

# Non-canonical vs. Canonical Functions of Heme Oxygenase-1 in Cancer

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Heme oxygenase-1 (HO-1) is a critical stress-responsive enzyme that has antioxidant and anti-inflammatory functions. HO-1 catalyzes heme degradation, which gives rise to the formation of carbon monoxide (CO), biliverdin, and iron. The upregulation of HO-1 under pathological conditions associated with cellular stress represents an important cytoprotective defense mechanism by virtue of the anti-oxidant properties of the bilirubin and the anti-inflammatory effect of the CO produced. The same mechanism is hijacked by premalignant and cancerous cells. In recent years, however, there has been accumulating evidence supporting that the upregulation of HO-1 promotes cancer progression, independently of its catalytic activity. Such non-canonical functions of HO-1 are associated with its interaction with other proteins, particularly transcription factors. HO-1 also undergoes post-translational modifications that influence its stability, functional activity, cellular translocation, etc. HO-1 is normally present in the endoplasmic reticulum, but distinct subcellular localizations, especially in the nucleus, are observed in multiple cancers. The nuclear HO-1 modulates the activation of various transcription factors, which does not appear to be mediated by carbon monoxide and iron. This commentary summarizes the non-canonical functions of HO-1 in the context of cancer growth and progression and underlying regulatory mechanisms.

**Key Words** Heme oxygenase-1, Cancer, Protein-protein interaction, Post-translational modification

## INTRODUCTION

Heme oxygenase-1 (HO-1) was first characterized in 1968 as a heme degradative enzyme by Tenhunen and colleagues [1]. Later on, many studies investigated the inducers of HO-1 such as heme, heavy metals, hydrogen peroxide, UV, hyperthermia, hyperoxia, sulfhydryl reagents, inflammatory cytokines, modified lipids, and growth factors [2-4]. The heme degradation catalyzed by HO-1 gives rise to release of carbon monoxide, biliverdin, and iron. Carbon monoxide and iron have anti-oxidant [5], anti-apoptotic [6], and anti-inflammatory activity [7].

Human HO-1 comprises 288 amino acids of which 22 hydrophobic residues are present in the C-terminus [8]. The structure of HO-1 with its bound substrate, heme, has been refined in a crystal form; the heme-binding pocket is formed by two helices termed as proximal and distal helices [9]. In

general, HO-1 is anchored to smooth endoplasmic reticulum through a C-terminal transmembrane domain, while the rest of the molecule is cytoplasmic. Endoplasmic reticulum anchored HO-1 faces the cytosol [10,11]. Alternatively, induction by heme and hypoxia promotes HO-1 translocation to plasma membrane [12,13], nucleus [14], and mitochondria [15]. The resistance to oxidative stress often observed in cancer cells is, in part, attributable to the upregulation of HO-1. The HO-1 overexpression correlates with poor prognosis in several malignancies. These include breast cancer [16], renal cancer [17], hepatocellular carcinoma [18], melanoma [19], and pancreatic cancer [20]. The upregulation of HO-1 is associated with cancer cell growth, resistance to anticancer therapy invasiveness, metastasis, angiogenesis, and metabolic reprogramming (Fig. 1).

Besides its canonical role in heme degradation as part of cellular cytoprotection, HO-1 directly regulates the signaling

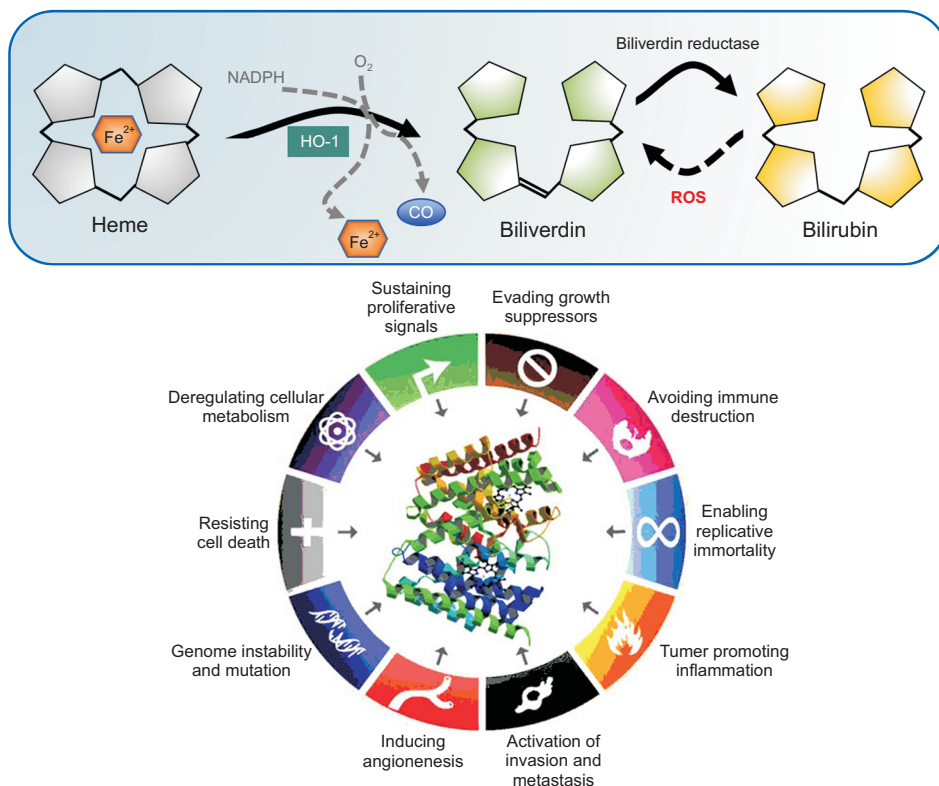
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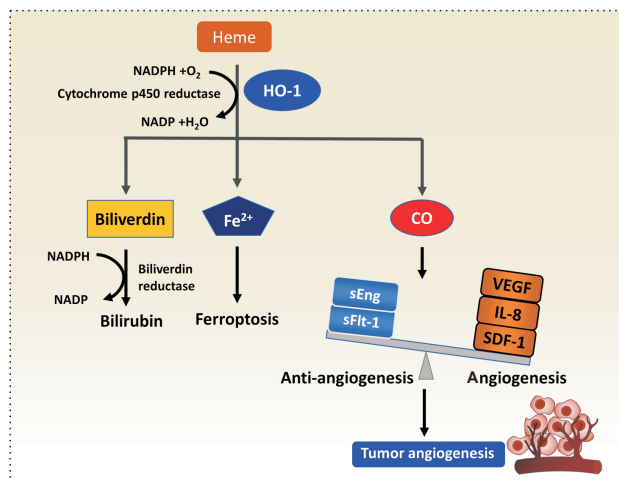
**Figure 1. Hallmarks of cancer modulated by heme oxygenase-1 (HO-1).** HO-1 degrades heme to produce carbon monoxide, iron, and biliverdin. Besides this canonical function, HO-1 is involved in manifestation of cancer hallmarks. HO-1 activates a number of oncogenic events involved in cancer cell growth, survival, and progression. ROS, reactive oxygen species.

molecules and events [21-23]. The overexpression of HO-1 in melanoma activates the B-Raf-extracellular signal-regulated kinases (ERK) pathway, thereby stimulating the proliferative signals [24]. HO-1 induces autophagy via the PI3K/AKT signaling pathway, which contributes to pharvorubicin resistance in breast cancer cells [25]. HO-1 promotes the survival signals in renal cancer cells by modulating apoptosis and autophagy regulating molecules [26]. Further, HO-1 induces the epithelial-mesenchymal transition by upregulating expression of metastatic marker genes, *snail* and *twist* in prostate cancer [27]. This mini-review highlights the non-canonical functions of HO-1 in the context of regulating redox balance and promoting cancer progression though a canonical mechanism is also involved to some extent.

**CANONICAL MECHANISM**

**Carbon monoxide**

As one of the by-products of HO-1, carbon monoxide can mediate various canonical signaling events involved in promoting tumorigenesis and contribute to poor prognosis in various cancers [28]. Carbon monoxide is a diffusible gas that regulates the synthesis of angiogenic mediators such as VEGF, interleukin 8 (IL-8), and stromal cell-derived factor 1, as shown in Figure 2 [29]. Carbon monoxide also functions as a second messenger that activates soluble guanylyl cyclase followed by cyclic-GMP production, enhancing cellular proliferation [28]. It also modulates the soluble p38 mito-



**Figure 2. Canonical functions of heme oxygenase-1 (HO-1).** HO-1 catalyzes the breakdown of heme, resulting in the formation of carbon monoxide (CO), iron, and biliverdin. CO stimulates VEGF production which promotes angiogenesis. Iron promotes ferroptosis and may play a role in cancer progression. IL-8, interleukin-8; SDF-1, stromal cell-derived factor.

gen-activated protein kinase (MAPKs), ERK, and c-Jun N-terminal kinase signaling pathways in cancer cells [28].

**Iron**

Iron is the only metallic by-product of heme degradation catalyzed by HO-1. Iron is essential for various biological process-

es such as oxygen transport, oxidative phosphorylation, DNA biosynthesis, and xenobiotic metabolism [30]. Iron undergoes transition between ferric ( $\text{Fe}^{3+}$ ) and ferrous ( $\text{Fe}^{2+}$ ) ion via oxidation-reduction reactions [30]. The ferrous iron is stored in ferritin, and the ferritin-bound iron is degraded by autophagy. The iron released during autophagy-mediated ferritin degradation induces ferroptosis [31].

Iron recycling and homeostasis are among the physiological functions of HO-1, but iron overproduction also plays a role in pathological conditions. Intracellular iron levels are maintained by ferroportin and transferrin receptor [32]. Excessive HO-1 expression decreases ferroportin 1 expression, or increased transferrin receptor expression enhances the labile iron pool, resulting in ferroptosis induction [32]. In the cytosol, iron accumulates in the form of ferrous ion as a consequence of heme degradation by HO-1. The ferrous ion reduces hydrogen peroxide to form hydroxyl radical ( $\text{HO}^\cdot$ ) via the Fenton reaction [33]. The increase in hydroxyl radical formation can cause genomic instability, oxidative DNA damage, and destruction of other biomolecules. The induction of ferroptosis, mitophagy, and ferritinophagy is attributable to overloaded iron (Fig. 2) [34]. Ferroptosis is a regulated cell death primarily caused by iron-mediated oxidative damage to the cell membrane [34]. Hemin and carbon monoxide releasing molecule (CORM), but not biliverdin or bilirubin, accelerate ferroptosis induced by Erastin [35]. Additionally, hemin and CORM induce HO-1 expression and increase membranous lipid peroxidation in the presence of Erastin. Thus, HO-1 is required for iron-dependent lipid peroxidation associated with ferroptosis [35]. The excessive iron accumulated in mitochondria contributed to the proliferation, migration and invasion of osteosarcoma, as well as stimulation of the Warburg effect [36].

## NON-CANONICAL MECHANISM

An emerging hypothesis suggests that the biological and cellular functions of HO-1 may in part relate to specific subcellular compartmentalization, and/or may transcend the catalytic breakdown of heme, with certain effector functions that are independent of its enzymatic reaction products [37].

### Protein-protein interactions

The HO-1 protein was first crystallized in 1990 [9]. It contains a heme-binding pocket at distal and proximal helices, and C-terminus contains a hydrophobic tail that is essential for membrane binding [38]. It also contains hydrophilic polar residues that regulate oncogenic signaling pathways. The protein-protein interactions were evaluated in clusters by mass spectroscopy and bioinformatics tools.

The protein-protein interactions dictate stability [39], oligomerization [40], and subcellular localization [41] of HO-1 as well as its functions. It is generally accepted that heat shock proteins, functioning as chaperones for tumor antigens, elicit tumor-specific adaptive immune responses [42]. CD91 is a

receptor for some heat shock proteins, including HSP70 and GP96 [43]. Based on the HO-1 amino acid sequence homology with Hsp70, CD91 was suggested as a putative binding partner of HO-1 [44]. The HO-1 sequence homology was analyzed using Patch Dock molecular docking algorithm and Fire Dock analysis against CD91 [23]. There are polar residues found in HO-1 including Glu63, Tyr78, Glu81, Glu82, His84, Lys86, Glu90, Gln91, and Gln102 [23]. The polar residues found in CD91 are Arg571, Thr576, Thr536, Arg553, Trp556, and Ser565 [23]. Hydrogen bond interactions are observed between Glu90 (HO-1) and Gly571 (CD91), between Glu63 (HO-1) and Thr576 (CD91), and between Gln102 (HO-1) and Thr536 (CD91). Furthermore, hydrophobic-hydrophobic interactions are observed between Tyr55, Val59 (HO-1) and Val535 (CD91) [23]. Salt bridges are found between Lys177 (HO-1) and Glu332 (CD91) [23].

Self-assembly to form dimers and higher oligomers is a common phenomenon in many membrane proteins, such as receptors, enzymes, neurotransmitter transporters, and ion channels, in which oligomerization is crucial for their proper cellular localization and function [40]. The stabilization of HO-1 through oligomerization in the endoplasmic reticulum was investigated [40]. HO-1 forms dimers/oligomers in the endoplasmic reticulum, which was not observed with a truncated HO-1 lacking the C-terminal transmembrane sequence (amino acids 266-285) [40]. As Trp 270 within the transmembrane sequence is conserved in vertebrate HO-1, it may contribute to a strong thermodynamic force in the interface and plays a prime role in the oligomeric state of HO-1. By utilizing a molecular modeling, Trp 270 was predicted as a potential interfacial residue of transmembrane  $\alpha$ -helices essential for protein-protein interactions. The W270N mutation significantly reduced microsomal HO-1 catalytic activity [40]. The decreased Fluorescence Resonance Energy Transfer (FRET) assay efficiency indicates that the W270N mutation appears to disrupt the oligomeric state. These findings indicate that oligomerization is critical for the HO-1 stability and function.

Cleavage of C-terminal transmembrane segment facilitates the nuclear translocation of HO-1 and cancer progression. In HeLa and H1299 cells, overexpression of a truncated HO-1 lacking the transmembrane sequence promoted proliferation, migration, and invasion of these cancer cells [45]. Inhibiting nuclear localization of truncated HO-1 abolished its tumorigenic effect [46]. In alveolar macrophages, the endoplasmic resident HO-1 translocates to the plasma membrane and binds to the caveolin scaffolding domain of caveolin [47].

The association of HO-1 with STAT3 in prostate cancer hampers the nuclear localization of STAT3. Thus, HO-1 interaction inhibits the androgen receptor/STAT3 signaling pathway [48]. 14-3-3 family proteins interact with HO-1, thereby enhancing its stability and activating the STAT3 signaling pathway in hepatocellular carcinoma [39]. The cholesterol-induced hypoxia triggers the association of HO-1 with emopamil-binding protein and activates the AKT signaling pathway

[49]. The LNCaP prostate cancer cells express the truncated 28-kDa HO-1 that is predominantly localized in the nucleus as a complex with nuclear factor-erythroid factor 2-related factor 2 (Nrf2) [50]. Notably, nuclear HO-1 rescued Nrf2 from glycogen synthase kinase 3 beta (GSK3β)-mediated degradation. Further, cells enriched with nuclear HO-1 exhibited a significant increase in transcription of Nrf2 target genes, such as NAD(P)H:quinone oxidoreductase and glucose 6-phosphate dehydrogenase [50]. Thus, the preferential activation of cytoprotective mechanisms through stabilization of Nrf2 by nuclear HO-1 confers long-term tolerance to oxidative injury and cell survival of cancer as well as cells. HO-1 interacts with glucocorticoid receptors, which is responsible for the poor prognosis of prostate cancer [51]. The acetylation of nuclear HO-1 by p300/CREB-binding protein (CBP) histone acetyltransferase facilitates its interaction with Jun D, leading to transcriptional activation of AP-1 [45]. The resulting acetylated HO-1 enhanced growth, migration, and invasiveness of HeLa and H1299 cells, and xenograft tumor growth and metastasis [45].

### Post-translational modification

The post-translational modifications represent the key covalent conjugation of proteins. Phosphorylation, acetylation, and ubiquitination are the major post-translational modifications. The principal post-translational modifications of HO-1 and their functional significance are summarized below.

#### 1) Phosphorylation

Phosphorylation is one of the most important post-translational modifications observed in diverse pathophysiologic conditions. It regulates cancer signaling pathways by modulating the activity or stability of target proteins, their interaction with other proteins, subcellular localization, etc. [52]. Based on screening the proteomic data by mass spectrometry, the serine 229 residue of HO-1 was found to be a putative site phosphorylated by TANK binding kinase-1 [53]. Salinas et al. [54] found Ser 188 as the potential site for phosphorylation of HO-1 by AKT/PKB. The phosphorylation sites of HO-1 were predicted by utilizing by in silico bioinformatic tools and public databases such as the human protein database (<https://www.hprd.org/>). There are about five sites of serine/threonine-proline phosphorylation (Table 1). HO-1 is likely to be phosphor-

ylated by diverse kinases including AKT, Src kinase, GSK3β, casein kinase 1, ERK, and other MAPKs.

#### 2) Acetylation

Acetylation is another important post-translational modification that regulates the various cellular processes. It influences the protein dynamics such as protein folding, stability, and localization [55]. The HO-1 acetylated by p300/CBP interacts with Jun D at Lys 243 and induces AP-1 transcriptional activity [45]. To explore global acetylation of HO-1, we utilized an algorithm Group-based Prediction System (GPS). We evaluated the potent internal acetylation, using a computational approach called GPS-based prediction of acetylation on internal lysines (PAIL). It evaluates the possible histone acetyltransferases through a BLOSUM 64, amino acid substitution matrix. The algorithm compares the query sequence to an experimental acetylation of a known peptide [56]. As a result, we estimate that among the possible acetylated sites, Lys 143 and Lys 149 are more potential acetylation sites of HO-1 (Table 2). The residues were measured based on a high score with a specific threshold (<http://pail.biocuckoo.org/online.php>).

**Table 2.** The prediction of acetylated sites by GPS-PAIL database

Peptide	Position	Score	Threshold
DLSEALKEATKEV	18	0.76	0.2
ALKEATKVHTQA	22	1.11	0.2
VTRDGFKLVMASL	48	1.14	0.2
PEELHRKAALEQD	86	0.63	0.2
AMQRYVKRLHEVG	116	1.67	0.2
SGGQVLKIAQKA	148	1.38	0.2
GGQVLKIAQKAL	149	2.14	0.2
LKKIAQKALDLPS	153	2.27	0.2
ASATKFKQLTRSR	177	1.46	0.2
RVIEEAKTAFLLN	204	0.81	0.2
RQRASNKVQDSAP	243	0.59	0.2
VETPRGKPLNTR	256	0.93	0.2

GPS, Group-based Prediction System; PAIL, prediction of acetylation on internal lysines. **K** refers the site for N<sup>ε</sup>-lysine acetylation. Higher score indicates the higher probability of acetylation.

**Table 1.** The prediction of consensus phosphorylation of HO-1 using experimentally curated functional WW domains in phosphorylation site data base

Position in query protein	Sequence in query protein	Corresponding motif described in the literature	Features of motif described in the literature
71-72	SP	[pS/pT]P	WW domain binding motif
229-230	SP	[pS/pT]P	WW domain binding motif
108-109	TP	[pS/pT]P	WW domain binding motif
192-193	TP	[pS/pT]P	WW domain binding motif
252-253	TP	[pS/pT]P	WW domain binding motif

HO-1, heme oxygenase-1; S, serine; T, threonine; P, proline; WW, proline rich short sequence.

### 3) Ubiquitination

Ubiquitination is a universal post-translational modification, mediated through ubiquitin conjugation at a specific lysine residue of the target protein destined to be degraded by 28S proteasomes. It regulates cellular processes such as DNA repair, inflammation, and endocytosis [57]. Ubiquitination is a critical post-translational modification for destabilizing a protein and regulating various cancer signaling pathways. The translocation in renal carcinoma, chromosome 8 (TRC8), an E3 ubiquitin ligase, interacts with HO-1 and ubiquitinates it, and the lack of *trc8* stabilizes HO-1 and elevates its expression, promoting the tumor progression [58]. We implemented the Bayesian decision theory algorithm [59] to investigate potential ubiquitinated residues of HO-1. As a result, it was predicted that Lys 18, Lys 116, Lys 149, and Lys 153 might be potential sites for ubiquitination of HO-1 (Table 3). This computational prediction is useful to analyze more diligent outcomes (<http://bdmpub.biocuckoo.org/prediction.php>).

### Sub-cellular localization

HO-1 was initially purified as a 32-kDa microsomal protein mainly found in the smooth endoplasmic reticulum. However, HO-1 can also translocate into the plasma membrane, mitochondria, and nucleus under some stress conditions [12,60,61]. Caveolin 1 is a scaffolding protein and the main component of flask-shaped plasma membrane invaginations called caveolae. HO-1 binds to caveolin 1, and thereby induces the polarization of M1 to M2 macrophages and down-regulates IL-6 and other inflammatory cytokines [47]. The mitochondrial translocation of HO-1 induces mitophagy and promotes ferroptosis in breast cancer cells [32]. A 28-kDa HO-1 protein with truncation of the C-terminal amino acids lacking the membrane-bound domain was found in the nucleus [41,46].

**Table 3.** The prediction of ubiquitinated sites in HO-1 by BDT algorithm

Peptide	Position	Score	Threshold
QDLSEALKEATKEVH	18	2.14	0.3
EALKEATKEVHTQAE	22	1.65	0.3
QVTRDGFKLVMASLY	48	1.26	0.3
FPEELHRKAALEQDL	86	0.92	0.3
PAMQRYVKRLHEVGR	116	2.27	0.3
LSGGQVLKKAQKAL	148	1.34	0.3
SGGQVLKKAQKALD	149	2.40	0.3
VLKKAQKALDLPSS	153	2.69	0.3
PNIASATKFKQLYRS	177	0.46	0.3
QRVIEEAKTAFLLNI	204	0.49	0.3
LRQRASNKVQDSAPV	243	0.47	0.3
PVETPRGKPLNTRS	256	0.78	0.3

HO-1, heme oxygenase-1; BDT, Bayesian decision theory; K: Lysine residues potentially ubiquitinated. Higher score indicates the higher probability of ubiquitination.

### 1) Mitochondrial HO-1

Mitochondria is one of the key generators of reactive oxygen species. HO-1 translocates to the mitochondria in a truncated form. The mitochondrial HO-1 accumulates in the presence of cellular stress such as smoking, oxidative stress, and hypoxia [15,60,62]. Mitochondrial HO-1 induces mitophagy and ferroptosis in breast cancer cells treated with BAY117085, an NF- $\kappa$ B inhibitor [32]. Mitochondrial HO-1 accumulation alters mitochondrial protein profiles, regulates redox potential, and promotes tumorigenesis [15].

### 2) Nuclear HO-1

HO-1 is prone to undergo intramembrane proteolysis which facilitates its migration from the endoplasmic reticulum to the nucleus to promote cancer growth and invasiveness regardless of its enzymatic activity [45]. The transmembrane sequence region of HO-1 is located at the carboxyl end, and HO-1 is subjected to proteolytic cleavage to produce a truncated form that consists of 237 amino acids and 28 kDa fragments [41]. Although bioinformatic analysis predicted a monopartite nuclear localization sequence at position 111 and a bipartite nuclear localization sequence at position 196 for HO-1, it is unknown whether an importin-related mechanism mediates nuclear HO-1 import [23]. On the other hand, a lysine-rich region is highly homologous to a nuclear export motif on the HO-1 protein, and its function establishes through interaction with chromosomal maintenance region protein 1 [41].

While devoid of heme-degrading activity, nuclear HO-1 has been considered as a regulator of nuclear transcription factors [37]. For instance, the HO-1 protein in the nucleus inhibits DNA binding activity of NF- $\kappa$ B while activating core-binding protein (CBF), Brain-specific homeobox/POU domain protein 3, and AP-2 transcription factors [41]. Interestingly, depending on the type of stimulus, truncated HO-1 can either protect or kill cells. For example, both full-length HO-1 and truncated HO-1 expression protect cells from death when exposed to oxidative conditions [41].

Sacca et al. [61] reported that nuclear HO-1 might be involved in the development of prostate cancer. The truncated HO-1 promotes autophagy, thereby inducing chemoresistance in Her2 targeted therapy [63]. The truncated HO-1 acetylated by p300/CBP in nucleus interacts with JunD and stimulates AP-1 transcriptional activity in H1299 and HeLa cells, promoting their growth and migration [45]. The expression and subcellular localization of HO-1 increased with tumor progression in a mouse model of squamous cell carcinoma and head and neck squamous cell carcinoma [64]. Taken together, these findings suggest that aberrant HO-1 overexpression in head and neck squamous cell carcinoma and its nuclear localization is associated with malignant progression. Birrane et al. [65] have reported that cigarette smoke-induced nuclear translocation of HO-1 promotes the secretion of VEGF in prostate cancer cells.

The DNA G4 quadruplexes (G4) structures comprise the members of helicase superfamilies such as SF1, Pif1, or SF2, RecQ, Fanconi anemia group J protein, Bloom syndrome protein, and Werner syndrome protein [66]. The G4 structures are key regulators of oncogenes and cancer driving genes such as *c-MYC*, *K-RAS*, *PDGF-A*, and *VEGF-A* [67]. The nuclear HO-1 interacts with G4 structures, and thereby promotes genome instability and DNA damage responses [68]. The proximal ligation assay data verifies the interaction between HO-1 and quadruplex DNA [68].

### Clinical significance of HO-1

Nuclear HO-1 has been shown to be expressed aberrantly in many cancers. HO-1 is localized in the nuclear compartment of metastatic prostate cancer but not in benign prostatic hypertrophy [61]. Similarly, HO-1 immunoreactive protein is detected in the nucleus of malignant head and neck cancer; nuclear localization of HO-1 is associated with tumor progression of head and neck squamous cell carcinomas [64].

HO-1 bound to an Nrf2 immunoreactive fragment and altered cellular metabolism by preferentially inducing specific Nrf2 downstream genes such as glucose-6-phosphate dehydrogenase. The LNCaP prostate cancer cells are constitutively enriched with the 28-kDa truncated form of HO-1 that resides in the nucleus as a complex with Nrf2 [50]. Nuclear HO-1 stabilizes Nrf2 by protecting it from GSK3β-mediated degradation. As a result, it is associated with an increase in resistance to glucose deprivation, implying a preference for the hexose monophosphate shunt pathway [50]. The nuclear retention and activation of Nrf2 induce transcriptional regulation of antioxidant enzymes and may enable a feed forward adaptive reprogramming for recovery and a survival advantage of cancer cells under oxidative stress [50].

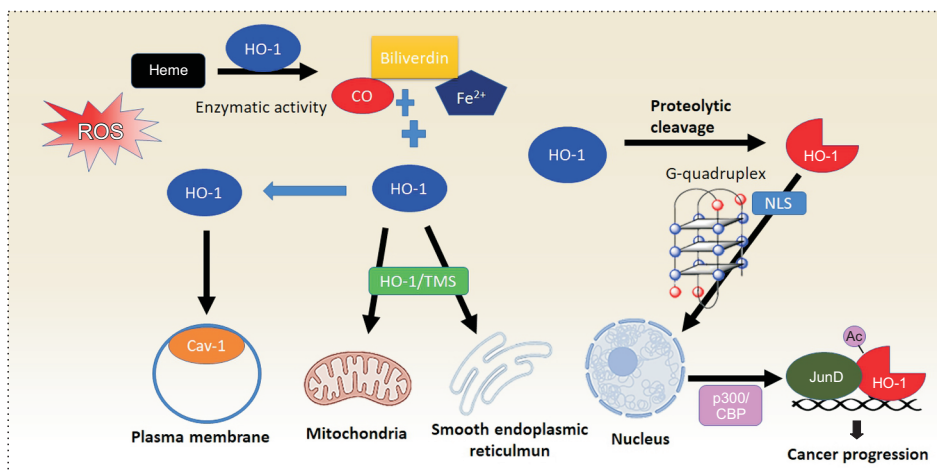
Though HO-1 overexpression plays a role in tumor progression, it also has an opposite function in some cancer cells. STAT3 mediates the activation of the androgen receptor which is a key step in the development of prostate cancer. Elguero et al. [48] demonstrated that the HO-1 protein could interact with STAT3 and enhance the cytoplasmic retention of this transcription factor, leading to repression of its transcriptional activity in prostate cancer cells. Given that STAT3 regulates cell proliferation, migration, and invasion via androgen receptor signaling involved in inflammation and angiogenesis, blockage of STAT3-androgen receptor axis by HO-1 through direct interaction with STAT3 represents a novel function for HO-1 in prostate cancer, beyond its conventional role in heme degradation [48].

Another mechanism by which HO-1 modulates tumor growth and progression may involve suppression of NF-κB activation and subsequent transcription of angiogenic as well as inflammatory genes, particularly those encoding VEGF, HIF-1α, and α5β1 integrin [69]. These findings propose HO-1 as a novel modulator of the angiogenic switch in prostate cancer [69]. So clinical application of HO-1 inhibitors or inducers/activators is controversial due to its dual functions in proliferation and progression of cancer cells.

### CONCLUSION AND FUTURE PERSPECTIVES

Carbon monoxide and iron accumulated during HO-1-mediated heme degradation play a pivotal role in signal transduction in various malignancies. The upregulation of carbon monoxide production promotes angiogenesis in several cancers. The iron mediates mitophagy, ferroptosis, and ferritinophagy, that modulate cancer development and progression.

The non-canonical mechanisms underlying oncogen-



**Figure 3. The non-canonical functions of heme oxygenase-1 (HO-1) involved in the cancer progression.** HO-1 localizes in the smooth endoplasmic reticulum. However, HO-1 can also translocate into plasma membrane, mitochondria, and nucleus. HO-1 is cleaved by a proteolytic enzyme and translocated into the nucleus with nuclear localization sequence. Truncated HO-1 interacts with G-quadruplex and enhances gene instability. The truncated HO-1 acetylated by p300/CBP in the nucleus interacts with JunD and enhances tumor progression. ROS, reactive oxygen species; CO, carbon monoxide; CBP, CREB-binding protein; TMS, transmembrane sequence; NLS, nuclear localization signal; Cav-1, Caveolin 1.

ic functions of HO-1 include protein-protein interactions, post-translational modifications, and sub-cellular localization (Fig. 3). HO-1 binds to other proteins and activates signaling pathways involved in growth, survival, migration, invasion, and metastasis of cancer cells. The post-translational modification of HO-1, such as acetylation, ubiquitination, and phosphorylation, modulates the stability function, and sub-cellular localization of this enzyme. The translocation of HO-1 into the nucleus modulates activity of transcription factors and their regulators involved in tumorigenesis.

We investigated potential residues for principal post-translational modifications of HO-1 by using *in silico* techniques. The proteomics analysis can also facilitate prediction of putative sites for post-translational modification in HO-1. The non-canonical mechanisms of HO-1 merit further investigations as a novel therapeutic target.

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## CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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