). While it remains uncertain whether protease-mediated cleavage of the TMS region of HMOX1 is essential for nuclear translocation, nonspecific inhibition of cysteine proteases blocked nuclear translocation of HMOX1 in one study (101). Studies of truncated rat HMOX1 indicate that a highly conserved leucine-rich nuclear shuttling sequence enables nuclear translocation (101) (Fig. 2). This leucine-rich sequence is partially conserved between human and mouse, and, to a lesser extent, chicken.

Once in the nucleus, truncated HMOX1 may protect against stress by different mechanisms, including the activation of transcription factors (52, 101). Consistent with this, we reported that yeast cells transfected with human HMOX1 and treated with oxidants showed nuclear translocation of HMOX1 and increased expression of antioxidant genes such as γ-glutamylcysteine synthetase, glutathione peroxidase, catalase, and methionine sulfoxide reductase (27). Whether the biological functions of nuclear HMOX1 require enzymatic activity is a matter of debate. As previously mentioned, truncated human HMOX1 retains enzymatic activity (143, 144, 181). Evidence against nuclear HMOX1 being enzymatically active comes from studies performed by Dennery and coworkers. These authors reported HMOX activity in 3T3 fibroblasts transfected with human *EGFP-HMOX1*Δ_{23} to be similar to that in non-transfected control cells, whereas transfection with full-length *EGFP-HMOX1* increased enzymatic activity $\sim$10 fold (101). In addition, transfection of cells with an enzymatically inactive HMOX1 mutant significantly altered the expression of various transcription factors (101). Thus, enzymatic activity does not appear to be required for HMOX1 to affect gene transcription in model systems, although the biological relevance of this remains to be established.

If enzymatically active, the resulting CO itself may modulate antioxidant gene transcription *via* the activation of the Nrf2 transcription factor that is a key initiator of *HMOX1* gene transcription (91). Thus, the HMOX1 response can be amplified. This amplification may be kept in check by CPR, which promotes oligomerization of HMOX1 and prevents cleavage and nuclear translocation of the truncated protein. In addition, HMOX1 protein has been shown to auto-regulate itself, independent of enzymatic activity (102). HMOX1 and CO can induce the transcription factor Yin Yang 1 (YY1) (9) that suppresses SMC proliferation without affecting EC proliferation (138). Up-regulation of YY1 by the HMOX1 inducer probucol was necessary for the inhibition of intimal hyperplasia in a rat model (9). Moreover, overexpression of YY1 can up-regulate HMOX1 expression (9). Similarly, both vascular endothelial growth factor (VEGF) and the pro-angiogenic chemokine stromal cell-derived factor-1 (SDF-1) can induce HMOX1 or, in turn, be induced by HMOX1 (37, 99). Exogenous administration of CO has also been shown to activate DNA repair signaling in HEK293 cells (126). This coordinate response to stress involving HMOX1, its associated machinery, and metabolites underlies the importance of heme degradation in cellular homeostasis.

The possibility of enzymatically active HMOX1 being located in the nucleus raises a number of intriguing questions, including the source of the substrate heme and how it is transported into the nucleus; the source and mode of transfer of reducing equivalents required for enzymatic activity; the fate of the Fe^{2+} released as a consequence of heme catabolism; and whether biliverdin IXα is converted to bilirubin IXα within the nucleus. There are some data to address these questions. Of note, Fe^{2+}-sequestering ferritin has been observed in the nucleus in a number of cell types, including astrocytes, where it was shown to protect DNA from Fe^{2+}-induced oxidative damage (165). Maines and coworkers also reported the presence of BVR in the nucleus, and they proposed that BVR might act as a transporter for heme into the nucleus (95). While CPR is not expected to be present in the nucleus, ascorbate could conceivably donate electrons to truncated HMOX1 in the nucleus. These data and our own that demonstrate the presence of bilirubin IXα in the nucleus of hypoxia-treated rat SMC as assessed by immunohistology (J Ni, pers. comm.) would seem to indicate that in addition to the activation of transcription factors, nuclear HMOX1 may retain some heme detoxification activity.

The effect of hypoxia on the regulation of HMOX1 is complex. For example, hypoxia increases HMOX1 expression in human dermal fibroblasts (127), while hypoxia is associated with decreased HMOX1 expression in human coronary artery EC, human umbilical vein EC, and immortalized human microvascular EC (88, 106, 119). The findings in human tissues are reminiscent of the tissue-specific effects of HMOX1 induction on cell proliferation, for example, inhibition in SMC

(9, 42) and stimulation in EC growth (36). By contrast, in rodents, hypoxia increases HMOX1 expression in rat pulmonary aortic EC (83), immortalized mouse EC (55), and rat aortic SMC (92). These species-specific differences in hypoxia-mediated HMOX1 induction may be explained, in part, by the presence or absence of Bach1 (hypoxia-induced repressor of Nrf2 and HMOX1 gene transcription) (106) or other transcription factors in human EC.

HMOX1 As a Therapeutic Target for the Treatment of Human Disease

HMOX1 is recognized as a promising therapeutic target for a broad range of conditions, including cardiovascular, metabolic, neurodegenerative, and other inflammatory diseases (Fig. 5A). The next section will focus on cardiovascular diseases. The catabolism of heme provides protection to cells *via* multiple avenues, including the induction of ferritin to store redox-active Fe (43), the antioxidant actions of biliverdin and bilirubin (154), and the anti-inflammatory and anti-apoptotic effects of CO (151). Avenues relevant to cardiovascular diseases are summarized in Figure 5B. Potential treatment modalities include pharmacological induction, gene delivery of *HMOX1*, or direct delivery of CO, biliverdin, and bilirubin. The ability of progenitor cells to specifically home to sites of vascular injury (187) also raises the possibility that HMOX1 or heme degradation products could be delivered by autologous stem cell therapy. In this section, we will discuss insights amassed from the *Hmox1*$^{-/-}$ mouse and the therapeutic utility of HMOX1 and heme degradation products as determined from animal models of human disease. Evidence for the association of HMOX1 with cardiovascular disease and diabetes is summarized in Table 2.

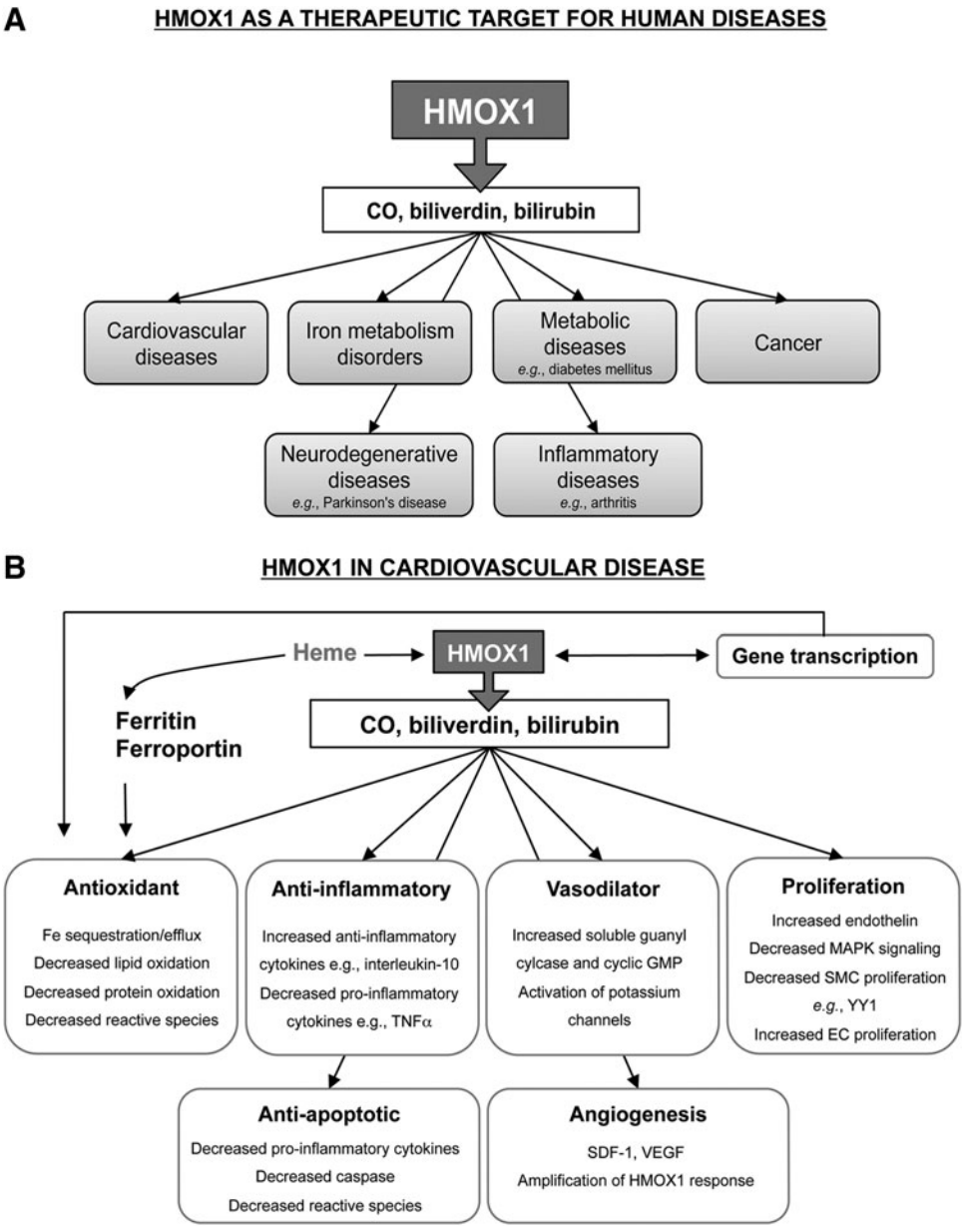

FIG. 5. Protective properties of HMOX1 in cardiovascular diseases. (A) HMOX1 is a therapeutic target for a broad range of human diseases. **(B)** In cardiovascular diseases, HMOX1 and heme catabolism products have antioxidant, anti-inflammatory, anti-apoptotic, vasodilatory, and anti-proliferative properties. The antioxidant effects of heme include the activation of transcriptional machinery that induces a range of anti-oxidant genes.

Table 2. Evidence for the Protective Effect of Heme Oxygenase-1 in Cardiovascular Disease and Diabetes

Evidence	*References*
Cardiovascular disease	
Longer GT repeats associated with increased risk of coronary artery disease and coronary events	(38, 79)
Longer GT repeats associated with increased atherosclerosis burden and plaque rupture	(20)
Longer GT repeats associated with increased restenosis	(38)
Longer GT repeats associated with increased inflammation	(141)
Longer GT repeats or low bilirubin associated with increased risk of cerebral ischemia	(87)
Low bilirubin concentrations associated with increased coronary artery disease	(145)
Low bilirubin concentrations associated with endothelial dysfunction, increased carotid intima-media thickness, or arterial stiffness	(45, 200)
High bilirubin concentrations associated with coronary collateral growth	(200)
Diabetes mellitus	
Long GT repeats and low serum bilirubin concentrations in diabetes mellitus associated with increased risk of coronary artery disease	(150)
High levels of HMOX1 reduce the risk of gestational diabetes mellitus	(131)
High bilirubin concentrations are associated with reduced HbA1c or lower incidence of diabetes	(21)

HMOX1 deficiency

To date, only two cases of human *HMOX1* deficiency have been reported (133, 188). In the first reported case, a 6-year-old boy had severe retardation, Fe loading in the kidneys and liver, vascular injury, and hyperlipidemia (188). Similarly, in the second case, a 2-year-old girl presented with reduced growth, Fe metabolism disorders, asplenia, hepatomegaly, nephritis, leukocytosis, and vascular injury (133, 134).

The apparently low penetrance of this gene deficiency may be explained by the fact that *Hmox1* gene deletion in mouse embryos is lethal in most instances (130). This embryonic lethality could be explained by defects in the placental vasculature. Recent studies using $Hmox1^{+/-}$ heterozygous mice demonstrated that partial *Hmox1* deficiency was associated with malformed vasculature and impaired spiral artery remodeling in the placenta (198). In these investigations, the maternal allele was identified to be responsible for the placental defects. These observations may account for the reduced numbers of $Hmox1^{-/-}$ pups born. It is also possible that partial *HMOX1* deficiency in a mother may underpin repeated failures at pregnancy or early miscarriage, and this could be the reason that only two cases of human *HMOX1* deficiency have been reported to date.

Many of the features of human HMOX1 deficiency are reflected in the $Hmox1^{-/-}$ mouse. The latter displays a profound inflammatory phenotype with features of human iron overload syndrome, including tissue iron deposition, splenomegaly, hepatomegaly, hepatic fibrosis, growth retardation, and premature death (130). Moreover, $Hmox1^{-/-}$ mice have increased numbers of leukocytes, activated $CD4^+$ T cells, proinflammatory cytokines, and oxidized proteins and lipids (80, 124).

HMOX1 promoter variation in cardiovascular diseases and diabetes mellitus

There is some variation in the transcriptional activity of the *HMOX1* gene by virtue of a microsatellite of GT repeats within the promoter. *In vitro* studies in human SMC have shown that shorter GT repeats lead to increased *HMOX1* transcriptional activity (18). In human EC, shorter GT repeats lead to reduced oxidative stress and proinflammatory cytokines, and increased responsiveness to VEGF-induced proliferation (158). In humans, longer GT repeats have been associated with increased inflammation after balloon angioplasty (46, 141) and increased in-stent restenosis (38). These data are reflected in the seminal observation that low plasma bilirubin levels are associated with an increased risk of coronary artery disease (66, 145). Not all studies have shown an association between the microsatellite and restenosis or coronary disease (44, 166). However, in two of these studies, shorter GT repeats were associated with higher bilirubin levels and a healthy lipid profile (44), or reduced inflammation (141). The underlying reasons for this disparity could include patient ethnicity and variations in the severity of the diseases examined.

There is continued debate regarding the association of longer GT repeats with an increased risk of developing Type 2 diabetes mellitus (5, 6, 23). Similar to cardiovascular diseases, the overall inconsistent results may be explained by differences in ethnicity and disease severity. HMOX1 expression is reduced in peripheral blood mononuclear cells of diabetes patients (150). In contrast, high serum levels of HMOX1 in early pregnancy may reduce the risk of developing gestational diabetes mellitus (131).

HMOX1 in vascular health and disease

As mentioned earlier, $Hmox1^{-/-}$ mice are not born in Mendelian ratios and partial *Hmox1* deficiency is associated with malformed placental vasculature (198), implicating HMOX1 in angiogenic processes. $Hmox1^{-/-}$ mice produce higher levels of the angiogenic inhibitors soluble VEGF and soluble endoglin (30). They also have impaired wound healing and wound neovascularization compared with wild-type littermates (57). *Hmox1* deficiency is associated with more damage from myocardial ischemia-reperfusion injury (77, 197). Conversely, cardiac-specific overexpression of the human *HMOX1* transgene in the mouse led to improved cardiac function and increased numbers of newly formed blood vessels (104). Similarly, adenoviral overexpression of rat *Hmox1* resulted in improved blood flow recovery and limb function in a rat hind limb ischemia model (157). In *ex vivo* aortic ring sprouting angiogenesis assays, *Hmox1* deficiency impairs VEGF- and SDF-1-induced angiogenesis (37). This impairment in angiogenesis could be attenuated by administration of exogenous CO.

HMOX1 is strongly implicated in vascular diseases such as atherosclerosis. HMOX1 protein is expressed in atherosclerotic lesions in both *apolipoprotein E* (*Apoe*) and *low-density lipoprotein receptor-deficient mice*, where it is thought to protect from disease (72, 178). This interpretation is supported by

studies demonstrating that adenoviral overexpression of human *HMOX1* is associated with decreased atherosclerosis in *Apoe*$^{-/-}$ mice (76). Moreover, mice deficient in *Hmox1* and *Apoe* have increased atherosclerosis and vein graft stenosis compared with *Apoe*$^{-/-}$ mice (191).

HMOX1 is also associated with vascular remodeling and endothelial function. In a pig model of arterial injury, adenoviral overexpression of *Hmox1* led to a decrease in SMC proliferation and improved vascular reactivity (42). In rats, *Hmox1* gene delivery or HMOX1 induction *via* hemin treatment attenuates vascular remodeling and neointimal hyperplasia after balloon injury (169, 170). Adenoviral overexpression of *HMOX1* has also been demonstrated to attenuate the development of graft arteriosclerosis in a rat aortic transplant model (40), and to improve graft survival in a rat aorta chronic rejection model (12). Further, HMOX1 induction by heme arginate leads to decreases in proinflammatory cytokines and improved endothelial function in *low-density lipoprotein receptor-deficient mice* (82). Outside the systemic circulation, HMOX1 is implicated in diseases of the pulmonary circuit such as pulmonary hypertension. Reduced expression of HMOX1 was found in lung tissues of newborns suffering from congenital diaphragmatic hernia and pulmonary hypertension (149). Compared with wild-type littermates, *Hmox1*$^{-/-}$ mice exposed to chronic hypoxia have features that are consistent with pulmonary hypertension, including exaggerated right heart hypertrophy, ventricular infarcts, and thrombi (192). Conversely, tissue-specific overexpression of human *HMOX1* in the lungs of transgenic FVB/N mice decreases pulmonary hypertension in response to chronic hypoxia (115). In rats, the administration of CO (41) or the induction of HMOX1 by hemin or nickel chloride (25) inhibits the development of pulmonary hypertension. Data in humans are lacking at this point in time but are important to obtain, particularly as sufferers of pulmonary hypertension have a poor diagnosis. Inhalation of CO is currently undergoing clinical trials in idiopathic pulmonary hypertension (*e.g.*, http://clinicaltrials.gov/ct2/show/NCT01214187) and severe pulmonary arterial hypertension (*e.g.*, http://clinicaltrials.gov/ct2/show/NCT01523548).

HMOX1 in diabetes

There is increasing evidence for a role of HMOX1 in experimental diabetes and associated complications. In diabetic mice, vascular injuries are exacerbated compared with nondiabetic controls; HMOX1 up-regulation is beneficial, although the impact of diabetes on the expression and activity of HMOX1 is inconsistent between different tissues (47, 132). *Hmox1*$^{-/-}$ mice treated with streptozotocin have increased oxidative stress and infarct size after myocardial ischemia reperfusion compared with nondiabetic *Hmox1*$^{-/-}$ mice (104). In addition, in the *db/db* mouse model, impaired wound healing and wound neovascularization is ameliorated by adenoviral overexpression of rat *Hmox1* (57). Similarly, overexpression of murine *Hmox1* attenuates the immune response in NOD mice and slows the progression to diabetes *via* a mechanism that involves CO (67). Furthermore, selective overexpression of murine *Hmox1* in the pancreas of NOD mice was associated with a decrease in proinflammatory mediators, and these mice were less likely to develop diabetes and had improved graft survival after islet transplantation compared with control animals (68).

The induction of HMOX1 by cobalt protoporphyrin IX has been reported to improve insulin sensitivity and adipose remodeling in the Zucker diabetic rat (123). Cobalt protoporphyrin IX has also been shown to regulate adiposity in male mice (15). In both female and male mice, HMOX1 induction was further associated with lower blood pressure and proinflammatory cytokines, with increased serum adiponectin and expansion of insulin-sensitive adipocytes (15). Similarly, hemin-mediated induction of HMOX1 has been shown to increase insulin sensitivity and glucose metabolism in a range of models of diabetes, including the obese Zucker rat, Goto-Kakizaki rat, and streptozotocin-treated rats (120–122).

Pharmacological inducers of HMOX1 protein expression

A number of pharmacological agents induce HMOX1 expression, and those in use or trialed for the treatment of cardiovascular diseases or diabetes are listed in Table 3, along with the proposed mode of action for inducing HMOX1. Interestingly, this list includes three classes of lipid-lowering drugs, that is statins, probucol (and its analog succinobucol), and fenofibrate.

Simvastatin, pravastatin, artovastatin, and fluvastatin were demonstrated to increase HMOX1 in RAW264.7 murine macrophages *via* protein kinase G, ERK, and p38 MAPK signaling (17). Simvastatin and pravastatin also increase HMOX1 in vascular EC (173) and renal epithelial cells (16). In human EC (63) as well as in human and rat vascular SMC (93), simvastatin increased HMOX1 *via* the PI3K-Akt pathway, although potential phosphorylation of HMOX1 at S188 by Akt (see above) was not explored. Similarly, fluvastatin increased the PI3K-Akt pathway in coronary artery SMC, resulting in enhanced HMOX1 expression *via* transcription factor Nrf2 (111). However, it remains to be clearly established whether this activity translates to pharmacologically relevant concentrations of statins [see *e.g.*, (105)].

Probucol increases HMOX1 expression in EC and vascular SMC *in vitro* and *in vivo*, and these are associated with a ~2-fold increase in HMOX activity in the arterial wall of animal models of atherosclerosis (15, 35, 160, 186). Increasing HMOX1 by a systemic administration of probucol has the dual benefit of inhibiting SMC proliferation while simultaneously increasing EC proliferation (9, 90, 186). This is associated with the inhibition of intimal hyperplasia and the promotion of re-endothelialization after arterial balloon injury in rabbits and rats, and in-stent re-endothelialization after femoral stenting in rabbits (160). Succinobucol, a more water-soluble mono-succinate derivative of probucol, increases HMOX1 in balloon-injured rabbit aortas and decreases neointimal hyperplasia (187). Similar to probucol, succinobucol increases HMOX1 expression in SMC *in vitro*, and this is associated with decreased cell proliferation. Unlike probucol, however, the anti-proliferative effect of succinobucol appears to be *via* the promotion of apoptosis rather than increased HMOX activity (114). Both probucol and succinobucol enhance the mobilization of progenitor cells to sites of vascular injury (167, 187). In clinical studies of atherosclerosis and restenosis, probucol and succinobucol have yielded mixed

Table 3. Heme Oxygenase-1 Inducers and Heme Degradation Products in Cardiovascular Diseases and Diabetes

Pharmacological agent	*Model or cell type*	*Mode of HMOX1 induction*	*References*
Lipid modulating drugs			
Fibrates			
Fenofibrate	Human umbilical vein EC, human vascular SMC	PPARα agonist	(89)
Phenolics			
Probucol	Rabbit aorta after balloon injury	Anti-inflammatory, antioxidant, and beneficial lipid profile, transcription factor Yin Yang 1	(9, 35, 186)
Succinobucol	Rat vascular smooth muscle cells after balloon injury	Apoptosis	(114)
	Human coronary events and new onset of diabetes	Anti-inflammatory and antioxidant mechanisms	(162)
Statins			
Atorvastatin	Murine RAW264.7 macrophages	PKG, ERK, and p38 MAPK signaling activation	(17)
	Human umbilical vein EC, human aortic EC	Induction of Krüppel-like factor 2	(3)
	Rat aortic SMC	Inhibition of NF-κB translocation/anti-inflammatory	(59)
Fluvastatin	Murine RAW264.7 macrophages	PKG, ERK, and p38 MAPK signaling activation	(17)
	Human coronary artery SMC	Increased Nrf2 *via* PI3K-Akt signaling	(111)
Pravastatin	Murine RAW264.7 macrophages	Protein kinase G, ERK, and p38 MAPK signaling activation	(17)
	Rat renal tubular epithelial cells	PPARα binds to peroxisome proliferator response element in the *HMOX1* promoter	(16)
Simvastastin	Murine RAW264.7 macrophages	Protein kinase G, ERK, and p38 MAPK signaling activation	(17)
	Human and rat aortic SMCs, mouse aorta	p38 MAPK and PI3K/Akt signaling	(93)
	Human EC	PI3K/Akt signaling	(63)
Anti-proliferative drugs			
Paclitaxel	Drug-eluting stent inhibits rat vascular SMC proliferation	JNK, ERK, and p38 MAPK signaling activation	(22)
Sirolimus (Rapamycin)	Drug-eluting stent, rabbit endothelialization, and human aortic EC	Prevents binding of PPARγ to *HMOX1* promoter	(49)
Anti-inflammatory drugs			
Aspirin	Human umbilical vein EC	NO-dependent pathway	(58)
Anti-diabetic drugs			
Rosiglitazone	Rat cardiomyoblast cell line, rat model of pre-eclampsia	PPARγ agonist	(89)

Agent	*Indication*	*Status*	*References*
Clinical trials			
Heme arginate	Cardiac injury after myocardial ischemia	Unclear	http://clinicaltrials.gov/ct2/show/NCT00483587
	Adenosine-induced vasodilation in atherosclerotic disease	Terminated	http://clinicaltrials.gov/ct2/show/NCT00856817
	Ischemia-reperfusion injury in renal transplants	Recruiting	http://clinicaltrials.gov/ct2/show/NCT01430156
Hemin	Gastroparesis in diabetic patients	Recruiting	http://clinicaltrials.gov/ct2/show/NCT01206582
Inhalants			
Carbon monoxide	Idiopathic pulmonary hypertension	Recruiting	http://clinicaltrials.gov/ct2/show/NCT01214187

(*continued*)

Table 3. Continued

Agent	*Indication*	*Status*	*References*
	Severe pulmonary arterial hypertension	Recruiting not commenced	http://clinicaltrials.gov/ct2/show/NCT01523548
	Pulmonary inflammatory response to endotoxin	Local inflammatory response not altered by inhaled CO	http://clinicaltrials.gov/ct2/show/NCT00094406 (108)

CO, carbon monoxide; EC, endothelial cell; HMOX1, heme oxygenase-1; SMC, smooth muscle cell.

results [reviewed in ref. (153)]. For example, while probucol failed to provide benefits in patients with femoral atherosclerosis (161, 162, 175), the drug was reported to decrease atherosclerosis in carotid arteries and associated cardiac events (140). Of potential importance, long-term treatment of probucol in combination with other cholesterol-lowering drugs prevents secondary cardiovascular events in patients with heterozygous familial hypercholesterolemia (190). In preclinical studies, fenofibrate, a PPARα agonist, was shown to increase HMOX1 in human umbilical vein EC and vascular SMC (89). Similarly, niacin increases HMOX1 and protects against vascular inflammation (184), similar to the manner in which apolipoprotein AI mimetic peptides have been shown to increase HMOX1 and to attenuate adipocyte dysfunction (174) and intimal hyperplasia, respectively (185).

Inducers of HMOX1 such as heme arginate and hemin are also the subjects of several clinical trials that are relevant to vascular disease (see Table 3). The indications include prevention of cardiac injury after myocardial ischemia, adenosine-induced vasodilation in atherosclerotic disease, ischemia-reperfusion injury in renal transplants (all heme arginate), and improvement of gastroparesis in diabetic patients (hemin).

Biliverdin, bilirubin, and CO

Biliverdin and bilirubin were originally considered waste products; however, these products of heme catabolism have antioxidant and cell-protective benefits in their own right (154). Low bilirubin levels are associated with an increased risk of coronary artery disease (66, 145) and an increased risk of metabolic diseases such as diabetes mellitus (21). In contrast, exogenous bilirubin has been shown to protect from ischemia-reperfusion injury (1) and to prevent neointimal hyperplasia in rats (128). In Gilbert syndrome, congenital hyperbilirubinemia is associated with a decrease in the development of diabetic vascular complications compared with non-Gilbert diabetic patients (71). The addition of bilirubin, or its precursor biliverdin, to culture media also prevents oxidant-induced cytotoxicity in vascular SMC (26) and the adhesion of leukocytes to EC (60).

More recently, HMOX1 induction or administration of heme degradation products has been found to have beneficial vascular effects, in part, *via* regulation of free heme and NADPH oxidases (116). NADPH oxidases generate superoxide and H_2O_2 derived from it that not only play important roles in host defense and cell signaling but can also lead to oxidative stress and inflammation. However, hemin-mediated induction of HMOX1 decreases NADPH oxidase activity in murine aorta *in vivo*, while bilirubin administration mimics the decrease of NADPH oxidase activity *in vitro* in rat vascular SMC (31) and human EC (73). In Spontaneous Hypertensive Rats, hemin treatment increased HMOX activity, improved endothelial function, and was associated with a decrease in NADPH oxidase-2 (96). In contrast, HMOX1 induction blocked vascular hypertrophy in *NADPH oxidase-4* null mice (142). These data suggest that HMOX1 may modulate individual NADPH oxidases independently of each other in different tissues or disease states.

CO is a signaling molecule with anti-inflammatory, anti-apoptotic, and anti-proliferative properties (135). CO inhibits LPS-induced increases in pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin-1β, and macrophage inhibitory protein-1β (125). Furthermore, CO can induce the release of anti-inflammatory interleukin-10 *via* p38 MAPK signaling (94). Finally, low doses of CO protect cells against inflammation-induced oxidative stress (14). In addition to the clinical trials of CO inhalation for pulmonary disease previously mentioned, CO inhalation has also been assessed with regard to its effect on the pulmonary inflammatory response to endotoxin (see *e.g.*, http://clinicaltrials.gov/ct2/show/NCT00094406).

The administration of non-toxic concentrations of CO has been shown to be beneficial in a number of animal models. It attenuates neointimal hyperplasia in balloon-injured rat carotid arteries (171) and, similar to increasing HMOX1, attenuates infarct size in a cerebral model of ischemia-reperfusion injury (176). Similarly, CO administration induces HMOX1 and reduces ischemic lung injury in *Hmox1*$^{-/-}$ mice (50). In addition, in aortic tissues from *Hmox1*$^{-/-}$ mice, CO has been demonstrated to rescue impairment of angiogenesis that is induced by SDF-1 (37).

There is also evidence that HMOX activity and its products may be relevant for transplantation-related cardiovascular diseases (148). Thus, inhibition of HMOX activity by tin protoporphyrin has been reported to enhance the rejection of mouse-to-rat heart transplants (139); while human *HMOX1* (13) or rat *Hmox1* overexpression prolongs cardiac allograft survival (4). These effects appear to be attributable to CO and/or biliverdin, as their administration also leads to marked inhibition of ischemia-reperfusion injury, with improved donor graft survival and recipient animal survival (118, 139).

Of note, increasing HMOX1 or administration of heme degradation products may augment HMOX1 expression *via* a positive feedback loop. Thus, CO can activate the transcription factor Nrf2, which binds to antioxidant response elements and induces *HMOX1* gene transcription, and this, in turn, amplifies the HMOX1 response (91) (Fig. 6).

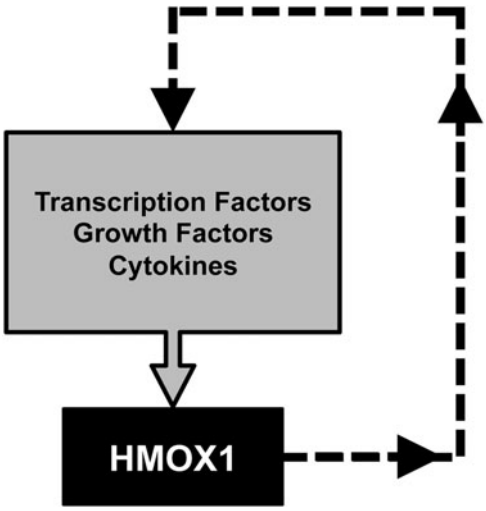

FIG. 6. HMOX1 may act downstream and upstream of transcription factors, growth factors, and cytokines. A new paradigm for HMOX1 as a cause and effect in gene transcription and cell signaling.

Amplification benefits have also been attributed to the biliverdin/bilirubin redox cycling (7) by BVR, but this notion has been questioned ever since (109, 113).

HMOX1 and cancer

While the potential benefits of inducing HMOX1 and heme degradation products to alleviate cardiovascular diseases are promising, due consideration to the multiple effects of HMOX1 is necessary. For example, HMOX1 expression is elevated in a variety of tumors and neoplasms [reviewed in ref. (180)]. HMOX1 may alternatively be over-expressed in cells supporting the immediate surroundings of the tumor, for example, macrophages (32). Within the tumor, or its microenvironment, HMOX1, CO, Fe, and bilirubin may encourage cell proliferation; while HMOX1 and CO have antiapoptotic effects that may improve tumor survival (180). In contrast to these observations, HMOX1 overexpression has been shown to block proliferation by increasing cell cycle arrest and apoptosis (62), and to prevent invasion (98) of the human breast cancer cell line MCF7 *in vitro*. Similar to the different effects of HMOX1 on EC and SMC proliferation, these data suggest that the role of HMOX1 in tumor growth may be complex. Nevertheless, HMOX1 inhibitors such as zinc protoporphyrin have shown promise in reducing tumor growth (64).

HMOX1 is a strong promoter of angiogenesis and neovascularization (75, 180) *via* its association with SDF-1 (37), a potent signal for progenitor mobilization and homing (2) supporting tumor growth. The promotion of angiogenesis may also facilitate metastasis. Furthermore, the rupture of malformed neovessels could lead to the release of hemoglobin (2), which, in turn, amplifies the HMOX1 response. Current treatment approaches such as UV irradiation and chemotherapy themselves induce HMOX1 expression, amplifying a pro-survival response that may reduce treatment efficacy (180). Moreover, HMOX1 and CO may inhibit dendritic cell effector responses that may impact T-regulatory cells and lead to immunosuppression [reviewed in ref. (11)]. Collectively, these data identify the potential obstacles to be overcome for inducers of HMOX1 to be successful therapeutics against cardiovascular disease.

Conclusions

In addition to degrading and detoxifying heme, HMOX1 is increasingly recognized as a protein that protects cells *via* multiple pathways. Increasing our understanding of the functions and post-translational modifications of HMOX1 in different subcellular localizations is paramount for our ability to exploit HMOX1 for the treatment of human diseases. At present, the application of HMOX1 inducers as treatment modality for cardiovascular and metabolic diseases should be carefully considered. This is due to the multiple activities of the enzyme that includes the regulation of transcription *via* an interplay with transcription factors such as YY1 (9) and hypoxia-inducible factor-1 (92), or growth factors/chemokines such as VEGF (74) and SDF-1 (37). Intriguingly, at least in the cases of VEGF and YY1, HMOX1 has been reported to be both downstream and upstream of key regulators of cellular metabolism and action. This raises the possibility that this action of HMOX1 may extend to other factors (Fig. 6). HMOX1 is elevated in a variety of cancers, and it can be associated with tumor angiogenesis and metastasis (64). Therefore, it will be of utmost importance to pay due consideration to the relationship between HMOX1 and specific diseases, and/or overlapping disease states.

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References

1. Adin CA, Croker BP, and Agarwal A. Protective effects of exogenous bilirubin on ischemia-reperfusion injury in the isolated, perfused rat kidney. *Am J Physiol Renal Physiol* 288: F778–F784, 2005.
2. Aicher A, Zeiher AM, and Dimmeler S. Mobilizing endothelial progenitor cells. *Hypertension* 45: 321–325, 2005.
3. Ali F, Hamdulay SS, Kinderlerer AR, Boyle JJ, Lidington EA, Yamaguchi T, Soares MP, Haskard DO, Randi AM, and Mason JC. Statin-mediated cytoprotection of human vascular endothelial cells: a role for Kruppel-like factor 2-dependent induction of heme oxygenase-1. *J Thromb Haemost* 5: 2537–2546, 2007.
4. Araujo JA, Meng L, Tward AD, Hancock WW, Zhai Y, Lee A, Ishikawa K, Iyer S, Buelow R, Busuttil RW, Shih DM, Lusis AJ, and Kupiec-Weglinski JW. Systemic rather than local heme oxygenase-1 overexpression improves cardiac allograft outcomes in a new transgenic mouse. *J Immunol* 171: 1572–1580, 2003.
5. Arredondo M, Jorquera D, Carrasco E, Albala C, and Hertrampf E. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with iron status in persons with type 2 diabetes mellitus. *Am J Clin Nutr* 86: 1347–1353, 2007.
6. Bao W, Song F, Li X, Rong S, Yang W, Wang D, Xu J, Fu J, Zhao Y, and Liu L. Association between heme oxygenase-1

gene promoter polymorphisms and type 2 diabetes mellitus: a HuGE review and meta-analysis. *Am J Epidemiol* 172: 631–636, 2010.
7. Baranano DE, Rao M, Ferris CD, and Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. *Proc Natl Acad Sci U S A* 99: 16093–16098, 2002.
8. Barone E, Di Domenico F, Sultana R, Coccia R, Mancuso C, Perluigi M, and Butterfield DA. Heme oxygenase-1 post-translational modifications in the brain of subjects with Alzheimer disease and mild cognitive impairment. *Free Radic Biol Med* 52: 2292–2301, 2012.
9. Beck K, Wu BJ, Ni J, Santiago FS, Malabanan KP, Li C, Wang Y, Khachigian LM, and Stocker R. Interplay between heme oxygenase-1 and the multifunctional transcription factor yin yang 1 in the inhibition of intimal hyperplasia. *Circ Res* 107: 1490–1497, 2010.
10. Bindu S, Pal C, Dey S, Goyal M, Alam A, Iqbal MS, Dutta S, Sarkar S, Kumar R, Maity P, and Bandyopadhyay U. Translocation of heme oxygenase-1 to mitochondria is a novel cytoprotective mechanism against non-steroidal anti-inflammatory drug-induced mitochondrial oxidative stress, apoptosis, and gastric mucosal injury. *J Biol Chem* 286: 39387–39402, 2011.
11. Blancou P and Anegon I. Editorial: heme oxygenase-1 and dendritic cells: what else? *J Leukoc Biol* 87: 185–187, 2010.
12. Bouche D, Chauveau C, Roussel JC, Mathieu P, Braudeau C, Tesson L, Soulillou JP, Iyer S, Buelow R, and Anegon I. Inhibition of graft arteriosclerosis development in rat aortas following heme oxygenase-1 gene transfer. *Transpl Immunol* 9: 235–238, 2002.
13. Braudeau C, Bouchet D, Tesson L, Iyer S, Remy S, Buelow R, Anegon I, and Chauveau C. Induction of long-term cardiac allograft survival by heme oxygenase-1 gene transfer. *Gene Ther* 11: 701–710, 2004.
14. Brouard S, Otterbein LE, Anrather J, Tobiasch E, Bach FH, Choi AM, and Soares MP. Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis. *J Exp Med* 192: 1015–1026, 2000.
15. Burgess A, Li M, Vanella L, Kim DH, Rezzani R, Rodella L, Sodhi K, Canestraro M, Martasek P, Peterson SJ, Kappas A, and Abraham NG. Adipocyte heme oxygenase-1 induction attenuates metabolic syndrome in both male and female obese mice. *Hypertension* 56: 1124–1130, 2010.
16. Chen HH, Chen TW, and Lin H. Pravastatin attenuates carboplatin-induced nephrotoxicity in rodents via peroxisome proliferator-activated receptor alpha-regulated heme oxygenase-1. *Mol Pharmacol* 78: 36–45, 2010.
17. Chen JC, Huang KC, and Lin WW. HMG-CoA reductase inhibitors upregulate heme oxygenase-1 expression in murine RAW264.7 macrophages via ERK, p38 MAPK and protein kinase G pathways. *Cell Signal* 18: 32–39, 2006.
18. Chen YH, Lin SJ, Lin MW, Tsai HL, Kuo SS, Chen JW, Charng MJ, Wu TC, Chen LC, Ding YA, Pan WH, Jou YS, and Chau LY. Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients. *Hum Genet* 111: 1–8, 2002.
19. Chen-Roetling J, Li Z, Chen M, Awe OO, and Regan RF. Heme oxygenase activity and hemoglobin neurotoxicity are attenuated by inhibitors of the MEK/ERK pathway. *Neuropharmacology* 56: 922–928, 2009.
20. Cheng C, Noordeloos AM, Jeney V, Soares MP, Moll F, Pasterkamp G, Serruys PW, and Duckers HJ. Heme oxygenase 1 determines atherosclerotic lesion progression into a vulnerable plaque. *Circulation* 119: 3017–3027, 2009.
21. Cheriyath P, Gorrepati VS, Peters I, Nookala V, Murphy ME, Srouji N, and Fischman D. High total bilirubin as a protective factor for diabetes mellitus: an analysis of NHANES data from 1999–2006. *J Clin Med Res* 2: 201–206, 2010.
22. Choi BM, Kim YM, Jeong YR, Pae HO, Song CE, Park JE, Ahn YK, and Chung HT. Induction of heme oxygenase-1 is involved in anti-proliferative effects of paclitaxel on rat vascular smooth muscle cells. *Biochem Biophys Res Commun* 321: 132–137, 2004.
23. Choi SW, Yeung VT, and Benzie IF. Heme oxygenase microsatellite polymorphism, oxidative stress, glycemic control, and complication development in type 2 diabetes patients. *Free Radic Biol Med* 53: 60–63, 2012.
24. Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen JV, and Mann M. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* 325: 834–840, 2009.
25. Christou H, Morita T, Hsieh CM, Koike H, Arkonac B, Perrella MA, and Kourembanas S. Prevention of hypoxia-induced pulmonary hypertension by enhancement of endogenous heme oxygenase-1 in the rat. *Circ Res* 86: 1224–1229, 2000.
26. Clark JE, Foresti R, Green CJ, and Motterlini R. Dynamics of haem oxygenase-1 expression and bilirubin production in cellular protection against oxidative stress. *Biochem J* 348: 615–619, 2000.
27. Collinson EJ, Wimmer-Kleikamp S, Gerega SK, Yang YH, Parish CR, Dawes IW, and Stocker R. The yeast homolog of heme oxygenase-1 affords cellular antioxidant protection via the transcriptional regulation of known antioxidant genes. *J Biol Chem* 286: 2205–2214, 2011.
28. Converso DP, Taille C, Carreras MC, Jaitovich A, Poderoso JJ, and Boczkowski J. HO-1 is located in liver mitochondria and modulates mitochondrial heme content and metabolism. *FASEB J* 20: 1236–1238, 2006.
29. Cruse I and Maines MM. Evidence suggesting that the two forms of heme oxygenase are products of different genes. *J Biol Chem* 263: 3348–3353, 1988.
30. Cudmore M, Ahmad S, Al-Ani B, Fujisawa T, Coxall H, Chudasama K, Devey LR, Wigmore SJ, Abbas A, Hewett PW, and Ahmed A. Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. *Circulation* 115: 1789–1797, 2007.
31. Datla SR, Dusting GJ, Mori TA, Taylor CJ, Croft KD, and Jiang F. Induction of heme oxygenase-1 *in vivo* suppresses NADPH oxidase derived oxidative stress. *Hypertension* 50: 636–642, 2007.
32. Deininger MH, Meyermann R, Trautmann K, Duffner F, Grote EH, Wickboldt J, and Schluesener HJ. Heme oxygenase (HO)-1 expressing macrophages/microglial cells accumulate during oligodendroglioma progression. *Brain Res* 882: 1–8, 2000.
33. Delaby C, Pilard N, Puy H, and Canonne-Hergaux F. Sequential regulation of ferroportin expression after erythrophagocytosis in murine macrophages: early mRNA induction by haem, followed by iron-dependent protein expression. *Biochem J* 411: 123–131, 2008.
34. Delaby C, Rondeau C, Pouzet C, Willemetz A, Pilard N, Desjardins M, and Canonne-Hergaux F. Subcellular localization of iron and heme metabolism related proteins at early stages of erythrophagocytosis. *PLoS One* 7: e42199, 2012.

35. Deng YM, Wu BJ, Witting PK, and Stocker R. Probucol protects against smooth muscle cell proliferation by upregulating heme oxygenase-1. *Circulation* 110: 1855–1860, 2004.
36. Deramaudt BM, Braunstein S, Remy P, and Abraham NG. Gene transfer of human heme oxygenase into coronary endothelial cells potentially promotes angiogenesis. *J Cell Biochem* 68: 121–127, 1998.
37. Deshane J, Chen S, Caballero S, Grochot-Przeczek A, Was H, Li Calzi S, Lach R, Hock TD, Chen B, Hill-Kapturczak N, Siegal GP, Dulak J, Jozkowicz A, Grant MB, and Agarwal A. Stromal cell-derived factor 1 promotes angiogenesis via a heme oxygenase 1-dependent mechanism. *J Exp Med* 204: 605–618, 2007.
38. Dick P, Schillinger M, Minar E, Mlekusch W, Amighi J, Sabeti S, Schlager O, Raith M, Endler G, Mannhalter C, Wagner O, and Exner M. Haem oxygenase-1 genotype and cardiovascular adverse events in patients with peripheral artery disease. *Eur J Clin Invest* 35: 731–737, 2005.
39. Dowal L, Yang W, Freeman MR, Steen H, and Flaumenhaft R. Proteomic analysis of palmitoylated platelet proteins. *Blood* 118: e62–e73, 2011.
40. Du D, Chang S, Chen B, Zhou H, and Chen ZK. Adenovirus-mediated heme oxygenase transfer inhibits graft arteriosclerosis in rat aortic transplants. *Transplant Proc* 39: 3446–3448, 2007.
41. Dubuis E, Potier M, Wang R, and Vandier C. Continuous inhalation of carbon monoxide attenuates hypoxic pulmonary hypertension development presumably through activation of BKCa channels. *Cardiovasc Res* 65: 751–761, 2005.
42. Duckers HJ, Boehm M, True AL, Yet SF, San H, Park JL, Clinton Webb R, Lee ME, Nabel GJ, and Nabel EG. Heme oxygenase-1 protects against vascular constriction and proliferation. *Nat Med* 7: 693–698, 2001.
43. Eisenstein RS, Garcia-Mayol D, Pettingell W, and Munro HN. Regulation of ferritin and heme oxygenase synthesis in rat fibroblasts by different forms of iron. *Proc Natl Acad Sci U S A* 88: 688–692, 1991.
44. Endler G, Exner M, Schillinger M, Marculescu R, Sunder-Plassmann R, Raith M, Jordanova N, Wojta J, Mannhalter C, Wagner OF, and Huber K. A microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with increased bilirubin and HDL levels but not with coronary artery disease. *Thromb Haemost* 91: 155–161, 2004.
45. Erdogan D, Gullu H, Yildirim E, Tok D, Kirbas I, Ciftci O, Baycan ST, and Muderrisoglu H. Low serum bilirubin levels are independently and inversely related to impaired flow-mediated vasodilation and increased carotid intima-media thickness in both men and women. *Atherosclerosis* 184: 431–437, 2006.
46. Exner M, Schillinger M, Minar E, Mlekusch W, Schlerka G, Haumer M, Mannhalter C, and Wagner O. Heme oxygenase-1 gene promoter microsatellite polymorphism is associated with restenosis after percutaneous transluminal angioplasty. *J Endovasc Ther* 8: 433–440, 2001.
47. Farhangkhoee H, Khan ZA, Mukherjee S, Cukiernik M, Barbin YP, Karmazyn M, and Chakrabarti S. Heme oxygenase in diabetes-induced oxidative stress in the heart. *J Mol Cell Cardiol* 35: 1439–1448, 2003.
48. Feron O, Saldana F, Michel JB, and Michel T. The endothelial nitric-oxide synthase-caveolin regulatory cycle. *J Biol Chem* 273: 3125–3128, 1998.
49. Finn AV, John M, Nakazawa G, Polavarapu R, Karmali V, Xu X, Cheng Q, Davis T, Raghunathan C, Acampado E, Ezell T, Lajoie S, Eppihimer M, Kolodgie FD, Virmani R, and Gold HK. Differential healing after sirolimus, paclitaxel, and bare metal stent placement in combination with peroxisome proliferator-activator receptor gamma agonists: requirement for mTOR/Akt2 in PPARgamma activation. *Circ Res* 105: 1003–1012, 2009.
50. Fujita T, Toda K, Karimova A, Yan SF, Naka Y, Yet SF, and Pinsky DJ. Paradoxical rescue from ischemic lung injury by inhaled carbon monoxide driven by derepression of fibrinolysis. *Nat Med* 7: 598–604, 2001.
51. Gandini NA, Fermento ME, Salomon DG, Blasco J, Patel V, Gutkind JS, Molinolo AA, Facchinetti MM, and Curino AC. Nuclear localization of heme oxygenase-1 is associated with tumor progression of head and neck squamous cell carcinomas. *Exp Mol Pathol* 93: 237–245, 2012.
52. Ghoreschi K, Bruck J, Kellerer C, Deng C, Peng H, Rothfuss O, Hussain RZ, Gocke AR, Respa A, Glocova I, Valtcheva N, Alexander E, Feil S, Feil R, Schulze-Osthoff K, Rupec RA, Lovett-Racke AE, Dringen R, Racke MK, and Rocken M. Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. *J Exp Med* 208: 2291–2303, 2011.
53. Giordano A, Nisoli E, Tonello C, Cancello R, Carruba MO, and Cinti S. Expression and distribution of heme oxygenase-1 and -2 in rat brown adipose tissue: the modulatory role of the noradrenergic system. *FEBS Lett* 487: 171–175, 2000.
54. Giorgi C, De Stefani D, Bononi A, Rizzuto R, and Pinton P. Structural and functional link between the mitochondrial network and the endoplasmic reticulum. *Int J Biochem Cell Biol* 41: 1817–1827, 2009.
55. Gloria MA, Cenedeze MA, Pacheco-Silva A, and Camara NO. The blockade of cyclooxygenases-1 and -2 reduces the effects of hypoxia on endothelial cells. *Braz J Med Biol Res* 39: 1189–1196, 2006.
56. Gottlieb Y, Truman M, Cohen LA, Leichtmann-Bardoogo Y, and Meyron-Holtz EG. Endoplasmic reticulum anchored heme-oxygenase-1 faces the cytosol. *Haematologica* 97: 1489–1493, 2012.
57. Grochot-Przeczek A, Lach R, Mis J, Skrzypek K, Gozdecka M, Sroczynska P, Dubiel M, Rutkowski A, Kozakowska M, Zagorska A, Walczynski J, Was H, Kotlinowski J, Drukala J, Kurowski K, Kieda C, Herault Y, Dulak J, and Jozkowicz A. Heme oxygenase-1 accelerates cutaneous wound healing in mice. *PLoS One* 4: e5803, 2009.
58. Grosser N, Abate A, Oberle S, Vreman HJ, Dennery PA, Becker JC, Pohle T, Seidman DS, and Schroder H. Heme oxygenase-1 induction may explain the antioxidant profile of aspirin. *Biochem Biophys Res Commun* 308: 956–960, 2003.
59. Haloui M, Meilhac O, Jandrot-Perrus M, and Michel JB. Atorvastatin limits the pro-inflammatory response of rat aortic smooth muscle cells to thrombin. *Eur J Pharmacol* 474: 175–184, 2003.
60. Hayashi S, Takamiya R, Yamaguchi T, Matsumoto K, Tojo SJ, Tamatani T, Kitajima M, Makino N, Ishimura Y, and Suematsu M. Induction of heme oxygenase-1 suppresses venular leukocyte adhesion elicited by oxidative stress: role of bilirubin generated by the enzyme. *Circ Res* 85: 663–671, 1999.
61. Higashimoto Y, Sugishima M, Sato H, Sakamoto H, Fukuyama K, Palmer G, and Noguchi M. Mass spectrometric identification of lysine residues of heme oxygenase-1 that are involved in its interaction with NADPH-cytochrome P450 reductase. *Biochem Biophys Res Commun* 367: 852–858, 2008.
62. Hill M, Pereira V, Chauveau C, Zagani R, Remy S, Tesson L, Mazal D, Ubillos L, Brion R, Asghar K, Mashreghi MF, Kotsch K, Moffett J, Doebis C, Seifert M, Boczkowski J,

Osinaga E, and Anegon I. Heme oxygenase-1 inhibits rat and human breast cancer cell proliferation: mutual cross inhibition with indoleamine 2,3-dioxygenase. *FASEB J* 19: 1957–1968, 2005.
63. Hinkelmann U, Grosser N, Erdmann K, Schroder H, and Immenschuh S. Simvastatin-dependent up-regulation of heme oxygenase-1 via mRNA stabilization in human endothelial cells. *Eur J Pharm Sci* 41: 118–124, 2010.
64. Hirai K, Sasahira T, Ohmori H, Fujii K, and Kuniyasu H. Inhibition of heme oxygenase-1 by zinc protoporphyrin IX reduces tumor growth of LL/2 lung cancer in C57BL mice. *Int J Cancer* 120: 500–505, 2007.
65. Hori R, Kashiba M, Toma T, Yachie A, Goda N, Makino N, Soejima A, Nagasawa T, Nakabayashi K, and Suematsu M. Gene transfection of H25A mutant heme oxygenase-1 protects cells against hydroperoxide-induced cytotoxicity. *J Biol Chem* 277: 10712–10718, 2002.
66. Horsfall LJ, Nazareth I, and Petersen I. Cardiovascular events as a function of serum bilirubin levels in a large, statin-treated cohort. *Circulation* 126: 2556–2564, 2012.
67. Hu CM, Lin HH, Chiang MT, Chang PF, and Chau LY. Systemic expression of heme oxygenase-1 ameliorates type 1 diabetes in NOD mice. *Diabetes* 56: 1240–1247, 2007.
68. Huang SH, Chu CH, Yu JC, Chuang WC, Lin GJ, Chen PL, Chou FC, Chau LY, and Sytwu HK. Transgenic expression of haem oxygenase-1 in pancreatic beta cells protects non-obese mice used as a model of diabetes from autoimmune destruction and prolongs graft survival following islet transplantation. *Diabetologia* 53: 2389–2400, 2010.
69. Huber WJ, 3rd and Backes WL. Expression and characterization of full-length human heme oxygenase-1: the presence of intact membrane-binding region leads to increased binding affinity for NADPH cytochrome P450 reductase. *Biochemistry* 46: 12212–12219, 2007.
70. Hwang HW, Lee JR, Chou KY, Suen CS, Hwang MJ, Chen C, Shieh RC, and Chau LY. Oligomerization is crucial for the stability and function of heme oxygenase-1 in the endoplasmic reticulum. *J Biol Chem* 284: 22672–22679, 2009.
71. Inoguchi T, Sasaki S, Kobayashi K, Takayanagi R, and Yamada T. Relationship between Gilbert syndrome and prevalence of vascular complications in patients with diabetes. *JAMA* 298: 1398–1400, 2007.
72. Ishikawa K, Sugawara D, Wang X, Suzuki K, Itabe H, Maruyama Y, and Lusis AJ. Heme oxygenase-1 inhibits atherosclerotic lesion formation in ldl-receptor knockout mice. *Circ Res* 88: 506–512, 2001.
73. Jiang F, Roberts SJ, Datla, Sr., and Dusting GJ. NO modulates NADPH oxidase function via heme oxygenase-1 in human endothelial cells. *Hypertension* 48: 950–957, 2006.
74. Jozkowicz A, Huk I, Nigisch A, Weigel G, Dietrich W, Motterlini R, and Dulak J. Heme oxygenase and angiogenic activity of endothelial cells: stimulation by carbon monoxide and inhibition by tin protoporphyrin-IX. *Antioxid Redox Signal* 5: 155–162, 2003.
75. Jozkowicz A, Was H, and Dulak J. Heme oxygenase-1 in tumors: is it a false friend? *Antioxid Redox Signal* 9: 2099–2117, 2007.
76. Juan SH, Lee TS, Tseng KW, Liou JY, Shyue SK, Wu KK, and Chau LY. Adenovirus-mediated heme oxygenase-1 gene transfer inhibits the development of atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 104: 1519–1525, 2001.
77. Juhasz B, Varga B, Czompa A, Bak I, Lekli I, Gesztelyi R, Zsuga J, Kemeny-Beke A, Antal M, Szendrei L, and Tosaki A. Postischemic cardiac recovery in heme oxygenase-1 transgenic ischemic/reperfused mouse myocardium. *J Cell Mol Med* 15: 1973–1982, 2011.
78. Jung NH, Kim HP, Kim BR, Cha SH, Kim GA, Ha H, Na YE, and Cha YN. Evidence for heme oxygenase-1 association with caveolin-1 and -2 in mouse mesangial cells. *IUBMB Life* 55: 525–532, 2003.
79. Kaneda H, Ohno M, Taguchi J, Togo M, Hashimoto H, Ogasawara K, Aizawa T, Ishizaka N, and Nagai R. Heme oxygenase-1 gene promoter polymorphism is associated with coronary artery disease in Japanese patients with coronary risk factors. *Arterioscler Thromb Vasc Biol* 22: 1680–1685, 2002.
80. Kapturczak MH, Wasserfall C, Brusko T, Campbell-Thompson M, Ellis TM, Atkinson MA, and Agarwal A. Heme oxygenase-1 modulates early inflammatory responses: evidence from the heme oxygenase-1-deficient mouse. *Am J Pathol* 165: 1045–1053, 2004.
81. Kawai Y, Garduno L, Theodore M, Yang J, and Arinze IJ. Acetylation-deacetylation of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) regulates its transcriptional activity and nucleocytoplasmic localization. *J Biol Chem* 286: 7629–7640, 2011.
82. Kawamura K, Ishikawa K, Wada Y, Kimura S, Matsumoto H, Kohro T, Itabe H, Kodama T, and Maruyama Y. Bilirubin from heme oxygenase-1 attenuates vascular endothelial activation and dysfunction. *Arterioscler Thromb Vasc Biol* 25: 155–160, 2005.
83. Kim HP, Wang X, Galbiati F, Ryter SW, and Choi AM. Caveolae compartmentalization of heme oxygenase-1 in endothelial cells. *FASEB J* 18: 1080–1089, 2004.
84. Kim KM, Pae HO, Zheng M, Park R, Kim YM, and Chung HT. Carbon monoxide induces heme oxygenase-1 via activation of protein kinase R-like endoplasmic reticulum kinase and inhibits endothelial cell apoptosis triggered by endoplasmic reticulum stress. *Circ Res* 101: 919–927, 2007.
85. Kim W, Bennett EJ, Huttlin EL, Guo A, Li J, Possemato A, Sowa ME, Rad R, Rush J, Comb MJ, Harper JW, and Gygi SP. Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol Cell* 44: 325–340, 2011.
86. Kim YS, Zhuang H, Koehler RC, and Dore S. Distinct protective mechanisms of HO-1 and HO-2 against hydroperoxide-induced cytotoxicity. *Free Radic Biol Med* 38: 85–92, 2005.
87. Kimm H, Yun JE, Jo J, and Jee SH. Low serum bilirubin level as an independent predictor of stroke incidence: a prospective study in Korean men and women. *Stroke* 40: 3422–3427, 2009.
88. Kitamuro T, Takahashi K, Ogawa K, Udono-Fujimori R, Takeda K, Furuyama K, Nakayama M, Sun J, Fujita H, Hida W, Hattori T, Shirato K, Igarashi K, and Shibahara S. Bach1 functions as a hypoxia-inducible repressor for the heme oxygenase-1 gene in human cells. *J Biol Chem* 278: 9125–9133, 2003.
89. Kronke G, Kadl A, Ikonomu E, Bluml S, Furnkranz A, Sarembock IJ, Bochkov VN, Exner M, Binder BR, and Leitinger N. Expression of heme oxygenase-1 in human vascular cells is regulated by peroxisome proliferator-activated receptors. *Arterioscler Thromb Vasc Biol* 27: 1276–1282, 2007.
90. Lau AK, Leichtweis SB, Hume P, Mashima R, Hou JY, Chaufour X, Wilkinson B, Hunt NH, Celermajer DS, and Stocker R. Probucol promotes functional reendothelialization in balloon-injured rabbit aortas. *Circulation* 107: 2031–2036, 2003.

91. Lee BS, Heo J, Kim YM, Shim SM, Pae HO, Kim YM, and Chung HT. Carbon monoxide mediates heme oxygenase 1 induction via Nrf2 activation in hepatoma cells. *Biochem Biophys Res Commun* 343: 965–972, 2006.
92. Lee PJ, Jiang BH, Chin BY, Iyer NV, Alam J, Semenza GL, and Choi AM. Hypoxia-inducible factor-1 mediates transcriptional activation of the heme oxygenase-1 gene in response to hypoxia. *J Biol Chem* 272: 5375–5381, 1997.
93. Lee TS, Chang CC, Zhu Y, and Shyy JY. Simvastatin induces heme oxygenase-1: a novel mechanism of vessel protection. *Circulation* 110: 1296–1302, 2004.
94. Lee TS and Chau LY. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. *Nat Med* 8: 240–246, 2002.
95. Lerner-Marmarosh N, Miralem T, Gibbs PE, and Maines MD. Human biliverdin reductase is an ERK activator; hBVR is an ERK nuclear transporter and is required for MAPK signaling. *Proc Natl Acad Sci U S A* 105: 6870–6875, 2008.
96. Li Z, Wang Y, and Vanhoutte PM. Upregulation of heme oxygenase 1 by hemin impairs endothelium-dependent contractions in the aorta of the spontaneously hypertensive rat. *Hypertension* 58: 926–934, 2011.
97. Li Volti G, Ientile R, Abraham NG, Vanella A, Cannavo G, Mazza F, Curro M, Raciti G, Avola R, and Campisi A. Immunocytochemical localization and expression of heme oxygenase-1 in primary astroglial cell cultures during differentiation: effect of glutamate. *Biochem Biophys Res Commun* 315: 517–524, 2004.
98. Lin CW, Shen SC, Hou WC, Yang LY, and Chen YC. Heme oxygenase-1 inhibits breast cancer invasion via suppressing the expression of matrix metalloproteinase-9. *Mol Cancer Ther* 7: 1195–1206, 2008.
99. Lin HH, Chen YH, Chang PF, Lee YT, Yet SF, and Chau LY. Heme oxygenase-1 promotes neovascularization in ischemic heart by coinduction of VEGF and SDF-1. *J Mol Cell Cardiol* 45: 44–55, 2008.
100. Lin PH, Lan WM, and Chau LY. TRC8 suppresses tumorigenesis through targeting heme oxygenase-1 for ubiquitination and degradation. *Oncogene* 32: 2325–2334, 2012.
101. Lin Q, Weis S, Yang G, Weng YH, Helston R, Rish K, Smith A, Bordner J, Polte T, Gaunitz F, and Dennery PA. Heme oxygenase-1 protein localizes to the nucleus and activates transcription factors important in oxidative stress. *J Biol Chem* 282: 20621–20633, 2007.
102. Lin QS, Weis S, Yang G, Zhuang T, Abate A, and Dennery PA. Catalytic inactive heme oxygenase-1 protein regulates its own expression in oxidative stress. *Free Radic Biol Med* 44: 847–855, 2008.
103. Linnenbaum M, Busker M, Kraehling JR, and Behrends S. Heme oxygenase isoforms differ in their subcellular trafficking during hypoxia and are differentially modulated by cytochrome P450 reductase. *PLoS One* 7: e35483, 2012.
104. Liu X, Wei J, Peng DH, Layne MD, and Yet SF. Absence of heme oxygenase-1 exacerbates myocardial ischemia/reperfusion injury in diabetic mice. *Diabetes* 54: 778–784, 2005.
105. Loboda A, Jazwa A, Jozkowicz A, Dorosz J, Balla J, Molema G, and Dulak J. Atorvastatin prevents hypoxia-induced inhibition of endothelial nitric oxide synthase expression but does not affect heme oxygenase-1 in human microvascular endothelial cells. *Atherosclerosis* 187: 26–30, 2006.
106. Loboda A, Stachurska A, Florczyk U, Rudnicka D, Jazwa A, Wegrzyn J, Kozakowska M, Stalinska K, Poellinger L, Levonen AL, Yla-Herttuala S, Jozkowicz A, and Dulak J. HIF-1 induction attenuates Nrf2-dependent IL-8 expression in human endothelial cells. *Antioxid Redox Signal* 11: 1501–1517, 2009.
107. Lynes EM, Bui M, Yap MC, Benson MD, Schneider B, Ellgaard L, Berthiaume LG, and Simmen T. Palmitoylated TMX and calnexin target to the mitochondria-associated membrane. *EMBO J* 31: 457–470, 2012.
108. Machado RF, Reda D, Tropea M, Gladwin MT, and Suffredini AF. The effects of inhaled carbon monoxide on endotoxin-induced pulmonary inflammation in humans. *Am J Respir Crit Care Med* 179: A5673, 2009.
109. Maghzal GJ, Leck MC, Collinson E, Li C, and Stocker R. Limited role for the bilirubin-biliverdin redox amplification cycle in the cellular antioxidant protection by biliverdin reductase. *J Biol Chem* 284: 29251–29259, 2009.
110. Maines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 37: 517–554, 1997.
111. Makabe S, Takahashi Y, Watanabe H, Murakami M, Ohba T, and Ito H. Fluvastatin protects vascular smooth muscle cells against oxidative stress through the Nrf2-dependent antioxidant pathway. *Atherosclerosis* 213: 377–384, 2010.
112. Marohnic CC, Huber Iii WJ, Patrick Connick J, Reed JR, McCammon K, Panda SP, Martasek P, Backes WL, and Masters BS. Mutations of human cytochrome P450 reductase differentially modulate heme oxygenase-1 activity and oligomerization. *Arch Biochem Biophys* 513: 42–50, 2011.
113. McDonagh AF. The biliverdin-bilirubin antioxidant cycle of cellular protection: missing a wheel? *Free Radic Biol Med* 49: 814–820, 2010.
114. Midwinter RG, Maghzal GJ, Dennis JM, Wu BJ, Cai H, Kapralov AA, Belikova NA, Tyurina YY, Dong LF, Khachigian L, Neuzil J, Kagan VE, and Stocker R. Succinobucol induces apoptosis in vascular smooth muscle cells. *Free Radic Biol Med* 52: 871–879, 2012.
115. Minamino T, Christou H, Hsieh CM, Liu Y, Dhawan V, Abraham NG, Perrella MA, Mitsialis SA, and Kourembanas S. Targeted expression of heme oxygenase-1 prevents the pulmonary inflammatory and vascular responses to hypoxia. *Proc Natl Acad Sci U S A* 98: 8798–8803, 2001.
116. Moraes JA, Barcellos-de-Souza P, Rodrigues G, Nascimento-Silva V, Silva SV, Assreuy J, Arruda MA, and Barja-Fidalgo C. Heme modulates smooth muscle cell proliferation and migration via NADPH oxidase: a counter-regulatory role for heme oxygenase system. *Atherosclerosis* 224: 394–400, 2012.
117. Morikawa T, Kajimura M, Nakamura T, Hishiki T, Nakanishi T, Yukutake Y, Nagahata Y, Ishikawa M, Hattori K, Takenouchi T, Takahashi T, Ishii I, Matsubara K, Kabe Y, Uchiyama S, Nagata E, Gadalla MM, Snyder SH, and Suematsu M. Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway. *Proc Natl Acad Sci U S A* 109: 1293–1298, 2012.
118. Nakao A, Neto JS, Kanno S, Stolz DB, Kimizuka K, Liu F, Bach FH, Billiar TR, Choi AM, Otterbein LE, and Murase N. Protection against ischemia/reperfusion injury in cardiac and renal transplantation with carbon monoxide, biliverdin and both. *Am J Transplant* 5: 282–291, 2005.
119. Nakayama M, Takahashi K, Kitamuro T, Yasumoto K, Katayose D, Shirato K, Fujii-Kuriyama Y, and Shibahara S. Repression of heme oxygenase-1 by hypoxia in vascular endothelial cells. *Biochem Biophys Res Commun* 271: 665–671, 2000.
120. Ndisang JF and Jadhav A. Heme oxygenase system enhances insulin sensitivity and glucose metabolism in

streptozotocin-induced diabetes. *Am J Physiol Endocrinol Metab* 296: E829–E841, 2009.
121. Ndisang JF and Jadhav A. Up-regulating the hemeoxygenase system enhances insulin sensitivity and improves glucose metabolism in insulin-resistant diabetes in Goto-Kakizaki rats. *Endocrinology* 150: 2627–2636, 2009.
122. Ndisang JF, Lane N, and Jadhav A. The heme oxygenase system abates hyperglycemia in Zucker diabetic fatty rats by potentiating insulin-sensitizing pathways. *Endocrinology* 150: 2098–2108, 2009.
123. Nicolai A, Li M, Kim DH, Peterson SJ, Vanella L, Positano V, Gastaldelli A, Rezzani R, Rodella LF, Drummond G, Kusmic C, L'Abbate A, Kappas A, and Abraham NG. Heme oxygenase-1 induction remodels adipose tissue and improves insulin sensitivity in obesity-induced diabetic rats. *Hypertension* 53: 508–515, 2009.
124. Orozco LD, Kapturczak MH, Barajas B, Wang X, Weinstein MM, Wong J, Deshane J, Bolisetty S, Shaposhnik Z, Shih DM, Agarwal A, Lusis AJ, and Araujo JA. Heme oxygenase-1 expression in macrophages plays a beneficial role in atherosclerosis. *Circ Res* 100: 1703–1711, 2007.
125. Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, and Choi AM. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med* 6: 422–428, 2000.
126. Otterbein LE, Hedblom A, Harris C, Csizmadia E, Gallo D, and Wegiel B. Heme oxygenase-1 and carbon monoxide modulate DNA repair through ataxia-telangiectasia mutated (ATM) protein. *Proc Natl Acad Sci U S A* 108: 14491–14496, 2011.
127. Panchenko MV, Farber HW, and Korn JH. Induction of heme oxygenase-1 by hypoxia and free radicals in human dermal fibroblasts. *Am J Physiol Cell Physiol* 278: C92–C101, 2000.
128. Peyton KJ, Shebib AR, Azam MA, Liu XM, Tulis DA, and Durante W. Bilirubin inhibits neointima formation and vascular smooth muscle cell proliferation and migration. *Front Pharmacol* 3: 48, 2012.
129. Ponka P. Tissue-specific regulation of iron metabolism and heme synthesis: distinct control mechanisms in erythroid cells. *Blood* 89: 1–25, 1997.
130. Poss KD and Tonegawa S. Heme oxygenase 1 is required for mammalian iron reutilization. *Proc Natl Acad Sci U S A* 94: 10919–10924, 1997.
131. Qiu C, Hevner K, Enquobahrie DA, and Williams MA. Maternal serum heme-oxygenase-1 (HO-1) concentrations in early pregnancy and subsequent risk of gestational diabetes mellitus. *PLoS One* 7: e48060, 2012.
132. Quan S, Kaminski PM, Yang L, Morita T, Inaba M, Ikehara S, Goodman AI, Wolin MS, and Abraham NG. Heme oxygenase-1 prevents superoxide anion-associated endothelial cell sloughing in diabetic rats. *Biochem Biophys Res Commun* 315: 509–516, 2004.
133. Radhakrishnan N, Yadav SP, Sachdeva A, Pruthi PK, Sawhney S, Piplani T, Wada T, and Yachie A. Human heme oxygenase-1 deficiency presenting with hemolysis, nephritis, and asplenia. *J Pediatr Hematol Oncol* 33: 74–78, 2011.
134. Radhakrishnan N, Yadav SP, Sachdeva A, Wada T, and Yachie A. An interesting tetrad of asplenia, inflammation, hemolysis, and nephritis. *Pediatr Hematol Oncol* 28: 723–726, 2011.
135. Ryter SW, Alam J, and Choi AM. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. *Physiol Rev* 86: 583–650, 2006.
136. Sacca P, Meiss R, Casas G, Mazza O, Calvo JC, Navone N, and Vazquez E. Nuclear translocation of haeme oxygenase-1 is associated to prostate cancer. *Br J Cancer* 97: 1683–1689, 2007.
137. Salinas M, Wang J, Rosa de Sagarra M, Martin D, Rojo AI, Martin-Perez J, Ortiz de Montellano PR, and Cuadrado A. Protein kinase Akt/PKB phosphorylates heme oxygenase-1 *in vitro* and *in vivo*. *FEBS Lett* 578: 90–94, 2004.
138. Santiago FS, Lowe HC, Bobryshev YV, and Khachigian LM. Induction of the transcriptional repressor Yin Yang-1 by vascular cell injury. Autocrine/paracrine role of endogenous fibroblast growth factor-2. *J Biol Chem* 276: 41143–41149, 2001.
139. Sato K, Balla J, Otterbein L, Smith RN, Brouard S, Lin Y, Csizmadia E, Sevigny J, Robson SC, Vercellotti G, Choi AM, Bach FH, and Soares MP. Carbon monoxide generated by heme oxygenase-1 suppresses the rejection of mouse-to-rat cardiac transplants. *J Immunol* 166: 4185–4194, 2001.
140. Sawayama Y, Shimizu C, Maeda N, Tatsukawa M, Kinukawa N, Koyanagi S, Kashiwagi S, and Hayashi J. Effects of probucol and pravastatin on common carotid atherosclerosis in patients with asymptomatic hypercholesterolemia. Fukuoka Atherosclerosis Trial (FAST). *J Am Coll Cardiol* 39: 610–616, 2002.
141. Schillinger M, Exner M, Mlekusch W, Ahmadi R, Rumpold H, Mannhalter C, Wagner O, and Minar E. Heme oxygenase-1 genotype is a vascular anti-inflammatory factor following balloon angioplasty. *J Endovasc Ther* 9: 385–394, 2002.
142. Schroder K, Zhang M, Benkhoff S, Mieth A, Pliquett R, Kosowski J, Kruse C, Luedike P, Michaelis UR, Weissmann N, Dimmeler S, Shah AM, and Brandes RP. Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase. *Circ Res* 110: 1217–1225, 2012.
143. Schuller DJ, Wilks A, Ortiz de Montellano P, and Poulos TL. Crystallization of recombinant human heme oxygenase-1. *Protein Sci* 7: 1836–1838, 1998.
144. Schuller DJ, Wilks A, Ortiz de Montellano PR, and Poulos TL. Crystal structure of human heme oxygenase-1. *Nat Struct Biol* 6: 860–867, 1999.
145. Schwertner HA, Jackson WG, and Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. *Clin Chem* 40: 18–23, 1994.
146. Shibahara S, Muller R, Taguchi H, and Yoshida T. Cloning and expression of cDNA for rat heme oxygenase. *Proc Natl Acad Sci U S A* 82: 7865–7869, 1985.
147. Slebos DJ, Ryter SW, van der Toorn M, Liu F, Guo F, Baty CJ, Karlsson JM, Watkins SC, Kim HP, Wang X, Lee JS, Postma DS, Kauffman HF, and Choi AM. Mitochondrial localization and function of heme oxygenase-1 in cigarette smoke-induced cell death. *Am J Respir Cell Mol Biol* 36: 409–417, 2007.
148. Soares MP, Lin Y, Anrather J, Csizmadia E, Takigami K, Sato K, Grey ST, Colvin RB, Choi AM, Poss KD, and Bach FH. Expression of heme oxygenase-1 can determine cardiac xenograft survival. *Nat Med* 4: 1073–1077, 1998.
149. Solari V, Piotrowska AP, and Puri P. Expression of heme oxygenase-1 and endothelial nitric oxide synthase in the lung of newborns with congenital diaphragmatic hernia and persistent pulmonary hypertension. *J Pediatr Surg* 38: 808–813, 2003.
150. Song F, Li X, Zhang M, Yao P, Yang N, Sun X, Hu FB, and Liu L. Association between heme oxygenase-1 gene promoter polymorphisms and type 2 diabetes in a Chinese population. *Am J Epidemiol* 170: 747–756, 2009.

151. Song R, Kubo M, Morse D, Zhou Z, Zhang X, Dauber JH, Fabisiak J, Alber SM, Watkins SC, Zuckerbraun BS, Otterbein LE, Ning W, Oury TD, Lee PJ, McCurry KR, and Choi AM. Carbon monoxide induces cytoprotection in rat orthotopic lung transplantation via anti-inflammatory and anti-apoptotic effects. *Am J Pathol* 163: 231–242, 2003.
152. Srivastava P and Pandey VC. Mitochondrial heme oxygenase of *Mastomys coucha*. *Int J Biochem Cell Biol* 28: 1071–1077, 1996.
153. Stocker R. Molecular mechanisms underlying the anti-atherosclerotic and antidiabetic effects of probucol, succinobucol, and other probucol analogues. *Curr Opin Lipidol* 20: 227–235, 2009.
154. Stocker R, McDonagh AF, Glazer AN, and Ames BN. Antioxidant activities of bile pigments: biliverdin and bilirubin. *Methods Enzymol* 186: 301–309, 1990.
155. Stocker R and Perrella MA. Heme oxygenase-1: a novel drug target for atherosclerotic diseases? *Circulation* 114: 2178–2189, 2006.
156. Sun Y, Rotenberg MO, and Maines MD. Developmental expression of heme oxygenase isozymes in rat brain. Two HO-2 mRNAs are detected. *J Biol Chem* 265: 8212–8217, 1990.
157. Suzuki M, Iso-o N, Takeshita S, Tsukamoto K, Mori I, Sato T, Ohno M, Nagai R, and Ishizaka N. Facilitated angiogenesis induced by heme oxygenase-1 gene transfer in a rat model of hindlimb ischemia. *Biochem Biophys Res Commun* 302: 138–143, 2003.
158. Taha H, Skrzypek K, Guevara I, Nigisch A, Mustafa S, Grochot-Przeczek A, Ferdek P, Was H, Kotlinowski J, Kozakowska M, Balcerczyk A, Muchova L, Vitek L, Weigel G, Dulak J, and Jozkowicz A. Role of heme oxygenase-1 in human endothelial cells: lesson from the promoter allelic variants. *Arterioscler Thromb Vasc Biol* 30: 1634–1641, 2010.
159. Taira J, Sugishima M, Kida Y, Oda E, Noguchi M, and Higashimoto Y. Caveolin-1 is a competitive inhibitor of heme oxygenase-1 (HO-1) with heme: identification of a minimum sequence in caveolin-1 for binding to HO-1. *Biochemistry* 50: 6824–6831, 2011.
160. Tanous D, Brasen JH, Choy K, Wu BJ, Kathir K, Lau A, Celermajer DS, and Stocker R. Probucol inhibits in-stent thrombosis and neointimal hyperplasia by promoting re-endothelialization. *Atherosclerosis* 189: 342–349, 2006.
161. Tardif JC, Gregoire J, Schwartz L, Title L, Laramee L, Reeves F, Lesperance J, Bourassa MG, L'Allier PL, Glass M, Lambert J, and Guertin MC. Effects of AGI-1067 and probucol after percutaneous coronary interventions. *Circulation* 107: 552–558, 2003.
162. Tardif JC, McMurray JJ, Klug E, Small R, Schumi J, Choi J, Cooper J, Scott R, Lewis EF, L'Allier PL, and Pfeffer MA. Effects of succinobucol (AGI-1067) after an acute coronary syndrome: a randomised, double-blind, placebo-controlled trial. *Lancet* 371: 1761–1768, 2008.
163. Tenhunen R, Marver HS, and Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc Natl Acad Sci U S A* 61: 748–755, 1968.
164. Tenhunen RMH and Schmid R. Microsomal heme oxygenase. Characterization of the enzyme. *J Biol Chem* 244: 6388–6394, 1969.
165. Thompson KJ, Fried MG, Ye Z, Boyer P, and Connor JR. Regulation, mechanisms and proposed function of ferritin translocation to cell nuclei. *J Cell Sci* 115: 2165–2177, 2002.
166. Tiroch K, Koch W, von Beckerath N, Kastrati A, and Schomig A. Heme oxygenase-1 gene promoter polymorphism and restenosis following coronary stenting. *Eur Heart J* 28: 968–973, 2007.
167. Tongers J, Knapp JM, Korf M, Kempf T, Limbourg A, Limbourg FP, Li Z, Fraccarollo D, Bauersachs J, Han X, Drexler H, Fiedler B, and Wollert KC. Haeme oxygenase promotes progenitor cell mobilization, neovascularization, and functional recovery after critical hindlimb ischaemia in mice. *Cardiovasc Res* 78: 294–300, 2008.
168. Torpey J and Ortiz de Montellano PR. Oxidation of the meso-methylmesoheme regioisomers by heme oxygenase. Electronic control of the reaction regiospecificity. *J Biol Chem* 271: 26067–26073, 1996.
169. Tulis DA, Durante W, Liu X, Evans AJ, Peyton KJ, and Schafer AI. Adenovirus-mediated heme oxygenase-1 gene delivery inhibits injury-induced vascular neointima formation. *Circulation* 104: 2710–2715, 2001.
170. Tulis DA, Durante W, Peyton KJ, Evans AJ, and Schafer AI. Heme oxygenase-1 attenuates vascular remodeling following balloon injury in rat carotid arteries. *Atherosclerosis* 155: 113–122, 2001.
171. Tulis DA, Keswani AN, Peyton KJ, Wang H, Schafer AI, and Durante W. Local administration of carbon monoxide inhibits neointima formation in balloon injured rat carotid arteries. *Cell Mol Biol (Noisy-le-grand)* 51: 441–446, 2005.
172. Turkseven S, Drummond G, Rezzani R, Rodella L, Quan S, Ikehara S, and Abraham NG. Impact of silencing HO-2 on EC-SOD and the mitochondrial signaling pathway. *J Cell Biochem* 100: 815–823, 2007.
173. Uchiyama T, Atsuta H, Utsugi T, Ohyama Y, Nakamura T, Nakai A, Nakata M, Maruyama I, Tomura H, Okajima F, Tomono S, Kawazu S, Nagai R, and Kurarbayashi M. Simvastatin induces heat shock factor 1 in vascular endothelial cells. *Atherosclerosis* 188: 265–273, 2006.
174. Vanella L, Li M, Kim D, Malfa G, Bellner L, Kawakami T, and Abraham NG. ApoA1: mimetic peptide reverses adipocyte dysfunction *in vivo* and *in vitro* via an increase in heme oxygenase (HO-1) and Wnt10b. *Cell Cycle* 11: 706–714, 2012.
175. Walldius G, Erikson U, Olsson AG, Bergstrand L, Hadell K, Johansson J, Kaijser L, Lassvik C, Molgaard J, Nilsson S, *et al.* The effect of probucol on femoral atherosclerosis: the Probucol Quantitative Regression Swedish Trial (PQRST). *Am J Cardiol* 74: 875–883, 1994.
176. Wang B, Cao W, Biswal S, and Dore S. Carbon monoxide-activated Nrf2 pathway leads to protection against permanent focal cerebral ischemia. *Stroke* 42: 2605–2610, 2011.
177. Wang J, Lad L, Poulos TL, and Ortiz de Montellano PR. Regiospecificity determinants of human heme oxygenase: differential NADPH- and ascorbate-dependent heme cleavage by the R183E mutant. *J Biol Chem* 280: 2797–2806, 2005.
178. Wang LJ, Lee TS, Lee FY, Pai RC, and Chau LY. Expression of heme oxygenase-1 in atherosclerotic lesions. *Am J Pathol* 152: 711–720, 1998.
179. Wang XM, Kim HP, Nakahira K, Ryter SW, and Choi AM. The heme oxygenase-1/carbon monoxide pathway suppresses TLR4 signaling by regulating the interaction of TLR4 with caveolin-1. *J Immunol* 182: 3809–3818, 2009.
180. Was H, Dulak J, and Jozkowicz A. Heme oxygenase-1 in tumor biology and therapy. *Curr Drug Targets* 11: 1551–1570, 2010.
181. Wilks A, Medzihradszky KF, and Ortiz de Montellano PR. Heme oxygenase active-site residues identified by heme-protein cross-linking during reduction of CBrCl3. *Biochemistry* 37: 2889–2896, 1998.

182. Wilks A and Ortiz de Montellano PR. Rat liver heme oxygenase. High level expression of a truncated soluble form and nature of the meso-hydroxylating species. *J Biol Chem* 268: 22357–22362, 1993.
183. Williams SE, Wootton P, Mason HS, Bould J, Iles DE, Riccardi D, Peers C, and Kemp PJ. Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel. *Science* 306: 2093–2097, 2004.
184. Wu BJ, Chen K, Barter PJ, and Rye KA. Niacin inhibits vascular inflammation via the induction of heme oxygenase-1. *Circulation* 125: 150–158, 2012.
185. Wu BJ, Chen K, Shrestha S, Ong KL, Barter PJ, and Rye KA. High density lipoproteins inhibit vascular endothelial inflammation by increasing 3β-hydroxysteroid-Δ24 reductase expression and inducing heme oxygenase-1. *Circ Res* 112: 278–288, 2013.
186. Wu BJ, Kathir K, Witting PK, Beck K, Choy K, Li C, Croft KD, Mori TA, Tanous D, Adams MR, Lau AK, and Stocker R. Antioxidants protect from atherosclerosis by a heme oxygenase-1 pathway that is independent of free radical scavenging. *J Exp Med* 203: 1117–1127, 2006.
187. Wu BJ, Midwinter RG, Cassano C, Beck K, Wang Y, Changsiri D, Gamble JR, and Stocker R. Heme oxygenase-1 increases endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 29: 1537–1542, 2009.
188. Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, Ohta K, Kasahara Y, and Koizumi S. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. *J Clin Invest* 103: 129–135, 1999.
189. Yamamoto N, Izumi Y, Matsuo T, Wakita S, Kume T, Takada-Takatori Y, Sawada H, and Akaike A. Elevation of heme oxygenase-1 by proteasome inhibition affords dopaminergic neuroprotection. *J Neurosci Res* 88: 1934–1942, 2010.
190. Yamashita S, Hbujo H, Arai H, Harada-Shiba M, Matsui S, Fukushima M, Saito Y, Kita T, and Matsuzawa Y. Long-term probucol treatment prevents secondary cardiovascular events: a cohort study of patients with heterozygous familial hypercholesterolemia in Japan. *J Atheroscler Thromb* 15: 292–303, 2008.
191. Yet SF, Layne MD, Liu X, Chen YH, Ith B, Sibinga NE, and Perrella MA. Absence of heme oxygenase-1 exacerbates atherosclerotic lesion formation and vascular remodeling. *FASEB J* 17: 1759–1761, 2003.
192. Yet SF, Perrella MA, Layne MD, Hsieh CM, Maemura K, Kobzik L, Wiesel P, Christou H, Kourembanas S, and Lee ME. Hypoxia induces severe right ventricular dilatation and infarction in heme oxygenase-1 null mice. *J Clin Invest* 103: R23–R29, 1999.
193. Yi L and Ragsdale SW. Evidence that the heme regulatory motifs in heme oxygenase-2 serve as a thiol/disulfide redox switch regulating heme binding. *J Biol Chem* 282: 21056–21067, 2007.
194. Yoshida T, Ishikawa K, and Sato M. Degradation of heme by a soluble peptide of heme oxygenase obtained from rat liver microsomes by mild trypsinization. *Eur J Biochem* 199: 729–733, 1991.
195. Yoshida T and Kikuchi G. Features of the reaction of heme degradation catalyzed by the reconstituted microsomal heme oxygenase system. *J Biol Chem* 253: 4230–4236, 1978.
196. Yoshida T and Kikuchi G. Purification and properties of heme oxygenase from pig spleen microsomes. *J Biol Chem* 253: 4224–4229, 1978.
197. Yoshida T, Maulik N, Ho YS, Alam J, and Das DK. H(mox-1) constitutes an adaptive response to effect antioxidant cardioprotection: a study with transgenic mice heterozygous for targeted disruption of the Heme oxygenase-1 gene. *Circulation* 103: 1695–1701, 2001.
198. Zhao H, Azuma J, Kalish F, Wong RJ, and Stevenson DK. Maternal heme oxygenase 1 regulates placental vasculature development via angiogenic factors in mice. *Biol Reprod* 85: 1005–1012, 2011.
199. Zhao S, Xu W, Jiang W, Yu W, Lin Y, Zhang T, Yao J, Zhou L, Zeng Y, Li H, Li Y, Shi J, An W, Hancock SM, He F, Qin L, Chin J, Yang P, Chen X, Lei Q, Xiong Y, and Guan KL. Regulation of cellular metabolism by protein lysine acetylation. *Science* 327: 1000–1004, 2010.
200. Zhu C, Xiong Z, Zheng Z, Chen Y, Chen X, and Qian X. Association of arterial stiffness with serum bilirubin levels in established coronary artery disease. *Intern Med* 51: 2083–2089, 2012.

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Abbreviations Used

Apoe = apolipoprotein E
BVR = biliverdin reductase
CAV = caveolin
CO = carbon monoxide
CPR = cytochrome P450 reductase
DAPI = 4′,6-diamidino-2-phenylindole
EC = endothelial cell
ER = endoplasmic reticulum
H_2O_2 = hydrogen peroxide
HMOX = heme oxygenase
LPS = lipopolysaccharide
MAM = mitochondrial-associated membrane
SDF-1 = stromal cell-derived factor-1
SMC = smooth muscle cell
TMS = transmembrane segment
VEGF = vascular endothelial growth factor
YY1 = Yin Yang 1