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Heat shock protein 27 downregulates the transferrin receptor 1-mediated iron uptake

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Abstract

It has been reported that over-expression of human heat shock protein 27 (hsp27) in murine cells decreased the intracellular iron level [Arrigo, A. P., Virot, S., Chaufour, S., Firdaus, W., Kretz-Remy, C., & Diaz-Latoud, C. (2005). Hsp27 consolidates intracellular redox homeostasis by upholding glutathione in its reduced form and by decreasing iron intracellular levels. *Antioxidants & Redox Signalling*, *7*, 412–422]. However, the mechanism involved is unknown. In this study, the regulation of transferrin receptor 1 (TfR1)-mediated iron uptake by human hsp27 was investigated in CCL39 cells by overexpression of human hsp27 and its dominant-negative mutant (hsp27-3G). The results showed that overexpression of hsp27 diminished intracellular labile iron pool, increased the binding activity of iron regulatory protein (IRP) to iron responsive element (IRE) and the cell surface-expressed TfR1s. However, the increased surface-expressed TfR1s resulted in decrease rather than increase of iron uptake. Further study revealed that overexpression of hsp27 decelerated transferrin endocytosis and recycling, while overexpressed hsp27-3G had a reversal effect. Moreover, flowcytometric analysis showed an enhanced actin polymerization in the cells overexpressing hsp27. In particular, fluorescence imaging of cytoskeleton displayed highly stabilized microfilaments and preferential localization of hsp27 in cortical area of the actin cytoskeleton. In contrast, disruption of actin cytoskeleton by cytochalasin B resulted in acceleration of the endocytosis and recycling of Tf, as well as increase of iron uptake. Meanwhile, the possible involvement of ferroportin 1 in down-regulation of intracellular iron level by overexpression of hsp27 was checked. However, the outcome was negative. Our findings indicate that hsp27 down-regulates TfR1-mediated iron uptake via stabilization of the cortical actin cytoskeleton rather than the classical IRP/IRE mode. The study may also imply that hsp27 protects cells from oxidative stress by reducing cellular iron uptake.

Keywords: Heat shock protein 27; Transferrin receptor-mediated iron uptake; Transferrin recycling; Iron regulatory protein; Actin polymerization

1. Introduction

Iron is an element required for many metabolic processes in all eukaryotes and most prokaryotes. Besides synthesis of hemoglobin, iron is also essential for cell cycling, synthesis of DNA and some other important enzymes (Lieu, Heiskala, Peterson, & Yang, 2001). Iron-deficiency causes many diseases, such as anemia. On

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the other hand, iron overload in human body causes cell death and tissue damage through iron-catalyzed Fenton chemistry (Halliwell & Gutteridge, 1990). In particular, abnormally high levels of iron in the brain have been found in a number of neurodegenerative disorders, including Parkinson's and Alzheimer's disease (Aisen, Wessling-Resnick, & Leibold, 1999). While the epidemiological and clinical studies on whether iron is a risk factor for heart disease have demonstrated conflicting results, it is still highly speculated that iron might play a role in cardiac disease (Patricia & Alpert, 2004). Therefore, understanding the molecular mechanisms of cellular iron uptake and homeostasis is of physiological importance.

Cells acquire iron via transferrin (Tf)-dependent and -independent pathways. Although the intestine absorbs iron from food via divalent metal transport 1 (DMT1) (Gunshin et al., 1997) and the differentiating epithelial cells in the earliest stages of embryonic development take up iron via neutral gelatinase-associated lipocalin-mediated iron delivery (Yang et al., 2002), the transferrin receptor-dependent pathway is still considered to be the main route of cellular uptake after iron is exported from intestine (Kaplan, 2002). There are two types of transferrin receptor, TfR1 and TfR2. TfR1 is ubiquitously expressed receptor for Tf that delivers iron to cells (Richardson & Ponka, 1997). TfR2 is a type II transmembrane protein and homolog of TfR1, and has limited tissue distribution (Fleming et al., 2000; Kawabata et al., 1999). Like TfR1, TfR2 binds Tf at neutral pH. However, differences in the activity, regulation, and expression of TfR1 and TfR2, and in the pathophysiology of disorders caused by their deficiency, indicate that they have different roles in iron homeostasis. The affinity of TfR2 for Tf at pH 7.5 is approximately 25-fold lower than that of TfR1 (West et al., 2000).

Unlike TfR1, TfR2 mRNA expression does not change in cells treated with $Fe_2(NO_3)$ or the iron chelator desferrioxamine (Kawabata et al., 2000), nor does it change in iron-deficient or iron-overloaded mice (Fleming et al., 2000). In humans homozygous for the Y250X TfR2 mutation and mice transgenic for the orthologous Y245X mutation, the liver accumulates iron, despite an absence of membrane-bound TfR2 and a reduction in TfR1 suggesting that the uptake of Tf-bound iron for use by the hepatocytes is not the principal role of TfR2 (Fleming et al., 2002).

It has been established that the level of intracellular iron regulates the expression of many key molecules that participate in iron metabolism in cells via a feedback regulatory mechanism. Two iron regulatory proteins, IRP1 and IRP2, were found to regulate the expression of TfR1 and ferritin by binding to the iron responsive elements (IREs) in the 3′-untranslated region in the mRNA of TfR1 (Mullner, Neupert, & Kuhn, 1989) and the 5′-untranslated region in the mRNA of ferritin (Goossen, Caughman, Harford, Klausner, & Hentze, 1990). Iron depletion enhances the binding of IRPs to IREs, inhibits ferritin mRNA translation and stabilizes TfR1 mRNA. These changes resulting from iron depletion lead to decreased iron sequestration into ferritin and enhanced iron uptake through TfR1. On the other hand, iron repletion inactivates IRP-1 and leads to degradation of IRP-2 which results in an efficient translation of ferritin mRNA and rapid degradation of TfR1 mRNA. Besides TfR1 and ferritin, some other iron metabolism-related proteins that possess IREs in their mRNAs are also regulated through the IRE/IRPs mode. These proteins include mitochondria aconitase (Kim, LaVaute, Iwai, Klausner, & Rouault, 1996), the iron–sulfur subunit of succinate dehydrogenase (Kohler, Henderson, & Kuhn, 1995) and DMT1 (Fleming et al., 1998). However, it is unclear whether the IRP/IRE regulatory mode is ubiquitous and the determinant regulation mechanism for intracellular iron homeostasis.

Heat shock protein 27 (hsp27) belongs to the family of stress proteins. Its expression increases following heat shock (Gething & Sambrook, 1992), oxidative stress (Marini, Frabetti, Musiani, & Franceschi, 1996) or stimulation by cytokines including tumor necrosis factor (Mehlen, Briolay et al., 1995; Mehlen, Preville et al., 1995) and basic fibroblast growth factor (Kozawa et al., 2001). In general, hsp27 has been thought to serve multiple functions in a variety of cell types. It acts as an ATP-independent chaperone that interacts with misfolded or oxidized polypeptides (Jakob, Gaestel, Engel, & Buchner, 1993) and up-regulates several key enzymes involved in the reactive oxygen species (ROS) (Arrigo, 2001; Preville et al., 1999). It protects cells from apoptosis induced by various environmental factors such as heat and H_2O_2 (Mehlen, Briolay et al., 1995; Mehlen, Preville et al., 1995). In addition to enhancing stress tolerance, hsp27 also plays an important role in regulation of actin cytoskeleton by phosphorylation (Schafer, Clapp, Welsh, Benndorf, & Williams, 1999).

Iron is a transition metal capable of generating $^{\bullet}OH$ radicals, the most potent reactive oxygen species. Based on epidemiological evidence citing excess iron as a risk factor for many diseases, the iron-induced oxidative stress has been considered as a key event in the pathogenic process of diseases (Reddy & Clark, 2004). Interestingly, tissue distribution comparisons demonstrated that high levels of hsp27 were present in heart and skeletal muscle (Voss et al., 2003). Very recently, it was