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The possible role of heat shock factor-1 in the negative regulation of heme oxygenase-1

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Abstract

We examined a possible role for heat shock factor-1 (HSF-1) in the negative regulation of *HO-1* gene expression in human Hep3B hepatoma cells responding to stimulation with 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) and arsenite. Overexpression of HSF-1 and heat-shock experiments indicated that HSF-1 repressed the 15d-PGJ_2-and arsenite-induced *HO-1* gene expression through directly binding to the consensus heat shock element (HSE) of the *HO-1* gene promoter. In addition, point mutations at specific HSE sequences of the HO-1 promoter-driven luciferase plasmid (pGL2/hHO3.2-Luc) abolished the heat shock- and HSF-1-mediated repression of reporter activity. Overall, it is possible that HSF-1 negatively regulates *HO-1* gene expression, and that the HSE present in the −389 to −362 region mediates HSF-1-induced repression of human *HO-1* gene expression.

Keywords: Heme oxygenase-1; Heat shock factor-1; Hepatoma; Arsenite; 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2

1. Introduction

Heme oxygenase (HO) is a microsomal enzyme that degrades protoheme IX by cleaving its α-methene bridge into carbon monoxide (CO), free divalent iron, and biliverdin-IXα (Maines, 1997; Ponka, 1999). Three isoforms transcribed from separate genes have been characterized. HO-2 is a constitutively active form found mainly in the brain and testes (McCoubrey & Maines, 1994), and HO-3 has only recently been described, with properties similar to those of HO-2 (McCoubrey, Huang, & Maines, 1997). HO-1, known

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as heat shock protein 32, is induced by stressors, including its substrate heme, cytokines, heavy metals, and oxygen free radicals (Elbirt, Whitmarsh, Davis, & Bonkosky, 1998; Keyse & Tyrrell, 1989; Lee & Chan, 2002; Vile, Basu-Modak, Waltner, & Tyrrell, 1994). Much evidence has suggested that up-regulation of HO-1 plays an important role in the cytoprotective defense response against oxidative stress and inflammatory stimuli (Choi & Alam, 1996; Rizzardini, Terao, Falciani, & Cantoni, 1993). High expression of HO-1 in the liver suggests that the HO/CO system might serve as a modulator of hepatobiliary function. For example, induction of HO-1 by heme resulting in increased delivery of heme to the liver occurred as a result of several processes including a splenectomy, hemolysis, and rhabdomyolysis (Rizzardini, Carelli, Cabello Porras, & Cantoni, 1994; Suematsu & Ishimura, 2000). The products of CO may function as a gaseous regulator of cytochrome P450-dependent biotransformation, such as bile acid synthesis and xenobiotic catabolism (Sano et al., 1997; Shinoda et al., 1998). In contrast to the beneficial roles, induction of HO-1 expression may be associated with endotoxic shock in vascular smooth muscle cells (Yet et al., 1997), and repression of HO-1 expression may represent a defense strategy developed in humans (Shibahara, Nakayama, Kitamuro, Udono-Fujimori, & Takahashi, 2003).

Regulation of *HO-1* expression has been extensively studied, and several cis-acting promoter elements involved in its expression have also been elucidated (Lavrovsky, Schwartzman, Levere, Kappas, & Abraham, 1994; Lu et al., 1998; Takahashi et al., 1999). Specific sequences of a putative heat shock element (HSE) have been identified to be present in the HO-1 promoter. However, HSE seems to play a discrepant role in different species and cell types. A difference in the heat-mediated induction of HO-1 exists between rats and humans. In the rat, exposure of cells to elevated temperatures causes a rapid increase in HO-1 expression, indicating that the HSE is a functional element in response to heat shock (Raju & Maines, 1994; Shibahara, Muller, & Taguchi, 1987). In contrast to the rat gene, the heat-mediated induction of human HO-1 seems to be observed only in certain cell lines (Keyse & Tyrrell, 1989; Mitani, Fujita, Sassa, & Kappas, 1990). The different expression patterns in various human cell lines caused by heat shock suggest that induction of HO-1 occurs in a cell line-dependent manner, and the HSE seems to be a functional element of the human *HO-1* gene.

Heat shock factor-1 (HSF-1) is known to have the unique ability to bind to the HSE in a heat shock-dependent manner. Upon treatment with stress inducers, such as heat shock, activated HSF-1 translocates to the nucleus, binds as trimers to multiple arrays of the HSE, which are located in the promoter region of genes, and then regulates gene expression (Mosser, Theodorakis, & Morimoto, 1988). The HSE has been found to be present in the promoter of *Hsp* genes and several non-*Hsp* genes, such as TNFα and IL-1β (Cahill, Waterman, Xie, Auron, & Calderwood, 1996; Singh, He, Calderwood, & Hasday, 2002). Recent studies indicated that HSF-1 negatively regulates TNFα and IL-1β expression for which the HSE might serve as a negative regulatory element. In human monocytic cells, HSF-1 bound to the IL-1β promoter and repressed its activity in a manner dependent upon the presence of an intact HSE (Cahill et al., 1996). Several studies have reported a negative regulatory region in human HO-1 (Deramaudt, da Silva, Remy, Kappas, & Abraham, 1999; Lu, Pepe, Gildemeister, Tyrrell, & Bonkovsky, 1997), and the HSE possibly playing a role as a negative regulatory element in the human *HO-1* gene has not been well investigated as yet.

In this study, we investigated the possible role of HSF-1 in repression of HO-1 expression in human Hep3B hepatoma cells. Due to the discrepant roles of the HSE in the human *HO-1* gene and HSE's ability to act as a negative regulatory element, we examined the possibility that HSF-1 negatively regulates HO-1 expression through binding to the HSE. Our results demonstrated that 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2)- and arsenite-induced upregulation of human HO-1 expression could be repressed by heat shock or overexpression of HSF-1. In another experiment, we used a mutated HO-1 promoter construct with specific point mutations in the HSE consensus sequences, which resisted repression by heat shock or the overexpression of HSF-1.

2. Materials and methods

2.1. Cell culture and treatments

Sodium arsenite and 15d-PGJ_2 were purchased from Biomol Research Laboratories (Plymouth Meeting,