

Selective inhibition of hemeoxygenase-1 as a novel therapeutic target for anticancer treatment

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Abstract

Effective and safe anticancer treatment remains a challenge to the scientific community. Major disadvantage inherent to current anticancer strategies is their lack of targeting tumour cells, or tissues resulting in severe dose limiting toxicity. Researches in the field of anticancer drug delivery are currently exploring the potentials of nanotechnology to realize the “magic bullet” professed by Paul Ehrlich at the turn of the 20th century. Heme oxygenase-1 (HO-1) is over expressed as a survival factor in tumour tissues to withstand adverse tumour micro environmental factors such as hypoxia, hypoglycaemia, and significant acidity. Inhibition of HO-1 activity thus can be a viable anticancer strategy. However HO-1 is essential for multiple physiological and adaptive responses in normal tissues of different organ systems. Utilizing nanotechnology advancement to selectively inhibit HO-1 activity in tumour tissue is being currently explored as a novel strategy for effective anticancer management. In this review we discuss the function of HO-1 in physiological conditions, its role in cancer progression and the potential therapeutic implication for selective inhibition of HO-1 in tumour tissues.

The physiology of hemeoxygenase, its isoenzymes and tissue of origin

Heme oxygenases are the rate limiting enzymes that catalyze the metabolism of heme into equimolar concentrations of carbon monoxide (CO), free iron and the bile pigment biliverdin. Biliverdin is further converted to bilirubin by biliverdin reductase [1,2]. CO, the product of heme degradation acts as a physiological stimulator of soluble guanylate cyclase (sGC) and regulates neuronal, vasodilatory and inflammatory signaling [3]. The functional role of CO was verified by using Zinc protoporphyrin (ZnPP), the competitive inhibitor of HO-1, which acts by inhibiting soluble guanylate cyclase (sGC) [4].

In humans there are three active isoforms of heme oxygenase namely, HO-1, HO-2 [1,5] with HO-3, the least active isoenzyme having 90% homology with HO-2 [6]. Both HO-1 and HO-2 isoenzymes are products of two distinct genes and share approximately 40% amino acid homology [7]. HO-1 is a 32 kDa protein also known as heat shock protein-32 (Hsp32) which was first purified from rat liver [8]. Subsequently, it was also identified in humans [9] and was found to be constitutively expressed in human renal inner medullary cells [10], Kupffer cells in the liver [11], purkinje cells in the cerebellum [12] and CD4+/CD25+ regulatory T lymphocytes [13] under normal physiological conditions. This wide range of expression of HO-1 in different organs was a clue of its important role for different organ functions. The expression of HO-1 can also be induced by variety of stimuli such as its own substrate heme, reactive oxygen species (ROS), hydrogen peroxide, heavy metals, hypoxia, NO, ultraviolet radiation, prostaglandins, cytokines, growth factors like insulin and lipopolysaccharide and certain therapeutic agents such as non-steroidal anti-inflammatory drugs, antidiabetic thiazolidinediones and statins [14-18]. HO-2 is a 36 kDa protein which is found to be expressed in testis, brain, endothelium, distal nephron segment, liver and gut myenteric plexus [1,2]. The biological functions of HO-1 are mainly associated with a basic adaptive and defensive response against oxidative and cellular stress and to maintain cellular homeostasis [19,20]. Numerous cell signaling pathways including extracellular signal-regulated kinases ERK1 and ERK2, c-jun-NH2-kinase (JNK) and p38 kinase, protein kinase C (PKC), phosphoinositol and protein kinase A mediate the transcription of HO-1, which ultimately regulates

cell survival and offers cytoprotection [21]. The central role of HO-1 in protection against oxidative stresses was demonstrated in HO-1 knockout mice [22] and also in a patient with an inherited HO-1 deficiency [23] where results showed a reduction in the protective responses against oxidant stress (Figure 1)

CO generated during heme catabolism assists in cytoprotective effects via anti-inflammatory, anti-proliferative and antiapoptotic activity [21]. Cross talk exists between HO system and NOS system [24]. It is evident that the NO/NOS system induces CO/HO system while CO/HO system reciprocally regulates the NO/NOS system [25]. HO can regulate the production of NO via multiple mechanisms (Maines, 1997). NO/HO-1 system has been shown to produce pro-tumoral effects through decrease cell growth inhibition and induction of cell survival [26].

Prelude to the protective effect of HO-1 in cancer cells were the various preclinical and clinical studies demonstrating a protective role of HO-1 in cardiovascular, renal disease and ischemia perfusion injury. Wang et al. [27] reported that sustained HO-1 upregulation in the failing heart serves to mitigate detrimental left ventricular (LV) remodeling via antioxidant, antihypertrophic, antifibrotic, and proangiogenic effects in mice [27]. Moreover, a clinical study in patients with peripheral artery disease showed that HO-1 genotype exerts protective effects against adverse coronary events [28]. Similarly, HO induction exerts a protective effect on renal function in animal models of rhabdomyolysis, cisplatin nephrotoxicity and nephrotoxic

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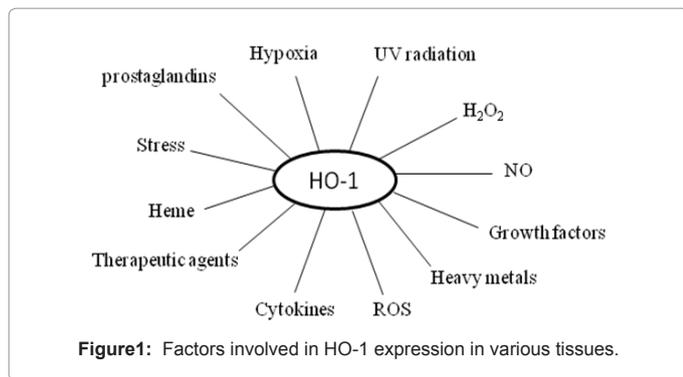


Figure 1: Factors involved in HO-1 expression in various tissues.

nephritis [29]. Furthermore, HO-1 derived CO provided protective effects in acute kidney injury and hypertension. In addition, HO-1 derived CO increased blood carboxyhemoglobin levels, renal blood flow and glomerular filtration [30]. *In vivo* expression of HO-1 protects kidneys from acute ischemic failure or ischemia/reperfusion injury [31] and cardiac xenografts from rejection [32]. Also, exposure of kidney graft recipients to low concentrations of HO-1 derived CO imparted significant protective effects against renal I/R injury and improve function of renal grafts [33]. The wealth of these studies warranted the evaluation of the role of HO-1 in solid tumors.

Role of HO-1 in tumor progression and tumor maintenance

While the HO-1 mediated cyto-protective effect plays an essential role in adaptive protection of different organs against oxidative stress, it can also shift the endogenous balance between apoptosis and proliferation towards an anti-apoptotic and anti-proliferative status, thereby promoting cancer formation and maintenance. HO-1 over expression was demonstrated in various cancer cells compared to surrounding healthy tissue leading to an increased survival of neoplastic cells [34]. In addition, HO-1 gene polymorphism was associated with an increased chance of cancer development [35].

HO-1 is over expressed in various tumor tissues derived from all the three germ layers such as brain tumors, lung cancer, hepatoma, colon carcinoma and prostate carcinoma [36-41]. HO-1 was induced in cancer cells by different stimuli. For example, in Kaposi sarcomas, HO-1 is induced by a Kaposi sarcoma-associated herpes virus (KSHV) [42] and in chronic myeloid leukemia and mast cell neoplasm it was induced by oncogenes BCR/ABL fusion kinase [43] and KIT D816V [44], respectively. Moreover, HO-1 was induced by NO in AH136B hepatoma [45] and by hemin and cadmium in human gastric cancer cells [46]. Finally, HO-1 can also be induced in cancer cells in response to chemotherapy, irradiation or photodynamic therapy [47-49]. The inherent over expression either in response to tumor microenvironment or in response to suboptimal anticancer therapy can thus provide a valuable therapeutic opportunity for treatment to both primary as well as refractory tumors (Figure 2).

Although, exact mechanism by which HO-1 causes increased proliferation and survival of cancerous cells is uncertain, some of the widely reported processes include, antiapoptotic effects, altered expression of cell cycle and promotion of angiogenesis [19,34]. HO-1 effect on the cell cycle is mainly mediated through the cell cycle regulatory protein, p21. HO-1 activation reduces the expression of p21 in endothelial cells, melanoma and colon carcinoma [50,34]. However, p21 expression was found to be up regulated in thyroid carcinoma [51] and gastric cancer [46].

The antiapoptotic effects of HO-1 have been documented in various cancer cells. HO-1 blocks apoptosis by three major pathways, namely, decreased intracellular pro-oxidant level, increased bilirubin level and elevated CO production [19]. Lin et al. [52] demonstrated that nuclear localization of HO-1 is an important signaling event in cancer cells which may up regulate genes that provide cytoprotection from oxidative stress [52]. In rat AH136B hepatoma cells, HO-1 exerted anti-apoptotic effects against oxidative stress induced by NO [45]. In melanoma cells, HO-1 overexpression caused resistance against oxidative stress and consequently leads to tumor growth *in vivo* [34]. The cytoprotective role of HO-1 has been shown to be dependent on p38 MAPK and PI3K/Akt signal transduction pathway which further modulate the expression of apoptosis related genes [5]. Specifically, antiapoptotic effects of HO-1 in gastric cancer cells are independent of p53 status in a p38 MAPK and ERK mediated pathway and show elevated caspase inhibitory protein2 (c-IAP2) and decreased caspase3 activity [46]. In addition, the increased activity of HO-1 was associated with increased nuclear localization of NFκB. The antiapoptotic effect of HO-1 was also reported in thyroid cancer cells [51]. This effect was mediated via activation of a p38 MAPK and ERK. Moreover, Busserolles et al. [50] reported that HO-1 produced resistance to apoptosis in colon cancer cells by modification of the Bcl-2/Bax ratio towards survival [50]. This effect was independent of p38 but mediated via the Akt pathway. In bladder cancer, HO-1 induced by hypericin-photodynamic therapy required functional p38 MAPK and PI3K pathways to confer a cytoprotective effect, probably through the control of the nuclear availability of the Nrf2 pool [48]. Furthermore, Banerjee et al. [53] reported the role of the Ras-Raf-ERK pathway that activates the expression of HO-1 in human renal cancer cells [53]. This further mediates anti-apoptotic signal leading to cancer cell survival. The cytoprotective action of HO-1 was also enhanced by supplementation of cultured cells with biliverdin or bilirubin as shown in hepatoma and colon carcinoma cells [45,50]. However, HO-1 derived CO was unable to provide cytoprotection in colon carcinoma, gastric cancer cells and chronic myelogenous leukemia [43,50].

Koiso et al. [54] reported the role of HO-1 in the modification of differentiation of human myeloid leukemia cells (K562) [54]. Similarly, Wang et al. [55] reported the association of high expression of HO-1 and tumor differentiation in gall bladder cancer [55]. Further, Mayerhofer et al. [56] reported that HO-1 is involved in BCR/ABL-dependent survival of CML cells [43].

Another mechanism by which HO-1 leads to cancer cell survival is by offering resistance to anticancer treatment as shown in pancreatic cancer [47], colon cancer, lung carcinoma [39] and chronic myeloid leukemia [43,56]. The enhanced sensitivity of cancer cells towards radiotherapy and chemotherapy was further explored by therapeutic inhibition of HO-1 in these cells.

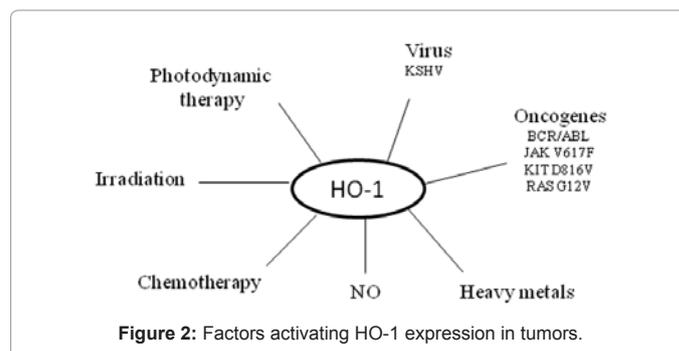


Figure 2: Factors activating HO-1 expression in tumors.

HO-1 may also play a role in tumorigenesis by reducing antitumor immunity and anticancer immunotherapy. It is established that HO-1 exerts T cell immune suppression thereby generating induced T regulatory cell (Treg) activities and helping cancer cells to escape immune response [57]. Importantly, HO-1-specific CD8+T cells were detected ex vivo and in situ among T lymphocytes from malignant melanoma, renal cell carcinoma and breast cancer patients which effectively suppressed cell immune responses [58]. HO-1 specific T cells isolated from the peripheral blood of cancer patients inhibited cytokine release, proliferation and cytotoxicity of other immune cells.

Recently, Tauber et al. [59] carried out gene expression profiling of HO-1 and reported its association with tumorigenesis. They demonstrated that the protein network downstream of HO-1 modulates adhesion, signaling, transport, and other critical cellular functions of neoplastic cells and therefore promotes tumor cell growth and dissemination [59]. The role of HO-1 gene promoter polymorphism is studied in various cancer patients and its association with cancer development is established. For example, Vashist et al. [60] evaluated the prognostic value of the transcription controlling GTn repeat germ line polymorphism in the promoter region of the HO-1 gene in curatively resectable pancreatic cancer patients. They found that the short GTn allele (SGTn) was associated with aggressive biological tumor behavior. Furthermore, SGTn had the worst disease-free and overall survival. They also reported a steadily increasing risk between LL, SL, and SS genotype patients for larger tumor size, presence of lymph node metastasis, poor tumor differentiation and higher recurrence rate [60]. Similarly in urothelial cancers, constitutive expressions of HO-1 were associated with the presence of SGTn [61]. Some studies have also reported the association of higher frequency of long GTn allele (LGTn) and greater risk of cancer as shown in patients with oral squamous cell carcinoma [62], lung adenocarcinoma [35], esophageal squamous cell carcinoma [63], breast cancer [64] and gastric adenocarcinoma [65].

Role of HO-1 in angiogenesis and metastasis

Tumor progression beyond 2 mm is totally dependent on efficient blood supply [66]. Further access of tumor cells to functional blood vessels is a prerequisite for its metastasis to distant organs. Angiogenesis is the process of formation of new blood vessels which supply nutrients for growing tumors. Tumor angiogenesis thus, is essential for the development and metastasis of tumors [66]. HO-1 has shown proangiogenic potential in addition to the cytoprotective effects. It was reported that genetic over expression of HO-1 in endothelial cells increased production of VEGF and consequently produced endothelial cell proliferation, migration and formation of capillary-like tube structure [67]. Soares et al. [32] first demonstrated that the overexpression of HO-1 prevents apoptosis in endothelial cells [32]. This anti-apoptotic effect was mediated via degradation of p38 α MAPK isoform [68].

HO-1 promotes endothelial cell proliferation and tumour vascularization in various types of cancers [69]. For example, expression of HO-1 increases the angiogenic potential of murine melanoma resulting into increased tumor vascularization [34]. In human gliomas and vertical growth melanomas, HO-1 expression was observed in infiltrating macrophages leading to increased vascular density and tumor vascularization [12,70]. Furthermore, in melanoma and oligodendroglioma, expression levels of HO-1 in macrophages correlated with tumor cell invasiveness and poor prognosis [36,70]. HO-1 stimulated *in vitro* tumor angiogenesis and increased endothelial cell survival in pancreatic carcinoma [69]. Recently, Miyake et al. [61] demonstrated that overexpression of HO-1 promotes angiogenesis

in urothelial carcinoma cells [71]. In addition, inhibition of HO-1 *in vivo* decreased tumor growth and micro vessel density (MVD) by suppressing angiogenic factors, particularly HIF-1 α and subsequently VEGF. Furthermore, the principal role of HO-1 in angiogenesis was confirmed through administration of HO-1 inhibitor or siRNA which showed decreased VEGF expression and cell survival as shown in endothelioma, hepatocellular carcinoma, lung carcinoma and in tumors formed by transformed fibroblasts [72,42,73,41]

As angiogenesis further leads to the metastasis, the effect of HO-1 expression on metastasis has also been studied. Was et al. [34] reported that expression of HO-1 in melanoma cells leads to the increased number of metastasis in lung which further shortened the survival of mice [34]. Similarly, pancreatic cancer cells over expressing HO-1 produced increased lung metastasis in mice [69]. In prostate carcinoma, silencing of the HO-1 gene reduced cell invasion *in vitro* and inhibited growth of primary and metastatic tumors *in vivo* [74]. Recently, Chong et al. [75] reported that overexpression of HO-1 can enhance tumor metastatic ability through cell invasiveness in patients with NSCLC [75].

However, the ability of HO-1 to produce metastatic effects remains controversial. For example, endogenous HO-1 inhibits migration and the invasive capacity of certain prostate cancer cells [76]. Furthermore, in MCF-7 breast cancer cells, HO-1 inhibited invasion induced by TPA [77]. Also, colorectal cancer patients expressing HO-1 showed lower rate of lymphatic tumor invasion and fewer lymph node metastases [78] and in oral carcinoma, HO-1 was suggested as a marker of low risk of metastasis. These data suggests that the role of HO-1 in metastasis is cell specific and in some cases it can paradoxically reduce the metastatic ability of cancer cells (Figure 3).

Therapeutic implication of HO-1 inhibition

Numerous studies have reported the therapeutic implications of HO-1 in various solid tumors. Berberat et al. [47] reported higher expression of HO-1 in human pancreatic tumors [47]. The targeted knockdown of HO-1 expression led to pronounced growth inhibition of the pancreatic cancer cells and increased sensitivity towards radiotherapy and chemotherapy [47]. Similarly, Sunamura et al. [69] demonstrated that HO-1 over expression leads to pancreatic cancer aggressiveness, by increasing tumor growth, angiogenesis and metastasis. The inhibition of HO-1 expression significantly decreased the tumor growth and lung metastasis in SCID mice inoculated with Panc-1/hHO-1 cells [69]. These studies show that administration of HO-1 inhibitors might be effective for the treatment of pancreatic cancers.

HO-1 upregulation was also reported in human hepatocellular carcinoma cells (HCC) where it was associated with poor prognosis

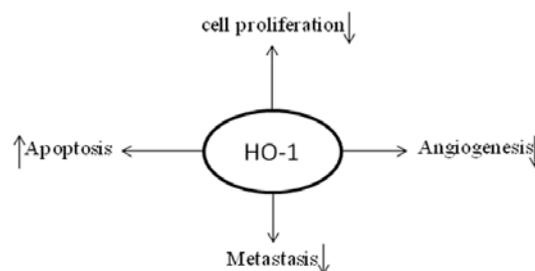


Figure 3: Possible antitumor mechanisms of HO-1 inhibition in tumors.

due to its protective and anti-apoptotic activity [41]. Down regulation of HO-1 resulted in cytotoxic effects in hepatoma cells both *in vitro* and *in vivo* [37,45]. Over expression of HO-1 contributes to tumor radio-resistance in HCC and indicates the potential therapeutic benefits of HO-1 inhibition in tumor tissues prior to hepatic irradiation [79].

Hill et al. [80] reported the higher expression of HO-1 in human breast cancer cells [80]. They showed that HO-1 inhibited human breast cancer cell proliferation. This study reported for the first time the anti-tumor activity of HO-1 in breast cancer cells and was contradictory to the anti-apoptotic effects of HO-1 in other types of cancers. In addition, HO-1 also inhibited the invasion and migration of breast carcinoma cells [77].

In addition to solid tumors, abnormal expression of HO-1 has been linked to oncogenesis and chemo resistance in hematological malignancies. It is reported that HO-1 is constitutively expressed in primary CML cells [43] and acts as a survival molecule in CML cells, as over expression of HO-1 inhibited apoptosis induced by BCR/ABL tyrosine kinase inhibitor imatinib (STI571). In another study, Mayerhofer et al. [43] showed that inhibition of HO-1 leads to the growth inhibition of imatinib-sensitive as well as imatinib-resistant CML cells [56]. HO-1 is also overexpressed in human primary acute myeloid leukemia (AML) cells where it offers protection from chemotherapy-induced apoptosis [81]. Interestingly, combined inhibition of HO-1 and NF- κ B significantly induced apoptosis in AML cells and thus provided a novel therapeutic approach to treat chemotherapy-resistant forms of AML [82].

In addition, HO-1 inhibition has been reported to have advantageous therapeutic effect on mast cell (MC) neoplasm. HO-1 was found to be overexpressed in neoplastic canine mast cells where it acts as a survival factor [83]. In human mast cells, HO-1 expression was induced by the mastocytosis-related oncoprotein KIT D816V and its inhibition led to the reduced expression of HO-1 and consequently decreased proliferation/survival in neoplastic MCs [44].

Selective inhibition of HO-1 as a new target for anticancer nanotechnology

As described before, HO-1 plays an important role in cancer progression therefore; selective inhibition of HO-1 has been explored as a novel anticancer therapy. The two main strategies used for selective inhibition of HO-1 are namely, siRNA and metalloporphyrins [16]. However, the greatest impediment in the therapeutic application of these strategies is poor solubility as well as their toxicity and poor delivery to the tumor. By using nanotechnology, various studies have shown targeted delivery of siRNA or protoporphyrins to tumors [84,85]. In the next section we discuss the therapeutic implications of both strategies and the attempted use of protoporphyrins for HO-1 inhibition by nanotechnology to address both short comings.

Selective inhibition via siRNA

Numerous studies have reported the association between decreased expression of HO-1 by siRNA and reduced cell survival in various human neoplasms both *in vitro* and *in vivo*. For example, siRNA induced knockdown of HO-1 led to increased apoptosis of cultured colon carcinoma cells, chronic and acute myeloid leukemia cells, lung cancer cells and hepatocarcinoma cells (HCC) [50,39,56,86,41]. In addition, in lung cancer cells, HO-1 siRNA increased the generation of ROS and augmented the cytotoxicity of cisplatin [39]. In pancreatic cancer cells, suppression of HO-1 expression by siRNA resulted in decreased cell proliferation and sensitization of pancreatic cells to

oxidative stress and gemcitabine or γ -radiation [47]. Importantly, HO-1 siRNA reduced growth of orthotopic hepatocellular tumors [41]. Alaoui-Jamali et al. [74] demonstrated an inhibition in the therapeutic activity of the HO-1 by using a small-molecule inhibitor OB-24, which was found to mimic the activity of HO-1 shRNA in prostate cancer cells [74]. OB-24 is a competitive and reversible inhibitor of the HO-1 enzyme which selectively inhibits HO-1 but not HO-2. OB-24 reduced cell proliferation, cell survival and cell invasion in prostate cancer cells *in vitro*. In addition, it also inhibited prostate tumor growth as well as lymph node and lung metastasis *in vivo*. Interestingly, OB-24 potentiated the anticancer activity of taxol.

Selective inhibition via proto porphyrin derivatives

Protoporphyrin (PP) IX is a heme metabolite and its iron-exchanged derivatives, such as zinc PPIX (ZnPPIX) and tin PPIX (SnPPIX), have been found to inhibit competitively *in vitro* and *in vivo* HO activity [87]. In contrast, hemin (FePP) and cobalt PPIX (CoPPIX) induce and activate HO-1, while copper PPIX (CuPPIX) does not affect HO-1 activity [87,88]. The pharmacological inhibition of HO-1 using protoporphyrins has been reported to exert cytotoxic effects in various cancer cells and thus has potential use for therapeutic treatment of cancer. For example, administration of the HO-1 inhibitor ZnPP via tumor feeding artery significantly suppressed the growth of hepatoma AH136B tumors [37] and this effect was mediated via induction of apoptosis [45]. Similarly, SnPP IX treatment also induced apoptosis in AH136B tumor cells [45]. However, SnPP IX treatment of the rats did not affect the blood flow in the tumor tissue whereas both ZnPPIX and CuPPIX decreased the blood flow to P22 carcinosarcoma tumors in rats [89]. The pretreatment of lung cancer A549 cells with ZnPP produced increased apoptosis in cisplatin-treated cells as compared with the cells treated with cisplatin alone which suggests the role of HO-1 in sensitizing lung cancer cells to cisplatin [39]. In addition, simultaneous treatment with ZnPP and cisplatin synergistically increased reactive oxygen species (ROS) generation and decreased the expression of HO-1 [39]. In colon cancer cells, Zn (II) PPIX exerted potent cytotoxic effect both *in vitro* and *in vivo* and this anticancer effect was mediated through a cell cycle arrest, caspase-3 dependent apoptosis induction and increased generation of ROS [90]. Finally, administration of ZnPP significantly inhibited progression of a B-cell leukemia/lymphoma 1 tumor in mice by specially targeting tumor cells and reported HO independent effects of ZnPP on tumorigenesis [91]. However, it is reported that the cytotoxic effect of ZnPP in rat hepatoma AH136B primary cells was reversed by the presence of bilirubin [45].

Although an inhibition of HO-1 by ZnPP has been widely used for drug development, some conflicting evidence has been reported. Nowis et al. [90] demonstrated that ZnPPIX was unable to restore cisplatin sensitivity in HO-1 over expressing melanoma cells [90]. Also, it didn't potentiate the antitumor effects of cisplatin, doxorubicin or 5-FU in C-26 colon adenocarcinoma; B16F10 melanoma and EMT6 breast adenocarcinoma models. The study warranted more selective and efficient delivery of HO-1 inhibitors to the tumor for combination therapies with chemotherapeutics.

Role of nanotechnology in targeted inhibition of HO-1 in tumors

HO-1, as evident from the above discussion, is an attractive target for inhibition of tumor progression on the cellular level. However, on tissue level many obstacles have to be overcome before the HO-1 inhibitors can reach its cellular targets at the cytoplasm of tumor cells. First, the HO-1 inhibitor needs to be water soluble so that it can be

administered as parenteral therapeutic. Second, the water soluble drug needs to reach the tumor tissues and concentrate their selectively in a therapeutically effective concentration, and finally preferably the drug can retain its therapeutic concentration for extended duration. Unfortunately, neither si RNA, nor metal protoporphyrins satisfy the abovementioned conditions. In this respect, nanotechnology comes into action to render these promising approaches into potential drug candidates. Tumor tissues are selectively permeable to macromolecules (drugs) of nanosize magnitudes due to their extensive vascular leakage [92]. Specifically, the macromolecules of size exceeding 7 nm, known as nanomedicine have an advantage of evading the tight junction in normal vasculature [92]. More importantly they escape renal clearance for being above the renal excretion threshold, thus they can attain longer circulator life in plasma [93]. As the circulatory half-life and the pharmacological effect are parallel to each other, nanomedicine tends to have prolonged and selective anticancer activity. Thus the chemical conjugation of poor water soluble HO-1 inhibitors into a long chain of high molecular weight polymeric carrier or encapsulating it into the core of a micellar carrier can impose all the advantages needed for clinical applications.

As discussed above, ZnPP is ideal for selective cancer cell toxicity, as it inhibits HO-1 which is overexpressed by cancer cells and is crucial to their survival. ZnPP has strong phototoxic properties in addition to its capability of radio-sensitization of tumor cells to megavoltage RT [94] (Figure 4). However, the pharmaceutical application of ZnPP is limited due to its poor water solubility [95]. Therefore, with the help of nanotechnology, a water-soluble micellar form of ZnPP was formulated by conjugating it with polyethylene glycol (PEG) [95]. The PEG-ZnPP micelles have a mean particle size around 180 nm [96]. This smaller size of micelles offers an advantage of higher vascular permeability at target tumor sites by diffusion mechanisms [97]. Thus, PEG-ZnPP selectively accumulated in tumor tissues utilizing the mechanism called enhanced permeability and retention (EPR) effect and exhibited targeted inhibition of HO in tumor tissue [98]. In addition, via encapsulation, micelles offer stability and thus improve pharmacokinetics and biodistribution of sparingly soluble anticancer agents [99]. Specifically, the pharmacokinetic profile of PEG-ZnPPIX nanoparticles showed a 40 fold longer plasma residence time compared to free ZnPPIX after intravenous administration [98]. Also, PEGylated ZnPP (PEG-ZnPP) exhibited the desired cytotoxic effects in various cancer cells *in vitro* and *in vivo*. For example, PEG-ZnPP induced oxidative stress, and consequently apoptotic death in colon cancer SW480 cells [98]. Interestingly, PEG-ZnPP preferentially accumulated in solid tumor tissue in a S180 murine model resulting in significant tumor suppression without any side effects [98]. This effect was mediated through targeted suppression of HO-1 and an induction of apoptosis in tumor cells. The similar effect was also observed when PEG-ZnPP was combined with another oxidative chemotherapeutic agent such as PEG-DAO/D-proline (PEG-conjugated D-amino acid oxidase with D proline) [100]. PEG-ZnPP pre-treatment significantly reduced the growth of S180 tumors in mice receiving PEG-DAO/D-proline compared to no PEG-ZnPP pre-treatment. In addition, PEG-ZnPP sensitized colon cancer cells to cytostatic/cytotoxic effects of camptothecin or doxorubicin and suggested the role of HO-1 inhibitor in potentiating the chemotherapeutic response of solid tumors [100].

SMA-ZnPP nanomicelles as a potential anticancer agent

In spite of having promising anticancer activity *in vitro* and *in vivo*, the poor drug (ZnPP) loading (1.5% ZnPP/PEG w/w ratio) was the critical shortcoming of PEG-ZnPP for its future biological applications

[101]. To overcome this problem, another highly water soluble micellar formulation of ZnPP was designed by the use of amphiphilic styrene-maleic acid copolymer (SMA), namely SMA-ZnPP [85]. SMA-ZnPP showed higher and more efficient intracellular uptake rate compared to PEG-ZnPP by endocytotic pathway followed by release of free ZnPP in the presence of membrane components [96]. After its release, ZnPP is mainly colocalized with HO-1 at endoplasmic reticulum (ER) compartment and inhibits HO-1 activity which leads to higher oxystress and cell death. SMA-ZnPP exist as nanoparticles in aqueous solution and tend to accumulate preferentially at tumor site by the EPR effect therefore it's been used in a variety of ways to induce its anticancer effect [102].

SMA-ZnPP micelles exhibited potent dose dependent HO-1 inhibitory potential as well as cytotoxic effects on KYSE-510 human esophageal cancer cells [101]. Importantly, HO-1 inhibitory potential of native ZnPP was not altered by its SMA-ZnPP formulation. In animal model, SMA-ZnPP showed potent antitumor effects without any apparent side effects [85]. Kondo et al. [44] reported that both SMA-ZnPP and PEG-ZnPP reduced the growth of mast cell leukemia HMC-1 cells in a dose dependent manner [44]. The growth inhibitory effects

	Reference
Cerebral glioblastoma and astrocytomas	(Hara <i>et al.</i> , 1996)
Oligodendroglioma	(Deiningner <i>et al.</i> , 2000)
lymphosarcoma	(Schacter <i>et al.</i> , 1982)
Malignant vertical growth melanoma	(Torisu-Itakura <i>et al.</i> , 2000)
Oral squamous cell carcinoma	(Chang <i>et al.</i> , 2004; Tsuji <i>et al.</i> , 1999)
Chronic myeloid leukemia	(Mayerhofer <i>et al.</i> , 2004)
Mast cell leukemia	(Kondo <i>et al.</i> , 2007)
Renal cell carcinoma	(Goodman <i>et al.</i> , 1997)
Prostate cancer	(Maines <i>et al.</i> , 1996)
Hepatoma	(Doi <i>et al.</i> , 1999; Sass <i>et al.</i> , 2008)
Kaposi sarcoma	(Marinissen <i>et al.</i> , 2006)
Pancreatic cancer	(Berberat <i>et al.</i> , 2005)
Colorectal cancer	(Becker <i>et al.</i> , 2007)
Lung cancer	(Hirai <i>et al.</i> , 2007)
Breast cancer	(Hill <i>et al.</i> , 2005)
Thyroid carcinoma	(Chen <i>et al.</i> , 2004)
Gall bladder cancer	(Wang <i>et al.</i> , 2010b)

Table1: Expression of HO-1 in different types of cancers

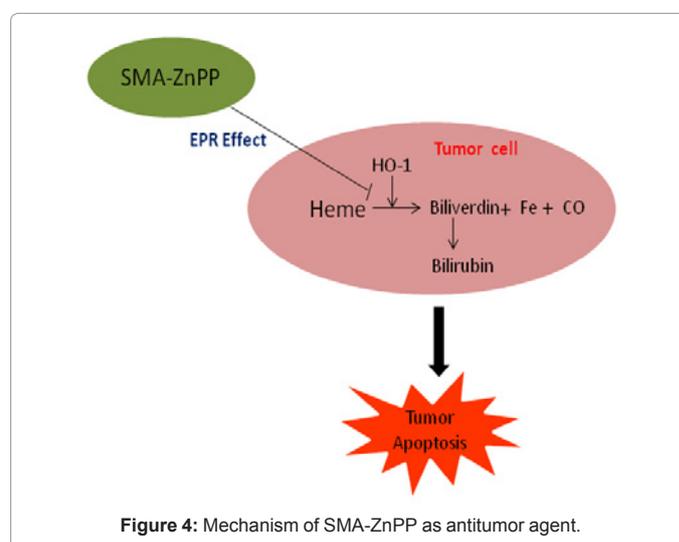


Figure 4: Mechanism of SMA-ZnPP as antitumor agent.

of SMA-ZnPP were associated with induction of apoptosis. Moreover, SMA-ZnPP showed powerful cytotoxic activity against primary CML cells obtained from patient's refractory to Gleevec therapy [56]. This response was associated with down-regulation of oncogene BCR-ABL dependent tyrosine kinase activity. Gleixner et al. [103] reported the cytotoxic effect of SMA-ZnPP in a variety of hematopoietic and non-hematopoietic (solid tumors) cells [103]. The cell death was associated with induction of apoptosis. In addition, SMA-ZnPP in combination with various targeted drugs or conventional drugs showed synergistic cytotoxicity in myeloid leukemia and various solid tumor cells *in vitro* [103].

The potential application of the SMA-ZnPP and PEG-ZnPP has also been explored in photodynamic therapy. Iyer et al. [85] reported higher cytotoxic effect of PEG-ZnPP in KYSE-510 human esophageal cancer cell line in the presence of light [85]. Similarly, Regehly et al. [94] reported that in Jurkat cells, SMA-ZnPP causes about five times higher phototoxicity compared to PEG-ZnPP due to higher uptake of ZnPP by tumor cells [94]. Furthermore, in ddY mice bearing S-180 tumors, 12mg/kg dose of SMA-ZnPP showed more effective tumor regression when irradiated by a tungsten-xenon light at a luminous intensity of 50,000 LUX for 5 min whereas, utilizing high intensity light (HIL) as a source of irradiation, SMA-ZnPP at 6 or 12 mg/kg showed marked reduction in tumor growth in DMBA induced mammary cancer model in female SD rats [85]. This effect was attributed to the synergistic effect of oxystress induced killing augmented by *in situ* free radical generation (in presence of light) by SMA-ZnPP.

Conclusion

It is now well established that HO-1 is constitutively expressed in various neoplastic cells where it acts as a survival factor and offers cytoprotection to developing tumors. In addition, over expression of HO-1 promotes angiogenesis and metastasis in tumors and advances resistance against conventional and targeted drugs in various malignancies. Numerous preclinical studies have reported that selective inhibition of HO-1 in tumors leads to reduced tumor growth and increased response to chemotherapy and radiotherapy. Targeted inhibition of HO-1 using nanotechnology has shown promising anticancer effect. The recent advancement in efficient delivery of HO-1 inhibitors to tumor sites presents a new paradigm furthering its future clinical application as anticancer agents.

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