Circulating heat shock protein 70 (HSPA1A) in normal and pathological pregnancies

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Abstract Heat shock proteins (Hsps) are ubiquitous and phylogenetically conserved molecules. They are usually considered to be intracellular proteins with molecular chaperone and cytoprotective functions. However, Hsp70 (HSPA1A) is present in the peripheral circulation of healthy nonpregnant and pregnant individuals. In normal pregnancy, circulating Hsp70 levels are decreased, and show a positive correlation with gestational age and an inverse correlation with maternal age. The capacity of extracellular Hsp70 to elicit innate and adaptive proinflammatory (Th1-type) immune responses might be harmful in pregnancy and may lead to the maternal immune rejection of the fetus.

Decreased circulating Hsp70 level, consequently, may promote the maintenance of immunological tolerance to the fetus. Indeed, elevated circulating Hsp70 concentrations are associated with an increased risk of several pregnancy complications. Elevated Hsp70 levels in healthy pregnant women at term might also have an effect on the onset of labor. In preeclampsia, serum Hsp70 levels are increased, and reflect systemic inflammation, oxidative stress and hepatocellular injury. Furthermore, serum Hsp70 levels are significantly higher in patients with the syndrome of hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) than in severely preeclamptic patients without HELLP syndrome. In HELLP syndrome, elevated serum Hsp70 level indicates tissue damage (hemolysis and hepatocellular injury) and disease severity. Increased circulating Hsp70 level may not only be a marker of these conditions, but might also play a role in their pathogenesis. Extracellular Hsp70 derived from stressed and damaged, necrotic cells can elicit a proinflammatory (Th1) immune response, which might be involved in the development of the maternal systemic inflammatory response and resultant endothelial damage in preeclampsia and HELLP syndrome. Circulating Hsp70 level is also elevated in preterm delivery high-risk patients, particularly in treatment-resistant cases, and may be a useful marker for evaluating the curative effects of treatment for preterm delivery. In addition, increased circulating Hsp70 levels observed in asthmatic pregnant patients might play a connecting role in the pathomechanism of asthmatic inflammation and obstetrical/perinatal complications. Nevertheless, a prospective study should be undertaken to determine whether elevated serum Hsp70 level precedes the development of any pregnancy complication, and thus can help to predict adverse maternal or perinatal pregnancy outcome. Moreover, the role of

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circulating Hsp70 in normal and pathological pregnancies is not fully known, and further studies are warranted to address this important issue.

Keywords Heat shock protein 70 · HSPA1A · Pregnancy · Preeclampsia · HELLP syndrome · Preterm delivery · Asthma

Introduction

Heat shock proteins (Hsps) are ubiquitous and phylogenetically conserved molecules, which indicate their functional importance. Hsps are traditionally classified on the basis of their molecular weight, but a recent guideline for the nomenclature of the human heat shock proteins is based on the systematic gene symbols that have been assigned by the HUGO Gene Nomenclature Committee (Kampinga et al. 2009). Their expression can be induced by several physiological (growth factors and hormones), pathological (infection, inflammation, ischemia, oxidant injury, and toxins), and environmental (thermal changes and heavy metals) conditions (Prohaszka and Fust 2004). Hsps utilize adenosine triphosphate-driven conformational changes to refold their targets, and they have been implicated in the molecular evolution of modern enzymes (Hartl 1996; Csermely 1997; Csermely 1999). The major classes of heat shock proteins play essential roles in the folding/unfolding of proteins, the assembly of multiprotein complexes, the transport/sorting of proteins into correct subcellular compartments, cell-cycle control and signaling, and the protection of cells against stress/apoptosis (Schlesinger 1990; Li and Srivastava 2004; Borges and Ramos 2005).

The human genome encodes 13 members of the Hsp70 (HSPA) family (Hageman and Kampinga 2009). The best known members are the stress-inducible form Hsp70/Hsp72 (HSPA1A), the constitutively expressed Hsc70/Hsp73/Hsc73 (HSPA8), the endoplasmic reticulum form, Grp78/BiP (HSPA5), and Hsp75/mtHsp70/mortalin/TRAP-1 (HSPA9), which is localized mainly to mitochondria (Tavaria et al. 1996). Of these, the cytosolic inducible Hsp70 can mediate cytoprotective, antiapoptotic, and immune regulatory effects, and is by far the most studied. Enhanced expression of Hsp70 in experimental models of stroke, sepsis, acute respiratory distress syndrome, renal failure, and myocardial ischemia, has been revealed to reduce organ injury and in some cases to improve survival (Weiss et al. 2002; Chen et al. 2003; Giffard and Yenari 2004; Jo et al. 2006). Furthermore, it has been reported that embryonal Hsp70 plays a role in normal development (processes such as apoptosis, cell cycle regulation) and protects against stressors at vulnerable embryonic stages (Luft and Dix 1999). This paper deals with the inducible Hsp72 (HSPA1A), and Hsp70 refers to this protein, if not otherwise specified.

Extracellular Hsp70

Heat shock proteins are usually considered to be intracellular proteins with molecular chaperone and cytoprotective functions (Hightower 1991). However, they can also be expressed on the cell surface (Multhoff and Hightower 1996; Soltys and Gupta 1997). In addition, Hsp60 and Hsp70 have been reported to be present in the serum and plasma of healthy nonpregnant individuals, but the source of circulating Hsps has not yet been completely determined (Pockley et al. 1998; Pockley et al. 1999; Lewthwaite et al. 2002). Recent data suggest that Hsp70 may be released from viable cells exposed to stressful insults into the extracellular environment by nonclassical (endoplasmic reticulum-Golgi-independent) protein transport mechanisms: within exosomes or lysosomes, as well as via intact lipid rafts (Broquet et al. 2003; Hunter-Lavin et al. 2004a; Lancaster and Febbraio 2005; Mambula and Calderwood 2006). However, Basu et al. (2000) and Saito et al. (2005) state that Hsp70 can also be discharged from damaged, necrotic cells in a passive manner. As Hsp70 can also be bound to the membrane or embedded in the membrane, microparticles released from cells by membrane shedding need also be taken into account as a potential source of extracellular Hsp70.

Although intracellular Hsp70 has anti-inflammatory effects, extracellular Hsp70 can act as an intercellular stress signaling molecule, representing an ancestral danger signal of a nonphysiological condition, such as cellular stress or damage, to elicit innate and adaptive proinflammatory immune responses (Pockley 2003). Extracellular Hsp70 acts through binding to surface receptors (CD14, CD36, CD40, CD91, LOX-1, Toll-like receptors 2 and 4) on antigen-presenting cells, stimulating their proinflammatory cytokine (tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β and IL-6), chemokine, and nitric oxide production, as well as costimulatory molecule expression (Asea et al. 2000; Basu et al. 2001; Asea et al. 2002; Asea 2005). Extracellular Hsp70 can induce the maturation, migration and activation of dendritic cells (DCs), converting them from tolerogenic to immunogenic (Basu et al. 2000; Wang et al. 2002; Millar et al. 2003; Asea 2005). It can also directly stimulate the migratory and cytolytic activity of natural killer (NK) cells, as well as can up-regulate γ/δ T cells (Lehner et al. 2000; Gastpar et al. 2005). Hsp70 expressed on the surface of tumor cells has been reported to induce tumor killing by γ/δ T cells (Wei et al. 1996; Thomas et al. 2000). Furthermore, our research group has previously demonstrated that Hsp70 is a potent activator of the classical pathway of the human complement system (Prohaszka et al. 2002). Hsp70 has also been implicated in the processing and presentation of exogenous antigens with chaperoning and transferring antigenic peptides to the class I and class II molecules of the major histocompatibil-

ity complexes (DeNagel and Pierce 1992; Delneste et al. 2002; Srivastava 2002).

Nevertheless, extracellular Hsp70 may also be cytoprotective, as exogenous Hsp70 increases the survival of stressed arterial smooth muscle cells in culture via cell surface binding (Johnson et al. 1990; Johnson and Tytell 1993). Hsp70 has also been demonstrated to protect cells from stress-induced apoptosis (Mosser et al. 2000). In vitro studies showed that Hsp70 can be released by glial cells, and that exogenous Hsc70/Hsp70 (HSPA8/HSPA1A) taken up by neuronal cells can enhance their tolerance to heat shock and staurosporine-induced apoptosis (Guzhova et al. 2001). Additionally, heat shock proteins possess dual immunoregulatory properties, and immune reactivity to endogenous (self-derived) Hsps seems to be anti-inflammatory (Pockley et al. 2008). The induction of T-cell reactivity to self-Hsp70 protected against autoimmune diseases in experimental models by a mechanism involving the development of Th2-type CD4⁺ T cells releasing immunosuppressive cytokines (Kingston et al. 1996; Tanaka et al. 1999; Wendling et al. 2000).

Circulating Hsp70 in normal pregnancy

We reported earlier that serum Hsp70 concentrations are significantly lower in healthy pregnant women than in healthy nonpregnant women (Molvarec et al. 2007a). Bloshchinskaya and Davidovich (2003) as well as Fukushima et al. (2005) also compared circulating Hsp70 concentrations between healthy pregnant and nonpregnant women, and they observed nonsignificantly higher circulating Hsp70 levels in pregnant than in nonpregnant women. However, in their studies, pregnant women with gestosis and preclinical gestosis or preterm delivery high-risk patients were primarily investigated, and there were only 16 and seven healthy nonpregnant women enrolled, respectively. As previous findings on the changes of circulating Hsp70 levels during the course of normal pregnancy had been controversial, we also addressed this issue in our study with a larger sample size. Fukushima et al. (2005) did not find significant differences in serum Hsp70 concentrations among the three trimesters. Conversely, in the study of Jirecek et al. (2002), serum Hsp70 levels tended to decrease, whereas in that of Bloshchinskaya and Davidovich (2003), Hsp70 concentrations in blood plasma tended to increase with advancing gestation. According to our results, serum Hsp70 concentration increases with advancing gestational age, while it decreases with increasing maternal age (Molvarec et al. 2007a).

The fetus is a semi-allograft, since half of its major histocompatibility complex antigens are derived from the father. Several mechanisms have been implicated in the maintenance of immunological tolerance to the embryo/fetus

in pregnancy. Pregnancy is characterized by a shift from T helper type 1 (Th1) towards Th2 cytokine production. Therefore, in normal pregnancy, the maternal immune response is biased towards humoral immunity, and the cell-mediated (cytotoxic) immune response, which could be harmful to the fetus, is suppressed (Wegmann et al. 1993). Dendritic cells (DCs) are known to be the most potent antigen-presenting cells. DCs at the maternal-fetal interface (decidual DCs), and in the peripheral circulation, seem to play a crucial role in controlling the maternal immune response to the semi-allogeneic fetus (Juretic et al. 2004; Adams et al. 2007). Natural killer cells and γ/δ T cells have also been implicated in the immunobiology of the maternal-fetal relationship (Szekeres-Bartho et al. 2001; Sargent et al. 2006).

The capacity of extracellular Hsp70 to elicit innate and adaptive proinflammatory immune responses (Pockley 2003)—involving the conversion of DCs from tolerogenic to immunogenic and the stimulation of the cytolytic activity of NK cells and γ/δ T cells—might be harmful in pregnancy, and may lead to the maternal immune rejection of the fetus. In addition, Hsps up-regulated in transplanted organs and anti-Hsp immune reactivity might also drive transplant rejection responses, but the overall influence of Hsps on graft outcome is complex (Pockley 2001; Pockley and Muthana 2005). On the other hand, exosomes present in sera and amniotic fluids of pregnant women have been supposed to protect the fetus against rejection by the maternal immune system (Taylor et al. 2006; Asea et al. 2008). Interestingly, amniotic fluid-derived exosomes have recently been demonstrated to contain Hsp70 (Asea et al. 2008).

We hypothesize that extracellular Hsp70 may be removed by innate immune mechanisms in physiological pregnancy as part of the tolerogenic changes of the immune system. Decreased circulating Hsp70 level, as a result, may promote the maintenance of immunological tolerance to the fetus. In normal pregnancy, components of the maternal innate immune system are activated systemically, involving a rise in number and activation of monocytes and granulocytes, activation of the complement system and production of acute phase proteins (Sacks et al. 1999). There are scavenger receptors on activated monocytes and macrophages such as LOX-1, which can potentially bind and take up Hsp70 (Delneste et al. 2002; Theriault et al. 2005). Another possible mechanism might be elimination by naturally occurring anti-Hsp70 antibodies (Molvarec et al. 2009a). However, anti-Hsp70 IgG markedly enhanced the capacity of Hsp70 to induce proinflammatory immunity (Yokota et al. 2006). The increased efficacy appeared to involve the aggregation of Hsp70 molecules by binding to the antibody. Moreover, anti-Hsp70 IgG may potentiate production of anti-microbial immune mediators in the amniotic cavity (Gelber et al. 2007). Additional studies are essential to assess the cause of decreased circulating Hsp70 concentrations observed in healthy pregnant women.

The increase in serum Hsp70 levels with advancing gestation in normal pregnancy might be explained by increasing shedding of this protein with trophoblast debris from the growing placenta into the maternal circulation (Redman and Sargent 2000). Indeed, trophoblast cells have been shown to express Hsp70 (Divers et al. 1995; Li et al. 1996; Shah et al. 1998; Ziegert et al. 1999). Nevertheless, the reports on alterations in placental expression of Hsp70 throughout pregnancy are contradictory. Divers et al. (1995) found that Hsp70 expression is constant throughout the third trimester of pregnancy in uteroplacental tissues. Li et al. (1996) did also not detect correlation between gestational age and placental expression of Hsp70. In contrast, Shah et al. (1998) observed that the intensity of immunostaining for Hsp70 decreases with advancing gestation in decidual and placental tissues. Earlier and recent findings suggest that elevated intracellular and extracellular Hsp70 levels at term might play a role in the onset of labor. In an animal model, dramatic tissue-specific increases in Hsp90 and Hsp70 messenger ribonucleic acid (mRNA) were observed in myometrium and endometrium during spontaneous and glucocorticoid-induced labor. The authors hypothesize that increased abundance of Hsp90 and Hsp70 at term may inhibit uterine progesterone receptor and stimulate estrogen receptor function in uterine tissues, which might lead to the onset of labor (Wu et al. 1996). Hsp70 is also present in the amniotic fluid, and term parturition was associated with elevated amniotic fluid Hsp70 concentrations (Jean-Pierre et al. 2006; Chaiworapongsa et al. 2008).

The age-related decrease in serum Hsp70 levels observed in healthy pregnant women is in agreement with previous findings in nonpregnant subjects, and it might reflect the reduced ability of cells to respond to stress and synthesize Hsps with increasing age (Rao et al. 1999; Rea et al. 2001; Jin et al. 2004).

Circulating Hsp70 in preeclampsia

Preeclampsia, characterized by hypertension and proteinuria occurring after mid-gestation, is a severe complication of human pregnancy with a worldwide incidence of 2–10% (Duckitt and Harrington 2005). It is one of the leading causes of maternal, as well as perinatal morbidity and mortality, even in developed countries. Despite intensive research efforts, the etiology and pathogenesis of preeclampsia are not completely understood. Increasing evidence suggests that an excessive maternal systemic inflammatory response to pregnancy with systemic oxidative stress and resultant endothelial damage plays a crucial role in the pathogenesis of the disease (Redman et al. 1999; Redman and Sargent 2005). The development of pre-

eclampsia is influenced by both genetic and environmental risk factors, suggesting its multifactorial inheritance (Roberts and Gammill 2005).

Several studies investigated circulating Hsp70 levels in preeclampsia. In a pilot study, Jirecek et al. (2002) found higher serum levels of Hsp70 in patients with early onset of severe preeclampsia. Fukushima et al. (2005) reported significantly higher Hsp70 serum levels in preeclampsia. Our research group observed that serum Hsp70 concentrations are elevated in transient hypertension of pregnancy, preeclampsia, and superimposed preeclampsia (Molvarec et al. 2006). On the contrary, Livingston et al. (2002) did not detect higher plasma levels of Hsp70 in women with severe preeclampsia. However, the scatter of plasma Hsp70 levels in their preeclamptic group was much higher than in their group of normotensive pregnant women. Furthermore, their control group included patients with premature rupture of membranes, premature labor, and placental abruptions.

In a recently published study of ours, increased serum Hsp70 levels showed significant correlations with serum C-reactive protein (CRP) levels, serum aspartate aminotransferase and lactate dehydrogenase (LDH) activities, as well as with plasma malondialdehyde levels in preeclampsia (Molvarec et al. 2009b).

The intracellular expression of Hsp70 can be induced by ischemia, reactive oxygen species, and inflammatory cytokines, as well as by hemodynamic stress (acute hypertension) (Prohaszka and Fust 2004). Placental ischemia and oxidative stress, an excessive maternal systemic inflammatory response with systemic oxidative stress, as well as hemodynamic stress have been implicated in the pathogenesis of preeclampsia. In vitro ischemia–reperfusion injury, as a suitable model for oxidative stress in preeclampsia, increased Hsp70 concentration in placental tissues (Hung et al. 2001). In addition, hypoxia-induced apoptosis was accompanied by increased expression of Hsp70 in trophoblast cells (Ishioka et al. 2007). Indeed, a significant up-regulation of Hsp70 was observed both in mRNA and in protein level in placental tissue of patients with placental vascular disease (preeclampsia and/or fetal growth restriction) in comparison to normal pregnancies (Liu et al. 2008). Conversely, there was no significant difference in immunostaining intensity for Hsp70 in placentas from preeclamptic compared with those from normotensive pregnancies. However, placental bed biopsies were not obtained in that study (Hnat et al. 2005). Acute hypertension induced Hsp70 gene expression in rat aorta (Xu et al. 1995). Moreover, Hsp70 expression induced by heat stress was found to be higher in peripheral blood lymphocytes from hypertensive humans compared with those from normotensives (Kunes et al. 1992).

According to our findings, systemic inflammation and oxidative stress seem to be responsible—at least in part—for increased circulating Hsp70 levels in preeclampsia, as

suggested by the significant positive correlations of serum Hsp70 levels with circulating levels of CRP (acute phase reactant) and malondialdehyde (lipid peroxidation product). Indeed, inflammatory cytokines have been reported to induce extracellular release of Hsp70 within exosomes (Bausero et al. 2005). Additionally, oxidative stress has been implicated in the exercise-induced circulating Hsp70 response, and the supplementation with vitamin C and the vitamin E isoform γ -tocopherol completely blunted this response (Fischer et al. 2006). Furthermore, the antioxidant folic acid, which reduces oxidative stress *in vivo*, significantly decreased serum Hsp70 levels in patients with type2 diabetes (Hunter-Lavin et al. 2004b).

Nevertheless, not only can Hsp70 be released from intact cells by active mechanisms, but it may also be discharged from damaged, necrotic cells in a passive manner (Basu et al. 2000). Circulating Hsp70 levels were found to be increased in several conditions where tissue damage is known to occur (Walsh et al. 2001; Dybdahl et al. 2002; Pittet et al. 2002; Kimura et al. 2004; Adewoye et al. 2005; Dybdahl et al. 2005; Fehrenbach et al. 2005; Suzuki et al. 2006). In our study, liver enzymes—particularly serum LDH activities—showed the strongest correlations with serum Hsp70 levels in preeclampsia, which suggests that release of Hsp70 from necrotic hepatic cells might substantially contribute to the elevation in circulating Hsp70 levels found in preeclampsia. However, hepatocellular necrosis occurs only in severe cases of preeclampsia, and it explained only 25% of the variance in serum Hsp70 concentration in our preeclamptic group. Indeed, Hsp70 may also be released from intact hepatic cells, as was observed during semi-recumbent cycling (Febbraio et al. 2002). Interestingly, a number of acute phase proteins, such as CRP, are also synthesized and released by the liver. Thus, the significant correlation between serum Hsp70 and CRP levels raises the possibility that Hsp70 may originate from this organ in preeclampsia in the absence of hepatocellular necrosis. Moreover, innate immune cells (monocytes and granulocytes), which are exaggeratedly activated in preeclampsia and produce both proinflammatory cytokines and reactive oxygen species, are also capable of releasing Hsp70 into the extracellular space (Hunter-Lavin et al. 2004a), and might be additional sources of circulating Hsp70 in preeclampsia. Interestingly, circulating levels of both CRP and malondialdehyde showed significant correlations with serum Hsp70 levels in preeclampsia, which may support this hypothesis. In addition, Hsp70 might also be released into the maternal circulation in preeclampsia from necrotic trophoblast cells with increased shedding of trophoblast debris or from damaged endothelial cells (Redman and Sargent 2001).

Elevated circulating Hsp70 level may not only be a marker of preeclampsia, but might also play a role in its pathogenesis. Extracellular Hsp70 derived from stressed and damaged, necrotic cells can elicit innate and adaptive

proinflammatory (Th1) immune responses (Pockley 2003). The maternal systemic inflammatory response, which seems to be responsible for the signs and symptoms of preeclampsia, involves a rise in number and activation of leukocytes (monocytes and granulocytes) with production of proinflammatory cytokines leading to Th1 bias, as well as the activation of the complement system and the production of acute phase proteins (Redman et al. 1999). The relationship between increased serum Hsp70 and CRP levels found in our study suggests that circulating Hsp70 might be involved in the development of the maternal systemic inflammatory response in preeclampsia. Indeed, elevated circulating Hsp70 levels have already been observed to be associated with inflammatory responses in several pathological conditions, such as in acute infections, after liver resection and coronary artery bypass grafting, as well as following myocardial infarction (Dybdahl et al. 2002; Njemini et al. 2003; Kimura et al. 2004; Dybdahl et al. 2005). However, Hsp70 can also have anti-inflammatory effects (Kingston et al. 1996; Tanaka et al. 1999; Wendling et al. 2000; House et al. 2001), and it might therefore also be involved in the resolution of inflammation. As extracellular Hsp70 has been reported to bind to endothelial cells (Theriault et al. 2005), and Hsp70 has recently been found to be associated with endothelial activation in placental vascular diseases (Liu et al. 2008), circulating Hsp70 might also directly contribute to endothelial activation or injury in preeclampsia. Nevertheless, recent preliminary findings have demonstrated internalization of Hsp70 by human endothelial cells, suggesting an underlying mechanism for its atheroprotective properties (Pockley et al. 2009).

Circulating Hsp70 in HELLP syndrome

The syndrome of hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) is a severe variant of preeclampsia with substantial maternal and perinatal morbidity and mortality (Baxter and Weinstein 2004). The incidence of HELLP syndrome among women with severe preeclampsia or eclampsia is 10–20% (Sibai et al. 1993). The syndrome is characterized by microvascular intimal damage, primarily in the liver, which is followed by platelet activation and aggregation with formation of microthrombi and fibrin deposits, ultimately resulting in distal ischemia and hepatocellular necrosis, as well as platelet consumption or destruction. The microangiopathic red blood cell damage leads to intravascular hemolysis with typical presence of fragmentocytes on the peripheral blood smear (Weinstein 1982; Curtin and Weinstein 1999; Baxter and Weinstein 2004).

We demonstrated for the first time in the literature that serum Hsp70 levels are significantly higher in patients with HELLP syndrome than in severely preeclamptic patients

without HELLP syndrome (Molvarec et al. 2007b). Furthermore, serum Hsp70 levels showed a very strong correlation to the markers of hemolysis (plasma free hemoglobin level, serum LDH activity and total bilirubin level) and of hepatocellular injury (serum aminotransferase activities) in HELLP syndrome. Circulating Hsp70 concentration reflected also the severity of the disease, as was expressed by the significant inverse correlation to the lowest platelet count (Madach et al. 2008).

Multiple studies suggest that both active and passive routes of Hsp70 release might occur in HELLP syndrome. First, hemolysis (in patients with healthy bone marrow) induces a strong reticulocyte response, and reticulocytes are known to release Hsp70-containing exosomes during their maturation (Mathew et al. 1995). Additionally, the concomitant release of Hsp70 and markers of hemolysis and tissue damage has been reported after an Ironman triathlon race (Suzuki et al. 2006). The group of Alexzander Asea demonstrated the elevation of Hsp70 levels in the circulation of patients with sickle cell disease, a disorder characterized also by hemolysis and tissue damage (Adewoye et al. 2005). Hsp70 was also suggested to be released during liver resection in response to surgical stress and liver injury (Kimura et al. 2004). Finally, our research group has recently revealed that hepatocellular injury is associated with increased serum Hsp70 levels in chronic heart failure (Gombos et al. 2008).

In addition to being a marker of tissue damage (hemolysis and hepatocellular injury) and disease severity in HELLP syndrome, we speculate that circulating Hsp70 might also be involved in the pathogenesis of the disorder. Extracellular Hsp70 can stimulate proinflammatory cytokine (TNF- α , IL-1 β , and IL-6) production of antigen-presenting cells (Asea et al. 2000). Hsp70 can also activate the classical complement pathway (Prohaszka et al. 2002). Interestingly, the complement system has been reported to be activated, and plasma levels of TNF- α and IL-6 were found to be increased in HELLP syndrome (Haeger et al. 1990; Haeger et al. 1996). Indeed, a maternal cell-mediated immune response to the semi-allogeneic fetus with cytokine-mediated endothelial damage might play a crucial role in the pathogenesis of HELLP syndrome (Curtin and Weinstein 1999). As serum Hsp70 concentrations were significantly higher in HELLP syndrome than in severe preeclampsia without HELLP syndrome, it is also possible that circulating Hsp70 accounts directly or indirectly—at least in part—for the more pronounced endothelial injury observed in HELLP syndrome (Paternoster et al. 1995; Liu et al. 2008).

Circulating Hsp70 in preterm delivery

Preterm delivery, defined as delivery before 37 completed gestational weeks, is a major problem of obstetrics all over the

world, even nowadays. It is the leading factor causing perinatal morbidity and mortality. Its short-term and long-term sequelae constitute a serious problem in terms of mortality, disability, and cost to society (Slattery and Morrison 2002). Approximately 5–10% of all births are premature. Preterm delivery is associated with preterm rupture of membranes, cervical incompetence, polyhydramnios, fetal and uterine anomalies, infections, social factors, stress, smoking, heavy work, and other risk factors (Haram et al. 2003).

Several factors implicated in the etiology and pathogenesis of this obstetrical complication are also known to induce Hsp70 expression (Prohaszka and Fust 2004). Indeed, both intracellular expression and extracellular levels of Hsp70, as well as immune reactivity towards this protein, have been reported to be enhanced in preterm delivery and predisposing conditions. The presence of Hsp70 in vaginal samples of mid-trimester pregnant women was found to be associated with bacterial vaginosis (Genc et al. 2005). Vaginal Hsp70 release in response to abnormal vaginal microflora may trigger nitric oxide production in an attempt to minimize the pathological consequences of this altered milieu (Genc et al. 2006). In addition, human amniochorionic membranes constitutively express Hsp70 mRNA, and bacterial lipopolysaccharide markedly stimulated Hsp70 gene transcription in fetal membranes (Menon et al. 2001). Hsp70 was observed to be released from cells in mid-trimester amniotic fluid as a consequence of Toll-like receptor 2 stimulation, and exogenous Hsp70 potentiated TNF- α production by amniotic fluid cells (Jean-Pierre et al. 2006). Intra-amniotic infection, histologic chorioamnionitis and term parturition have recently been shown to be associated with elevated amniotic fluid Hsp70 concentrations. The authors concluded that the mechanisms of preterm and term parturition in humans may involve extracellular Hsp70 (Chaiworapongsa et al. 2008). Furthermore, the presence of anti-Hsp60 and anti-Hsp70 antibodies in the serum and formation of Hsp60- and Hsp70-immune complexes in the placenta were also associated with preterm birth (Ziegert et al. 1999). However, investigating Hsp60, Hsp70, and Hsp90 expression in placental and decidual tissues, Divers et al. (1995) did not find an immunohistochemical evidence for a differential stress response in preterm labor.

Given the heterogeneous etiopathogenesis of preterm delivery, currently, there is no reliable single biomarker with appropriate sensitivity and specificity for predicting women at risk (Lockwood and Kuczyński 1999; Vogel et al. 2005; Yeast and Lu 2007). In this context, it is noteworthy that Fukushima et al. (2005) observed significantly higher serum Hsp70 levels in pregnant women at a higher risk for preterm delivery than in healthy pregnant women without any complication. Moreover, serum Hsp70 concentrations were significantly higher in preterm delivery high-risk patients who delivered preterm as compared with

those who had full-term delivery. Because Hsp70 levels were particularly high in treatment-resistant preterm delivery cases, the authors suggested that Hsp70 may be a useful marker for evaluating the curative effects of treatment for preterm delivery. In addition, Hsp70 might also be involved in the final common mechanism leading to myometrial activation and contractions, as was supposed during normal delivery (Wu et al. 1996; Chaiworapongsa et al. 2008).

Circulating Hsp70 in other pregnancy complications

In a recent study, we found increased circulating Hsp70 levels in pregnant asthmatics as compared with maternal and gestational age-matched healthy pregnant women (Tamasi et al. *in press*). In asthma, airway cells (epithelial cells and alveolar macrophages) as well as peripheral blood mononuclear cells showed increased expression of Hsp70 (Vignola et al. 1995; Tong and Luo 2000). Hsp70 has been suggested to play an important role in the initiation and modulation of the asthmatic immune response and the maintenance of the chronic bronchial inflammation in asthma (Bertorelli et al. 1998; Harkins et al. 2003). Asthmatic women are at an increased risk for several complications during pregnancy, including preeclampsia and preterm delivery (Liu et al. 2001). As elevated circulating Hsp70 levels were associated with a higher risk for these pregnancy complications, we hypothesize that circulating Hsp70 might play a connecting role in the pathomechanism of asthmatic inflammation and obstetrical/perinatal complications in asthmatic pregnant patients.

Hsp70 has been shown to have several important functions during mammalian embryogenesis, including embryo-protective effects (Luft and Dix 1999). Child et al. (2006) reported that serum anti-Hsp70 antibody levels are significantly elevated at 16 weeks of gestation in women who later gave birth to babies with birth defects (cleft lip or palate or neural tube defects), suggesting a prior increase in Hsp70 expression. Although the authors did not find a significant difference in serum Hsp70 levels between women whose babies were born with a birth defect and those who gave birth to healthy babies, they did not obtain blood samples in the first trimester. It is still also unclear whether Hsp70 is involved directly or indirectly in the pathogenesis of these anatomical malformations.

Altered intracellular Hsp70 expression has also been observed in other obstetrical complications. A sharp peak of expression of the inducible form of Hsp70 within the syncytiotrophoblast has been reported between 8 to 9 weeks in normal pregnancies. The authors concluded that a burst of oxidative stress occurs in the normal placenta as the maternal circulation is established. This may stimulate normal placental differentiation, but may also be a factor

in the pathogenesis of early pregnancy failure if antioxidant capacity is depleted (Jauniaux et al. 2000). Indeed, placental Hsp70 expression has been shown to be enhanced in first trimester missed miscarriages by several studies, and this increase may have an important implication on the miscarriage process (Hempstock et al. 2003; Jauniaux et al. 2003; Sotiriou et al. 2004). Additionally, increased Hsp70 levels in the lymphocytes were found to be associated with high risk of adverse pregnancy outcomes in early pregnancy, which also suggests that Hsp70 may be a marker of early pregnancy failure (Tan et al. 2007). There are conflicting reports in the literature regarding the changes in placental Hsp70 expression in intrauterine growth restriction (IUGR). In thrombus, excessive syncytial knots and avascular villi, the expression of Hsp70 was higher in placentas with IUGR in both syncytiotrophoblasts (ST) and cytotrophoblasts (CT). In contrast, Hsp70 expression decreased in both ST and CT around the infarction region (Wataba et al. 2004). In another study, while immunostaining for Hsp70 was revealed to be significantly less intense in the vascular endothelium from placentas of IUGR pregnancies, there was no significant difference in total Hsp70 expression in villous tissue as compared with normal pregnancies (Hnat et al. 2005). On the other hand, in pregnancies with placental vascular disease (preeclampsia and/or IUGR), the expression of Hsp70 in both mRNA and protein level was up-regulated in placental tissue and microvascular endothelial cells, and showed inverse correlation with fetal birth weight (Liu et al. 2008). Nevertheless, the source of circulating Hsps in healthy individuals, as well as in patients with pathological conditions has not been fully identified yet. It is also unknown whether circulating Hsp70 levels reflect intracellular concentration. Further studies are required to determine the association of circulating Hsp70 with spontaneous abortion and fetal growth restriction.

Concluding remarks

Hsp70 is present in the peripheral circulation of healthy pregnant women. Circulating Hsp70 levels are decreased in normal human pregnancy as compared with nonpregnant women, and show a positive correlation with gestational age and an inverse correlation with maternal age. The capacity of extracellular Hsp70 to elicit innate and adaptive proinflammatory (Th1) immune responses—involving the conversion of dendritic cells from tolerogenic to immunogenic and the stimulation of the cytolytic activity of NK cells and γ/δ T cells—might be harmful in pregnancy, and may lead to the maternal immune rejection of the fetal semi-allograft. We hypothesize that extracellular Hsp70 may be removed by innate immune mechanisms in physiological pregnancy as

part of the tolerogenic changes of the immune system. Decreased circulating Hsp70 level, as a result, may promote the maintenance of immunological tolerance to the fetus. Indeed, elevated circulating Hsp70 concentrations are associated with an increased risk of several pregnancy complications. Elevated Hsp70 levels in healthy pregnant women at term might also have an effect on the onset of labor. In preeclampsia, serum Hsp70 levels are increased in comparison to normal pregnancy, and reflect systemic inflammation, oxidative stress and hepatocellular injury. Furthermore, serum Hsp70 levels are significantly higher in patients with HELLP syndrome than in severely preeclamptic patients without HELLP syndrome. In HELLP syndrome, elevated serum Hsp70 level indicates tissue damage (hemolysis and hepatocellular injury) and disease severity. Increased circulating Hsp70 level may not only be a marker of preeclampsia and HELLP syndrome, but might also play a role in their pathogenesis. Extracellular Hsp70 derived from stressed and damaged, necrotic cells can elicit a proinflammatory (Th1) immune response, which might be involved in the development of the maternal systemic inflammatory response and resultant endothelial damage in preeclampsia and HELLP syndrome. Circulating Hsp70 level is also elevated in preterm delivery high-risk patients, particularly in treatment-resistant cases, and may be a useful marker for evaluating the curative effects of treatment for preterm delivery. In addition, increased circulating Hsp70 levels observed in asthmatic pregnant patients might play a connecting role in the pathomechanism of asthmatic inflammation and obstetrical/perinatal complications. However, a prospective study should be undertaken with sequential measurements from the preconceptional stage, throughout the pregnancy and continuing until the end of the puerperium to determine the changes in serum Hsp70 levels during the course of pregnancy. Such a longitudinal study could also assess whether elevated serum Hsp70 level precedes the development of any pregnancy complication, and thus can help to predict adverse maternal or perinatal pregnancy outcome. Moreover, membrane microparticles, especially syncytiotrophoblast-derived microparticles as a potential source of extracellular Hsp70 should also be investigated in future studies. Finally, it is also possible that elevated Hsp70 level is a consequence of adverse conditions such as preeclampsia, HELLP syndrome and preterm labor in an attempt to return to homeostasis. Further studies are therefore highly warranted to determine the role of circulating Hsp70 in normal and pathological pregnancies.

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