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Forum Review

Growth Factors and Heme Oxygenase-1: Perspectives in Physiology and Pathophysiology

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ABSTRACT

Growth factors are mediators of both normal homeostasis and pathophysiology through their effects on various cellular processes. Similarly, heme oxygenase-1 (HO-1) has a role in maintaining physiologic equilibrium, by which it can either alleviate or exacerbate disease, depending on several considerations, including amount, timing, and location of expression, as well as the disease setting. Thus, the synthesis and activities of growth factors and HO-1 are intricately regulated. Interestingly, several growth factors induce HO-1, and, conversely, HO-1 can regulate the expression of some growth factors. This review focuses on the influence of growth factors and HO-1 and potential physiologic effects of the growth factor(s)–HO-1 interaction. *Antioxid. Redox Signal.* 9, 2197–2207.

INTRODUCTION

Growth factors are naturally occurring proteins that act as signaling molecules and are capable of affecting a multitude of cellular processes, including proliferation, migration, and differentiation. They are significant mediators in both normal and abnormal physiology. Growth factors also interplay and can affect each other. For instance, increased transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF) have an inverse effect on the activity level of bone morphogenic protein-7 (BMP-7) and hepatocyte growth factor (HGF), leading to an epithelial-to-mesenchymal transition (EMT) of injured tubular epithelial cells and the progression of chronic kidney disease [reviewed in (121)]. Growth factors bind to specific high-affinity, low-capacity receptors on the surface of responsive cells, inducing a series of events, including phosphorylation by tyrosine kinases or serine/threonine kinases or both. The eventual outcome is an alteration in cellular activity and changes in the program of genes expressed within the responding cells. One such example is the heme oxygenase-1 (HO-1) gene, which is modulated by several growth factors and may be a mediator for the downstream effects of these factors.

The heme oxygenases are rate-limiting enzymes that catalyze the conversion of heme into carbon monoxide, free iron and biliverdin, which is subsequently converted to bilirubin by biliverdin reductase. It exists in two major isoforms, an inducible (HO-1) and constitutive (HO-2) isoform. The products of the HO reaction modulate important adaptive responses to oxidative stress, inflammation, apoptosis, angiogenesis and fibrosis [reviewed in (40, 106]. Numerous reviews exist for the individual growth factors, as well as for HO-1, and the reader is referred to these for more elaborate details that are beyond the scope of the present article. This review highlights the current knowledge regarding growth factor–mediated HO-1 expression and its potential consequences.

TRANSFORMING GROWTH FACTOR-β (TGF-β)

TGF-βs are members of a group of structurally related, multifunctional proteins known as the TGF-β superfamily, which also includes activins/inhibins and bone morphogenic proteins

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(BMP) [reviewed in (77, 105)]. TGF-βs are peptides consisting of three highly homologous isoforms, TGF-β1, 2, and 3 (96), distinguished for their ability to inhibit the growth of epithelial and hematopoietic cells and to regulate the production of extracellular matrix by mesenchymal cells. They act through autocrine, paracrine and endocrine modes to control the pathogenesis of diseases, especially events like fibrosis, autoimmunity, and carcinogenesis (77, 105). TGF-β has a critical role in normal homeostasis. TGF-β1–null mice die of wasting and multifocal inflammation within 3–4 weeks of age; TGF-β2–null mice exhibit a wide range of developmental defects in major organs, including heart and kidneys, and die near the time of birth; and TGF-β3–null mice exhibit cleft palates and pulmonary development problems and die within 20 h of birth (27). In response to injury, TGF-βs and other growth factors are released via autocrine or paracrine mechanisms or both to maintain cellular homeostasis. They enhance wound repair by stimulating the synthesis of connective tissue matrix proteins, structural proteins (collagen and fibronectin), and by inducing proliferative arrest and differentiation (105). TGF-βs play a dual role in tissue pathology: increasing cell proliferation and migration, as well as synthesis, and deposition of extracellular matrix (ECM) results in progressive fibrosis (17, 55, 61, 76, 102, 103, 128, 129, 132).

TGF-βs are synthesized as biologically inactive aggregates consisting of mature TGF-β, latency-associated peptide, and a latent TGF-β–binding protein. Active TGF-βs are produced by proteolytic action of plasmin and cathepsin D (77, 105) and initiate signaling through interactions with three types of TGF-β receptors [TβR; reviewed in (77)]. Activation of TβR results in activation of TβR-I kinase to initiate downstream signaling events (77). The molecular mechanism of TGF-β signal transduction from the cell surface to the nucleus has been identified to occur through a group of structurally related proteins called Smads (Fig. 1). Vertebrate Smads are subdivided, based on structural and functional considerations, into receptor-regulated Smads, common mediator Smads, and inhibitory Smads (33, 35, 45, 88). Receptor-regulated Smads (Smad 1, 2, 3, 5, and 8) are pathway specific and are directly phosphorylated by activated TGF-β family receptors. Phosphorylated receptor-regulated Smads oligomerize with the common mediator Smad (Smad 4) in the cytoplasm, and this heterocomplex translocates into the nucleus to interact directly with DNA or bind to transcription factors or both (77). Inhibitor Smads (Smad 6 and Smad 7) are antagonists of TGF-β–mediated signaling events. The presence of one or more Smad-binding elements (SBE), a β hairpin with the major groove of the sequence GTCT and CAGA, is required for several genes to respond to members of the TGF-β family (22, 34, 86, 120). Smads have been shown to interact with several different transcription factors, and, based on the availability of cofactors within a cell type, TGF-β elicits different gene responses [reviewed in (22)]. In addition to the Smad pathway, the activated TGF-β–receptor complex can signal through other pathways, including mitogen-activated kinases (MAPKs), phosphoinositol-3 kinase (PI3K), and PP2A/p70s6K (122). Cross-talk among a variety of pathways is necessary for maximal stimulation of TGF-β–regulated effects (13). Some of these pathways regulate Smad activation; however, others may induce responses independent of Smads or transcription (13). The MAPK signaling pathway plays a role in TGF-β–stimulated pro-α1(I) collagen (124), collagen I (10), and PAI-1 expression (65).

A mechanism by which TGF-β may maintain cellular homeostasis is through induction of the cytoprotective protein, HO-1 (40, 42, 56, 64, 89, 90, 110). TGF-β1 increases the expression of HO-1 in human retinal pigment epithelial cells, human renal proximal tubular epithelial cells, human pulmonary epithelial cells derived from a lung cell carcinoma (A549 cells), HaCaT human keratinocytes, and bovine choroid fibroblasts (42, 63, 64, 90). However, TGF-β1 does not induce HO-1 in all cell types, including HeLa or bovine corneal fibroblasts (64). Interestingly, in an LPS-induced rat model of endotoxemia, as well as in IL-1β–treated cultured rat vascular smooth muscle cells (VSMCs), wherein HO-1 is preinduced, the HO-1 mRNA and protein expression were reduced by TGF-β1 in the heart and lung (94). TGF-β1 downregulated HO-1 mRNA after its

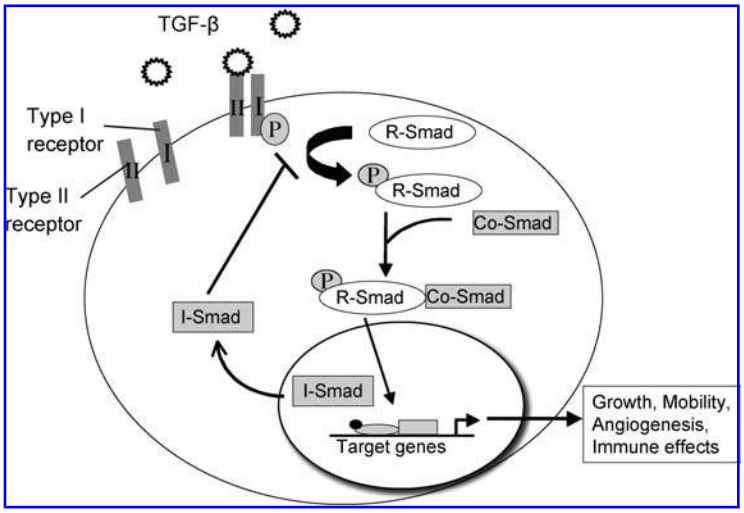

FIG. 1. Schematic diagram of the transforming growth factor-beta (TGF-β)-Smad signaling pathway. TGF-β binds to the type II TGF-β receptor, which results in the phosphorylation of the type I TGF-β receptor. The ligand-induced receptor complex phosphorylates receptor-regulated Smads (R-Smad), which includes Smad2 and Smad3 for the TGF-βs or activins and Smad1, Smad5, and Smad8 for bone morphogenic proteins (BMP) and related factors. Activated R-Smads then interact with the common mediator Smad (Co-Smad), which is Smad4 in mammals and is common for all the R-Smads. The Smad complex then translocates to the nucleus, where it can associate directly with DNA or DNA-binding cofactors as well as coactivators or co-repressors to select target genes and determine the transcriptional effect on these genes. One such gene is for the inhibitor Smad (I-Smad), which includes Smad7, which negatively regulates signaling by competing with R-Smads for either receptor or Co-Smad association. (Adapted from Massague J and Chen Y-G. Controlling TGF-β signaling. *Genes Dev* 14: 627–644, 2000).

induction by IL-1β in VSMCs (94). During endotoxemia, NO production is increased consequent to upregulation of inducible nitric oxide synthase (iNOS), and NO is a potent inducer of HO-1 (83). TGF-β1 suppresses iNOS and NO production (119), and this observation could explain the underlying mechanism for the downregulation of HO-1 by TGF-β1 during endotoxemia. TGF-β conferred a negative effect on HO-1, which contributed to the beneficial effects of TGF-β in endotoxic shock (94). Conversely, overexpression of HO-1, using retrovirus gene transfer, markedly inhibited TGF-β mRNA and protein in a rat lung microvessel endothelial cell line (1). Thus, TGF-β–mediated HO-1 induction may counteract the negative effects of TGF-β1 by blocking further TGF-β1 production or affecting cell proliferation, apoptosis, and deposition of extracellular matrix, or by a combination of these.

All three isoforms of TGF-β (1, 2 and 3), but not another member of the TGF-β superfamily, BMP-7, induce HO-1 protein in human renal proximal tubular epithelial cells (Fig. 2A and B). TGF-β1 augments HO-1 expression by increased gene transcription, is associated with an increase in HO activity, and does not involve increased mRNA stability (42, 117). Whereas inhibitors of the MAPK pathway have no effect on TGF-β1–stimulated HO-1 mRNA production in primary renal epithelial cells, it appears that p38 MAPK is a mediator in A549 cells as well as in human retinal pigment epithelial cells (32, 42, 90). Inhibitors such as *N*-acetylcysteine (NAC, an antioxidant) and quinacrine (phospholipase A_2 inhibitor) block induction of HO-1 by cytokines (TNF-α) in endothelial cells (115), and deferoxamine (DFO, an iron chelator) inhibits HO-1 induction by oxidized LDL and hyperoxia in endothelial cells (4, 25). However, TGF-β1–mediated induction of HO-1 mRNA in human renal proximal tubular epithelial cells is not affected by co-treatment with DFO, NAC, or quinacrine, suggesting that different pathways are involved (42).

HO-1 induction by TGF-β1 is also different from that of hemin. TGF-β1–mediated induction of HO-1 protein is not so persistent as that of hemin-induced (Fig. 2C), possibly because of a concomitant inhibitory Smad induction. TGF-β1 has been shown to upregulate the inhibitory Smad, Smad7, and overexpression of Smad7 inhibits the induction of the endogenous HO-1 gene by TGF-β1 in human renal epithelial cells (42). Furthermore, hemin requires the -4.5-kb promoter region and an enhancer, internal to the HO-1 gene, for maximal induction, and neither region is responsive to TGF-β1 (41). Recently, a $\sim$280-bp TGF-β1–responsive region between 9.1 and 9.4 kb of the human HO-1 promoter was identified that contains a putative SBE and Sp1 binding sites, both of which are involved in HO-1 induction by TGF-β1 (117). Taken together, regulation of HO-1 by TGF-β is complex and not completely understood.

Understanding the cellular effects and molecular mechanisms of HO-1 gene expression in response to TGF-β will be important in designing interventional strategies in TGF-β–mediated diseases. Increased expression of TGF-β leads to enhanced ECM deposition, resulting in fibrosis. TGF-β can also stabilize and attenuate tissue injury through the activation of cytoprotective proteins, which include HO-1 (Fig. 3). Induction of HO-1 is an adaptive response to provide a balance for some of the effects of TGF-β1, mediated through its reaction product(s); the production of antioxidants (bilirubin), degradation of pro-oxidants (heme), removal of free iron (through induction of ferritin), and production of CO (vasodilator and antiapoptotic). In addition, HO-1–catalyzed bilirubin and CO production have antifibrotic effects that may also balance the profibrotic effects of TGF-β (26, 30, 67, 74, 82, 123, 131). Perhaps persistently elevated TGF-β1 overwhelms this response, leading to fibrosis and progression of renal disease. HO-1 induction by TGF-β or the downstream mediators of HO-1 expression (CO, bilirubin, biliverdin, and/or biliverdin reductase) or both may be dysregulated in pathophysiologic states. Finally, late-stage metastatic disease is typically characterized by increased TGF-β levels and a concomitant reduction in responsiveness of tumor cells to its suppressor functions (122). The role of HO-1 in this setting is unclear. Upregulation of HO-1 has been associated with tumor growth, and perhaps HO-1 induction is responsible for the loss of tumor-suppressor functions of TGF-β (16, 20, 21, 38, 91). An appropriate level of HO-1 induction may be beneficial, whereas in the setting of cancer, its proangiogenic effects may potentiate the progression of tumor growth (20, 21, 43, 73, 75, 111, 126).

HEPATOCYTE GROWTH FACTOR (HGF)/SCATTER FACTOR

Hepatocyte growth factor/scatter factor (HGF/SF) is a mesenchymally derived, heparin-binding glycoprotein with mito-

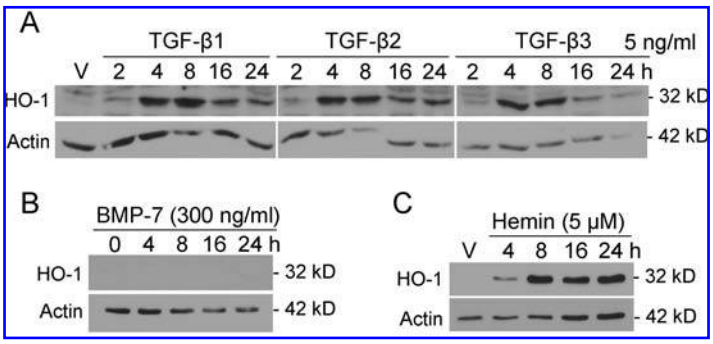

FIG. 2. HO-1 protein expression is upregulated by TGF-β isoforms in human renal proximal tubular epithelial cells. Confluent HK-2 cells, an immortalized human renal proximal tubular epithelial cell line, were treated with (**A**) TGF-β1 (5 ng/ml), TGF-β2 (5 ng/ml), TGF-β3 (5 ng/ml); (**B**) bone morphogenic protein-7 (300 ng/ml); (**C**) or hemin (5 μM) for 2–24 h. Total cell lysates were collected and analyzed with Western blotting. Membranes were incubated with anti-HO-1 (1:5,000) followed by a 1:10,000 dilution of peroxidase-conjugated goat anti-rabbit IgG antibody (1:10,000). Membranes were stripped and reprobed with actin as a loading control. Vehicle control (V) was BSA/HCl for TGF-β1 and BMP-7 or DMSO for hemin.

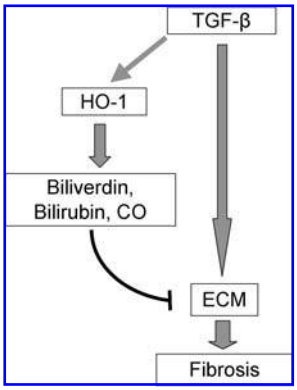

FIG. 3. Proposed model for the beneficial effects of TGF-β through HO-1 induction. Increased expression of TGF-β induces extracellular matrix (ECM) deposition and enhances fibrosis. This effect is balanced by TGF-β–mediated HO-1 induction. The HO-1 reaction products, carbon monoxide (CO), and bilirubin, possess antifibrogenic properties.

genic, motogenic, and morphogenic effects on a variety of cells [reviewed in (28)]. HGF/SF was originally identified and characterized as two different factors, one with growth-stimulating activity (HGF) and the other with scatter-factor activity (SF). Like TGF-β, HGF/SF has a dual role in tissue pathology. On the one hand, it participates in a wide range of biologic activities including growth motility, differentiation and morphogenesis, and processes involved in regeneration of injured tissues, such as promotion of matrix invasion and angiogenesis (69, 78, 112, 113). HGF/SF is also a potent, endogenous antifibrotic factor in a wide variety of animal models [reviewed in (70)]. On the other hand, aberrant HGF/SF signaling leads to the development of tumors and metastases (15, 24, 31, 47, 71, 113). For example, increased expression of c-Met in mesenchymal cells leads to carcinogenic transformation. In addition, inherited mutations in c-Met that result in constitutive activation of the HGF signaling pathway are associated with human renal papillary carcinoma.

HGF/SF is secreted as an inactive single-chain precursor [reviewed in (54, 69, 113)]. Several proteinases are capable of activating HGF, including HGF activator (HGFA), a coagulation factor XII–like serine protease that is the most potent (54). Other serine proteases, including urokinase, factor XIIa and tissue plasminogen activator, have been shown to activate HGF *in vitro*, but their biologic role in HGF activation is unknown. Biologically active HGF is generated by converting the single-chain precursor into a two-chain active form by a single proteolytic cleavage between Arg^{494}-Val^{495}, generating a heterodimer consisting of four kringles-containing α-chain and a serine protease-like β-chain (46, 54, 78, 87). Endogenous HGFA inhibitors are Kunitz-type serine protease inhibitors and have been used to study the regulation of HGFA activity (54, 58).

The sole HGF receptor, c-Met tyrosine kinase, is preferentially expressed in epithelial and endothelial cells and is normally activated by organogenesis (46, 54). c-Met expression is tightly controlled by cytokines, growth factors, and the extracellular environment (130). HGF and its receptor are cytoprotective against damage in various organs, including liver, kidney, and lungs in disease models (85). In the kidney, c-Met expression is rapidly and selectively upregulated in models of acute kidney injury (ischemia and nephrotoxins), and it plays a crucial role in accelerating tubular repair and renal regeneration (130). TGF-β1, through Sp1 and Smads, induces c-Met expression in renal epithelial cells (130). The inhibitory effect of TGF-β on epithelial cell proliferation is reversed by HGF. In endothelial cells, HGF stimulates expression of an angiogenic factor, VEGF, and its receptor [reviewed in (105)]. In the lung, basic fibroblast growth factor (FGF) stimulates macrophages and fibroblasts to synthesize HGF, which suppresses fibrosis and increases regeneration of bronchial and alveolar epithelial cells (105). Studies in the glycerol model of acute kidney injury indicate that pretreatment with HGF can improve renal function, possibly *via* HO-1 induction (Fig. 4) (85). Also, HGF induces HO-1 in HepG2 (human hepatoblastoma) and MDA-MB-231 cells (highly invasive breast carcinoma) but not in MCF-7 cells (low-invasive breast carcinoma) (112, 113). HO-1 induction may act as a regulator against the HGF-mediated increased attachment of metastatic cells to the ECM, proteolysis of basement membranes, enhanced migration, and ability to colonize target organs, contributing to metastatic growth (112). Very little information is available regarding the molecular regulation and signaling pathway(s) involved in HGF-mediated HO-1 induction, except that HGF regulates HO-1 gene expression through activation of NF-κB and HIF-1α in HepG2 cells and MDA-MB-231 cells (112, 113).

A recent study showed that HGF prevents H_2O_2-induced mesangial cell apoptosis and this protective influence was also induced by hemin, an HO-1 inducer (95). HGF induces HO-1, perhaps as an adaptive response and through its reaction product(s), bilirubin (antioxidant), and CO (antiapoptotic), may account for the inhibition of apoptosis and necrosis of renal epithelial cells afforded by HGF pretreatment in the glycerol model

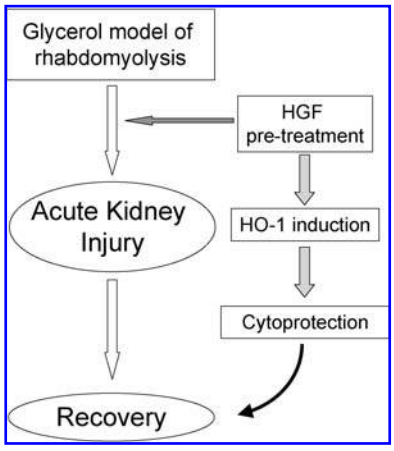

FIG. 4. Hepatocyte growth factor/scatter factor (HGF/SF) prevents acute kidney injury (AKI). Pretreatment with HGF/SF, in an animal model of glycerol-induced AKI, ameliorates the deterioration of renal function. HGF/SF increases the renal expression of HO-1 mRNA, which provides cellular protection potentially through the HO-1 products bilirubin, CO, and biliverdin.

of acute kidney injury (85, 112, 113). Therefore, understanding the molecular mechanisms and cellular effects involved in the induction of HO-1 by HGF requires further elucidation.

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

VEGF is relatively specific for endothelial cells and is a mediator of angiogenesis and vascular permeability by stimulating endothelial cell proliferation and differentiation and mediating endothelium-dependent vasodilation [reviewed in (39, 101, 105)]. Like TGF-β and HGF, VEGF is a fundamental player in both physiologic and pathologic angiogenesis (39, 101). VEGF is essential for development and is important in a number of postnatal angiogenic processes, including wound healing, maintenance of blood pressure, and pregnancy (39). Conversely, VEGF has been linked to several pathologic conditions associated with increased angiogenesis, including tumor progression (39).

The VEGF-related gene family consists of secreted glycoproteins: VEGF-A, -B, -C, -D, -E, and placenta growth factor-1 and -2 (39, 101). VEGF-A, referred to as VEGF, was originally identified as a vascular permeability–inducing factor secreted by tumor cells and initially known as vascular permeability factor (39). VEGF-A undergoes alternative splicing to yield at least six different homodimeric glycoproteins (39, 101). Some isoforms are soluble and freely secreted, whereas others are sequestered in the ECM and are activated by proteases (39). ECM-bound isoforms can be released in a diffusible form by plasmin cleavage to generate a bioactive fragment or by matrix metalloproteinase-9 to initiate angiogenesis (39). Expression patterns of certain VEGF isoforms are tissue specific and may have defined roles in vasculogenesis and probably tumor angiogenesis (39).

VEGF family members mediate their effects through several different receptors. The two best-described VEGF receptors, VEGFR-1 (also known as fms-like tyrosine kinase-1) and VEGFR-2 (also referred to as fetal liver kinase-1/KDR) are tyrosine kinase receptors and are expressed on endothelial cells and hematopoietic cell lineages in the adult (39, 101). A third, VEGFR-3, has been recently identified and found to be associated with lymphangiogenesis (39). VEGFR-1 and VEGFR-2 share ~44% homology and consist of seven extracellular immunoglobulin-like domains, a single transmembrane domain, and a consensus tyrosine kinase domain interrupted by a kinase insert domain (39). The various VEGF ligands have different binding specificities for each of these receptors, resulting in their distinct functions (39).

Several regulators of VEGF and VEGFR expression include growth factors and cytokines, oncogenes, tumor-suppressor genes, hypoxia [reviewed in (39)], and HO-1 (11, 14, 52, 72, 93). Prostaglandins, epidermal growth factor receptor, and insulin-like growth factor-I receptor are important regulators of VEGF regulation and angiogenesis in several tumor systems (39). For example, HGF, through its c-Met receptor, induces VEGF expression in normal and tumor cells (39). Platelet-derived growth factor (PDGF) regulates endothelial cell survival and pericyte/vascular smooth muscle cell recruitment by inducing VEGF in several model systems (39). Under conditions of hypoxia, hypoxia-inducible factor-1α (HIF-1α) dimerizes with HIF-1β, translocates into the nucleus, and binds to the VEGF promoter, to initiate transcription (39).

HO-1 induction, by NO, prostaglandins, prolactin, and hydrogen peroxide, upregulates VEGF (11, 14, 52, 72, 93). Conversely, VEGF regulates HO-1 production and induces HO-1 expression and activity in vascular smooth muscle cells and microvascular endothelial cells (8, 9, 18, 23, 51). Short treatment (6 h) with heme, followed by 18 h of incubation in heme-free media resulted in potent induction of VEGF expression and protein synthesis in human keratinocytes (49). HO-1 has also been shown to promote VEGF-induced angiogenesis by increasing endothelial cell survival, proliferation, and tube formation, possibly through CO (12, 50). Previous studies have reported that HO-1 induction with hemin in mouse aortic endothelial cells resulted in a modest increase in VEGF levels in both HO-$1^{+/+}$ and HO-$1^{-/-}$ cells, even though the basal levels of VEGF were lower in HO-$1^{-/-}$ cells (11). Furthermore, HO-1 inhibits leukocyte infiltration and promotes VEGF-driven noninflammatory angiogensesis, facilitating tissue repair (8). Inhibition of HO-1 abrogates VEGF-mediated angiogenesis (8). It has been proposed that HO-1 induced by VEGF promotes angiogenesis for the resolution of tissue injury, while inhibiting leukocyte adhesion and transmigration (Fig. 5) (8). Recent work by Siner and colleagues (107) showed that lung-specific overexpression of VEGF resulted in marked induction of HO-1 in the lung and was protective in a model of hyperoxia-induced lung injury *in vivo* through the HO-1 pathway (107). However, similar to TGF-β and HGF, VEGF-mediated HO-1 induction may promote pathologic angiogenesis in tumor progression. Little is known regarding the regulation of VEGF-mediated HO-1 induction. An increase in cytosolic calcium levels and activation of protein kinase C mediates HO-1 expression by VEGF in chick embryo chorioallantoic membranes (23). Clearly, a better understanding of the VEGF–HO-1 system could prove beneficial in developing therapeutic strategies when promotion of angiogenesis would be beneficial, as well as when preventing angiogenesis would prove efficacious.

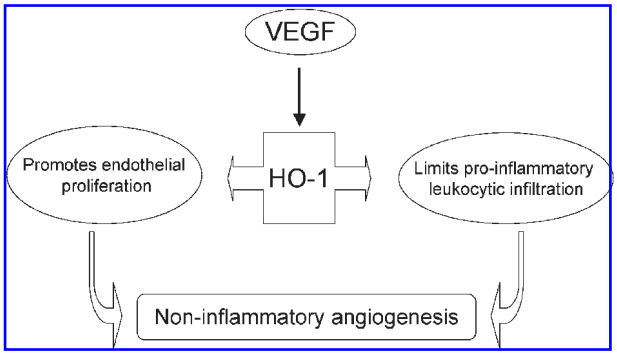

FIG. 5. Vascular endothelial growth factor (VEGF)-induced angiogenesis through HO-1 expression. HO-1 regulates VEGF-mediated angiogenesis by modulating leukocytic infiltration and endothelial cell proliferation, implicating HO-1 in the promotion of VEGF-driven non-inflammatory angiogenesis and tissue repair.

PLATELET-DERIVED GROWTH FACTOR (PDGF)

PDGFs are a family of disulfide-bonded dimeric isoforms (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD), synthesized as precursor molecules (A-, B-, C-, and D-polypeptide chains), and undergo proteolytic maturation [reviewed in (36, 37, 101)]. PDGF is secreted by many different cell types, including platelets, macrophages, and vascular cells at sites of inflammation and vascular injury (19, 37, 101). The expression of each precursor chain is independently regulated at the transcriptional and posttranscriptional level, by positive and negative regulatory elements (37). PDGF synthesis is increased in response to such external stimuli as low oxygen tension, thrombin, and various growth factors and cytokines (37).

PDGF has important functions in development, tissue homeostasis, and vascular response to injury [reviewed in (36, 37, 101)]. PDGF has potent mitogenic and weak angiogenic effects, and it modulates chemotaxis, vascular tone, and platelet aggregation (37, 101). PDGF has been implicated in the pathogenesis of atherosclerosis, hypertension, lung, liver and kidney fibrosis, as well as malignancies (19, 37).

PDGF exerts its cellular effects by binding to α- and β-protein tyrosine kinase receptors, resulting in receptor dimerization [reviewed in (36, 37)]. The PDGF isoforms induce different receptor complexes, depending on which receptor types are expressed on the target cell. -AA, -AB, -BB, and -CC PDGF isoforms induce $\alpha\alpha$ receptor homodimers (36, 37). -BB and -DD PDGF isoforms activate $\beta\beta$-receptor complexes, and -AB and -BB PDGF isoforms induce $\alpha\beta$ receptor heterodimers. The $\alpha\alpha$- and $\beta\beta$-receptor complexes regulate potent mitogenic signals; however, $\alpha\beta$-receptor complexes induce a more robust mitogenic effect. Whereas $\alpha\alpha$-receptor complexes inhibit chemotaxis induced by other agents, $\beta\beta$-receptor complexes stimulate it. Dimerization causes receptor autophosphorylation, leading to increased kinase activity and providing docking sites for signal-transduction molecules. Molecules that bind to the PDGF dimerized receptors include PI3-kinase, tyrosine kinase Src, tyrosine phosphatase SHP-2, and phospholipase Cγ. Receptor-bound signaling molecules are activated by phosphorylation, conformational change, or translocation to the inside of the cell membrane (36, 37).

PDGF intracellular signaling involves extensive cross-talk between signaling pathways (36, 37). Signal-transduction pathways induced by PDGF include PI3-kinase, which is important for cell migration, actin reorganization, and prevention of apoptosis (36, 37). The mitogenic effect of PDGF is mediated through Ras, which induces activation of the MAP kinase pathway, and the tyrosine kinase Src, an inducer of Myc transcription factor (36, 37).

Induction of HO-1, through its reaction products, may regulate some of the effects of PDGF. PDGF stimulates HO-1 gene expression in VSMCs and airway smooth muscle cells, but not in human retinal pigment epithelial cells (19, 64, 114). HO-1, through bilirubin production, acts in an autocrine negative-feedback manner to limit ROS-dependent phosphorylation of the ERK1/2 MAPK pathway and the concomitant PDGF-mediated proliferation of airway smooth muscle cells (114). Finally, hypoxia-induced HO activity, *via* CO, reduces PDGF expression in vascular smooth muscle cells and endothelial cells, and thus a regulatory loop between HO-1 and PDGF exists (Fig. 6) (62, 81). Interestingly, hypoxia induces HO-1 in rodent, bovine, and monkey cells, but represses HO-1 expression in several different human cell lines (60, 81, 104). The studies showing effects of hypoxia on HO activity were performed in co-cultures using rat vascular smooth muscle cells and human umbilical vein endothelial cells (81). Given the species-specific effects of hypoxia on HO-1 expression, it would be interesting to verify these results using co-cultures of cells from the same species.

In the adult, PDGF modulates interactions between connective tissue cells and ECM molecules to regulate interstitial fluid pressure (36). PDGF-mediated HO-1 induction provides an important cellular defense mechanism against tissue injury, as HO-1 induction in vascular cells leads to an increased resistance to oxidative stress, and HO-1 deficiency results in increased vascular cell injury (3, 84, 127). HO-1–catalyzed production of CO promotes blood flow and fluidity at the sites of vascular injury (19, 64, 114). PDGF also has a key role in wound healing, by stimulating mitogens and chemotactic agents affecting fibroblasts, smooth muscle cells, neutrophils, and macrophages and by stimulating the synthesis of ECM molecules, including fibronectin and collagen (36). HO-1–catalyzed bilirubin and CO production have antifibrotic effects that may counterbalance the profibrotic effects of PDGF (26, 30, 67, 82, 123, 131).

The molecular mechanism of PDGF-induced HO-1 gene expression is not clearly understood. In rat VSMCs, it is dependent on *de novo* RNA and protein synthesis and does not involve increased mRNA stability (19). Unlike TGF-β–mediated HO-1 induction in human renal epithelial cells, the antioxidant NAC attenuates HO-1 induction by PDGF, implying a role for reactive oxygen species (19). Understanding the cellular role and the mechanism of PDGF-stimulated HO-1 expression could prove beneficial in ameliorating PDGF-related diseases.

NERVE GROWTH FACTOR (NGF)

NGF, a pleiotropic factor that promotes cell growth, differentiation, survival, and cell death, is a member of a small fam-

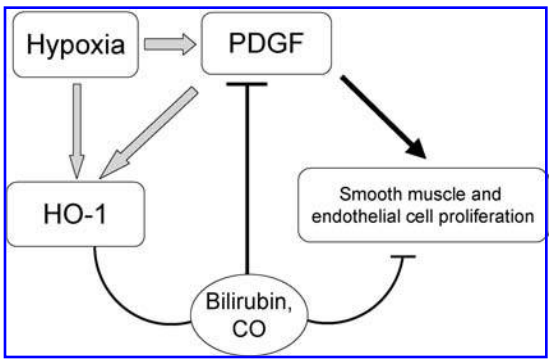

FIG. 6. The feedback circle of platelet-derived growth factor (PDGF) and HO-1. Hypoxia stimulates expression of PDGF in VSMCs and leads to the proliferation of smooth muscle and endothelial cells. At the same time, PDGF induces HO-1 gene expression in VSMCs, and hypoxia induces HO-1 expression. HO-1–derived bilirubin and CO can block PDGF and hypoxia-induced smooth muscle cell proliferation.

ily of neurotrophins (NTs) [reviewed in (79, 108)]. NGF, released by postsynaptic targets, acts on presynaptic neurons to enhance the function of neural circuits (108). However, the actions of NGF extend beyond development and nerve cells, and even outside the nervous system (108). NGF and its receptors are expressed by many cell types in the central and peripheral nervous system (CNS and PNS), immune and inflammatory system, and various tissues during development, adult life, and aging, suggesting multiple functions for NGF signaling (79, 108). NGF and NGF-receptor expression can be upregulated in response to injury (79, 108). NGF and NGF receptors are involved in the development and progression of neuronal diseases (including Alzheimer and Parkinson disease), as well as immune diseases (including autoimmunity and inflammation) (79).

The mechanisms that regulate NGF synthesis and release are not clearly understood [reviewed in (80)]. NGF is synthesized as pro-NGF and is cleaved intracellularly to generate biologically active mature NGF (79, 108). NGF is a polypeptide consisting of three subunits (β, γ_2, α_2) [reviewed in (80)]. The β-NGF subunit is responsible for the biologic activity; the γ-NGF subunit is a highly specific active protease that can process the pre-NGF to its mature form; and the α-NGF subunit is a blocked zymogen and is inactive (80). Active NGF appears to be similar in all tissues and consists of a dimer of polypeptide chains, connected by three intrachain disulfide bridges (108).

NGF signals are propagated *via* two distinct receptors: TrkA tyrosine kinase ($TrkA^{NGFR}$) and $p75^{NTR}$ receptor (79, 108). $TrkA^{NGFR}$, a member of the Trk gene family, is a single-pass transmembrane protein that elicits many of the classic neurotrophic actions of NGF (108). $p75^{NTR}$, a member of the tumor necrosis factor–receptor superfamily lacking intrinsic tyrosine domains, is a transmembrane glycoprotein that binds all members of the NT family with approximately equal affinity (108). Data from pheochromocytoma cells (PC12) suggest that NGF receptors may form three different receptor complexes: $TrkA^{NGFR}$ homodimers, $p75^{NTR}$ homo-oligomers, and a mixed complex of $TrkA^{NGFR}$ and $p75^{NTR}$ (79). The magnitude and duration of NGF signaling depends on the ratio of each receptor distributed on the cell surface (79).

Pro-NGF is a high-affinity ligand for $p75^{NTR}$, which induces a specific transduction pathway involving NF-κb, c-Jun kinase, and increased production of ceramide, leading to gene transcription or apoptosis (79). In the presence of $TrkA^{NGFR}$, $p75^{NTR}$ can participate in the formation of high-affinity binding sites, resulting in enhanced NGF responsiveness (80). Most of the biologic activities of NGF are mediated by ligand-dependent activation of $TrkA^{NGFR}$ tyrosine kinase activity (80). When the active form of NGF binds to $TrkA^{NGFR}$ tyrosine kinase receptor, it stimulates autophosphorylation and subsequent phosphorylation and activation of transduction cascades, including the MAPK-Ras-Erk pathway, PI3K, and phospholipase Cy1, to regulate differentiation/activation and proliferation/survival (79). Activation of PI3-k and its downstream effectors is a major mechanism involved in NGF-induced cell survival (7, 53).

NGF elicits a protective effect against oxidative stress, both in PC12 cells and in cultured neurons, by increasing the expression of catalase, glutathione peroxidase activity, and HO-1 (59, 68, 97–99, 125). NGF, by a mechanism that involves PI3K/Akt-dependent induction of HO-1, prevents the accumulation of reactive oxygen species in PC12 cells exposed to a Parkinson disease–related neurotoxin (97). The increase in HO-1 induction is sensitive to pretreatment with actinomycin D and cycloheximide, suggesting that the induction requires *de novo* transcription and protein synthesis (97). In serum-deprived PC12 cells, the NGF-mediated induction of HO-1 involves MEK activation and has antioxidative and antiapoptotic effects (68).

NGF and NTs are essential for the differentiation, survival, and functions of neuronal cells in the CNS, PNS, and immune and inflammatory system (80). Rat peritoneal mast cells synthesize, store, and secrete biologically active NGF (66, 80). Human basophils, T and B lymphocytes, monocyte/macrophages, and peripheral blood eosinophils express, release, and respond to NGF (5, 80, 109, 116). Plasma or serum levels of NGF or both are increased in several autoimmune, inflammatory, fibrotic, and allergic disorders, including systemic lupus erythematosus, psoriasis, Kawasaki syndrome, asthma, scleroderma, and rheumatoid arthritis (6, 80). Whether levels of HO-1 in these diseases or microsatellite polymorphisms in the HO-1 promoter correlate with disease severity in these disorders in which NGF is implicated would be of significant interest.

FIBROBLAST GROWTH FACTOR-1 (FGF-1)

Fibroblast growth factors (FGFs) represent a homologous family of at least 22 proteins associated with cell proliferation and differentiation. They range in molecular mass from 17 to 34 kDa and share 13–71% amino acid identity [reviewed in (92)]. They are highly conserved in both gene structure and amino acid sequence and a prime component of angiogenesis associated with organogenesis, tumor growth, and wound healing. The best-characterized FGF family members are the prototypes FGF-1 (acidic FGF) (48) and FGF-2 (basic FGF) (2). FGF-1 is a polypeptide growth factor that is expressed in motor neurons and has recently been shown to exert neuroprotective effects, possibly through the induction of HO-1 (118). FGFs mediate their effects through cell-surface receptors, which are members of the tyrosine kinase family. In humans, four different fibroblast growth factor receptors (FGFRs) have been identified: FGFR1, 2, 3, and 4. These receptors share common structural features and consist of an extracellular ligand-binding domain (containing three Ig-like loops), a unique acidic region, a transmembrane domain, and the cytoplasmic region (which contains the tyrosine kinase catalytic domain and kinase insert) (44). The FGF-FGFR interaction is very complex because cell-surface and extracellular matrix heparan sulfate proteoglycans are essential for binding of FGF-1 and FGF-2 to their cognate receptors (29). Ligand binding causes the FGFRs to dimerize and activate specific intracellular signaling pathways.

FGF-1 is upregulated in animal models of neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), and increased HO-1 expression has been shown in conditions such as Parkinson's and Alzheimer's disease (57, 100). Recent studies by Vargas *et al.* (118) showed that FGF-1 is a potent inducer of HO-1 in spinal cord astrocytes through increased transcription and *de novo* protein synthesis and required the Nrf2 transcription factor. Both Nrf2 and HO-1 were found to colocalize in reactive astrocytes in the spinal cord of rats with the ALS-

associated SOD1 G93A mutation, and Nrf2 overexpression resulted in increased motor neuron survival *in vitro*. These studies suggest that the HO-1 pathway is a protective response in neurodegenerative diseases, and mediators such as FGF-1 may exert their neuroprotective effect through HO-1–dependent mechanisms.

SUMMARY

Growth factors and HO-1 are intimately related. They have significant influences on a wide range of similar physiologic and pathophysiologic events. Perhaps HO-1 is an adaptive response providing a balance for the effects of growth factors. Conversely, it is possible that some of the effects associated with growth factors may be attributable to HO-1 induction. Regulation of HO-1 by each growth factor is complex, and none has been completely elucidated. Understanding the molecular mechanisms and the biologic outcomes of HO-1 induction by individual growth factors will be important in designing interventional strategies in several diseases in which growth factors are involved.

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ABBREVIATIONS

ALS, amyotrophic lateral sclerosis; BMP, bone morphogenic protein; CTGF, connective tissue growth factor; DFO, deferoxamine; ECM, extracellular matrix; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; HGF, hepatocyte growth factor; HGFA, hepatocyte growth factor activator; HIF, hypoxia-inducible factor; HO, heme oxygenase; MAPK, mitogen-activated kinases; NAC, *N*-acetylcysteine; NGF, nerve growth factor; NT, neurotrophin; PDGF, platelet-derived growth factor; PI3K, phosphoinositol-3 kinase; SBE, Smad binding elements; SF, scatter factor; TGF-β, transforming growth factor-β; TβR, transforming growth factor receptor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; VSMCs, vascular smooth muscle cells.

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