Minireview

Role of Hsp70 in regulation of stress-kinase JNK: implications in apoptosis and aging

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Abstract Cell protection from stresses by the major heat shock protein Hsp72 was previously attributed to its ability to prevent aggregation and to accelerate refolding of damaged proteins. This repair function of Hsp72 may play an important role in cell survival after extremely harsh protein damaging treatments leading to necrotic cell death. On the other hand, protein repair function of Hsp72 cannot explain how it protects cells from stresses which do not cause direct protein damage, e.g. some genotoxic agents. These stresses kill cells through activation of apoptosis, and Hsp72 increases cell survival by interfering with the apoptotic program. Recently it has been found that Hsp72 mediates suppression of a stress-activated protein kinase, JNK, an early component of stress-induced apoptotic signalling pathway. This finding provides the basis for the anti-apoptotic activity of Hsp72. These observations can explain increased stress sensitivity of aged cells in which compromised inducibility of Hsp72 leads to a loss of control of JNK activation by stresses and subsequently to a higher rate of apoptotic death.

Key words: Hsp72; JNK stress kinase; Apoptosis; Aging

1. Introduction

Heat shock proteins of Hsp70 family function as molecular chaperones in protein folding, transport and degradation (see [1] for review). Members of this protein family, including Hsp72, are induced in response to stresses and serve to protect cells against heat shock and other harmful conditions which cause massive protein damage and denaturation [2,3]. Since Hsp72 promotes protein folding, this heat shock protein was suggested to prevent and/or repair stress-induced protein damages. Indeed, there are many experimental data in the literature which support this suggestion. For example, overexpression of Hsp72 strongly decreased heat-induced aggregation of nuclear proteins as well as inactivation of a reporter enzyme, firefly luciferase [4–6]. Furthermore, accumulation of Hsp72 and other heat shock proteins due to the priming of cells with mild heat shock dramatically reduced aggregation of actin and other cytoskeletal constituents caused by severe heat shock [7,8]. Reduction of protein aggregation in cells expressing Hsp72 also correlated with an increased survival following treatment with protein damaging agents such as SH-reagents (arsenite-diamide) and ethanol [9]. It also correlated with the increased survival of cells following ischemia or oxidative stress (see [10] for review). These data taken together have led to a common and natural assumption that activities of Hsp72 in refolding, prevention of aggregation and of degradation of damaged polypeptides form the basis of its protective function.

On the other hand, another set of data in the literature suggests that Hsp72 plays a distinct role in cell protection. Several groups reported that Hsp72 prevents cell death after stresses that do not cause detectable protein damage. For example, overproduction of Hsp72 protected cells from death caused by UV irradiation [11], etoposide [12], doxorubicin [13], and from a death induced by signalling molecules, TNF [14] and NO [15] (Table 1). Furthermore, it was found that overproduction of Hsp72, while protecting cells from a DNA-damaging anticancer drug doxorubicin, did not reduce DNA lesions [13]. Therefore, a new distinct function of Hsp72 might exist that plays a major role in cell survival after stresses. To better understand the protective action of Hsp72, one should take a closer look at the mechanisms of cell killing by stresses.

2. Apoptosis as a novel mode of stress-induced cell death

Originally investigators distinguished two modes of cell death under hyperthermia and other stresses: (i) necrosis, which is associated with marked damage of intracellular structures and cytoplasmic membrane, followed by the leakage of the cellular constituents; and (ii) reproductive death, which is manifested by the loss of the colony-formation ability. After extreme stresses (e.g. heating at more than 46–47°C) mammalian cells usually die via necrosis, while milder stresses do not cause visual damage but nevertheless lead to the loss of reproductive ability. In addition, recently it became clear that cells die via the third mode of death – apoptosis after non-extreme heat shock (44–45°C), UV or ionizing irradiation, oxidative stress, exposure to heavy metals, and other stresses [16–18]. Apoptotic program activates membrane blebbing, mitochondrial damage, degradation of some proteins and nuclear DNA and other events, which ultimately culminate in cell death (see [19–21] for review). Recent data indicate that under pathological conditions such as heart or brain ischemia, Alzheimer's, Huntington's and other neurodegerative diseases, apoptosis is the major contributor to cell death [20,22].

In contrast to necrosis, in the apoptotic process initial stress-induced damage does not kill cells directly. Rather, it triggers an apoptotic signalling program that leads to the cell's suicide. It is of importance that suppression of this signalling pathway can completely prevent the loss of cell viability. The

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Table 1
Hsp72-mediated projection and JNK dependence of apoptotic cell death

Stimuli	Hsp72-mediated protection	JNK dependence of apoptosis	References
Heat shock	+	+	[26,30,31]
Ethanol	+	+	[28,31]
UV	+	+	[11,26,37]
Oxidative stress	+	+	[11,15,26]
Etoposide	+	+	[12,25]
Proteasome inhibitors	+	+	[28]
TNF	+/−[a]	+/−[a]	[14,26]
TCR/CD3 activation	−	−	[51,52]
Fas	−	−	[51,52]

[a]Absence of protection by Hsp72 and JNK-independent apoptosis was demonstrated in some cell lines [28,53].

apoptotic program involves several major players, including mitochondrial membrane proteins of the bcl-2 family, a family of cysteine proteinases (caspases), and stress-activated kinases; inactivation of these components by a number of methods dramatically raises survival of cells exposed to stresses [17,19–21]. For example, overproduction of anti-apoptotic proteins bcl-2 and bcl-x prevents cell death under many stressful conditions [21]. Similarly, specific inhibition of caspases, which are involved in execution of apoptosis, markedly improves cell survival after the stresses [19]. Likewise, inhibition of a stress-activated protein kinase JNK, another component of the apoptotic signalling pathway, prevents apoptosis induced by a variety of stresses, including heat shock, ethanol, UV irradiation, oxidative stress and others [23–27] (Table 1). This was demonstrated by interruption of the JNK signalling pathway either immediately upstream of JNK (by expression of dominant-negative mutants of the JNK activator SEK1) [23,26] or immediately downstream of JNK (by expression of a dominant negative mutant of JNK substrate, c-jun) [26,28].

3. Hsp72 regulates activity of stress kinase KNL

How does Hsp72 protect cells from stresses and other stimuli that do not cause direct protein damage ('non-proteotoxic' stresses) like UV irradiation, TNF, NO, etoposide and others (Table 1)? It is likely that Hsp72 can somehow interfere with the apoptotic program initiated by these stimuli, thus allowing cells to survive. Similarly, interference with the apoptotic program rather than repair of damaged proteins may explain the protective effect of Hsp72 against mild proteotoxic stresses, like heat shock, ethanol, amino acid analogs, etc.

The hypothesis that Hsp72, when present at an elevated level, inhibits apoptosis has been recently tested by several groups. It was clearly demonstrated that elevated levels of Hsp72 strongly reduced activation of caspases, and suppressed other manifestations of apoptosis such as membrane blebbing, mitochondrial damage, and nuclear fragmentation [29–32]. Using the lymphoid cell line PEER stably transfected with a plasmid which encodes Hsp72 under the regulation of an inducible promoter, our group and, independently, Mosser et al. [30] have found that Hsp72 affects the apoptotic pathway at a very early stage, namely it prevents activation of a stress kinase, JNK. In fact, activation of JNK in response to heat shock, ethanol and certain other stresses was dramatically reduced in cells with transiently increased levels of this heat shock protein [30,31]. Similar results were obtained with other cell lines including primary cultures of human fibroblasts, embryonic cardiomyocytes, and other cells which expressed Hsp72 transiently from an adenovirus-based vector ([33] and unpublished data). It is noteworthy that the same blockade of JNK activation was achieved when Hsp72 was induced by physiological conditions (mild heat shock) or by a drug, proteasome inhibitor MG132 [28,31]. Furthermore, there is a clear correlation between the dependence of apoptosis on activation of JNK and suppression of apoptosis by elevated expression of Hsp72. Indeed, as shown in Table 1, apoptosis after all the stresses which activate cell death via the JNK signalling pathway, could be suppressed by Hsp72. In contrast, JNK-independent apoptosis initiated by FAS, TCR or TNF (in certain cell lines) cannot be suppressed by Hsp72. These data indicate that JNK or regulators of JNK activity is the site(s) of inhibitory action of Hsp72 on the apoptotic program.

Although Hsp72 can suppress apoptosis induced by the stresses which do not cause detectable protein damage, there is still a remote possibility that such stresses cause a minor undetectable protein damage which is sufficient to activate JNK and subsequently apoptosis. At this scenario, Hsp72 repairs such damage, thus protecting cells, and hence the function of Hsp72 in suppression of JNK could be explained purely by Hsp72's chaperone activity. We think this possibility is unlikely, because the function of Hsp72 in suppression of JNK activation could be separated from its function in protein refolding. Indeed, we have observed that a mutant Hsp72 lacking the ATPase domain and therefore unable to promote refolding is still capable of suppressing JNK activation and heat-induced apoptosis (manuscript in preparation). Similarly, Dr. D. Mosser observed that a mutant Hsp72 lacking four C-terminal amino acids suppresses JNK activation (Mosser, personal communication), but is unable to bind damaged proteins and even inhibits protein refolding by normal Hsp72 [34]. Therefore, suppression of JNK does not require chaperone function, and Hsp72 plays a distinct role in JNK signalling.

The suppression of JNK by Hsp72 sheds new light on the phenomenon of acquired stress tolerance, i.e. that mild heat shock followed by the recovery period makes cells resistant to hyperthermia, doxorubicin, ethanol and other stresses [13,18]. Mild heat shock initiates two processes: it rapidly and transiently activates JNK to a relatively low level which is insufficient to turn on the apoptotic process, and induces slow (within several hours) accumulation of Hsp72 [31]. After a recovery period, the accumulated Hsp72 suppresses the activation of JNK following exposure of cells to stresses and cells do not undergo apoptosis.

Potentially, there could be other mechanisms of interference of Hsp72 with the apoptotic pathway, independent of suppression of JNK. This possibility was seemingly supported by the fact that in contrast to inducible expression of recombinant Hsp72, constitutive expression of this protein did not suppress JNK activation immediately following heat shock, although it protected cells from apoptosis and inhibited activation of caspases [30,32]. It was suggested therefore that Hsp72 has an additional target in the apoptotic program downstream of JNK and upstream of caspases. Although this is a plausible hypothesis, this experimental system is somewhat problematic. Hsp72 expressed at high levels constitutively is toxic for a cell [35], which could lead to selection of a variety of unpredictable adaptive changes in surviving cells. It is conceivable that during establishing cell lines that chronically overproduce Hsp72, resistant mutant variants with defects in the apoptotic machinery downstream of JNK were selected. In addition, using Rat-1 cells stably expressing human Hsp72 at high levels [29,36], we have found that although the initial levels of JNK activation following heat shock are not affected, JNK activity decays in these cells much faster than in control cells (manuscript in preparation). Prolonged JNK activation was shown by several groups to be critical for apoptosis (e.g. [37,38]) and therefore effects of Hsp72 on apoptosis could potentially be explained solely by its effects on JNK. Moreover, effects of the regulated acute overexpression of Hsp72 on JNK are identical to that of elevated levels of Hsp72 induced by mild heat shock (i.e. classical heat shock response), indicating that the JNK pathway is the physiologically relevant target of Hsp72 in protection of cells from apoptosis [28,31,32].

Another important implication of the new finding of Hsp72-mediated suppression of the JNK signalling pathway is in cell apoptosis following heart ischemia and stroke. Indeed, overproduction of Hsp72 was shown to strongly protect heart and brain cells from the ischemia/reperfusion injuries both in vitro and in animal models (see [10] for review). These findings stimulated a search for non-toxic Hsp72 inducers to be used for treatment of ischemia and related conditions. As mentioned above, the protective action of Hsp72 in ischemia-induced necrosis was previously explained by the Hsp72-mediated prevention of protein aggregation [7,39]. However, in the cells undergoing apoptosis after ischemia/reperfusion suppression of JNK is likely to play a major role in Hps72-mediated protection. First, ischemia/reperfusion strongly activates JNK and a homologous kinase p38 in cardiomyocytes [40,41], and their simultaneous activation is sufficient to cause apoptosis in these cells [42], and second, in H9c2 myogenic cells subjected to simulated ischemia/reperfusion, both JNK and p38 activities were inhibited after expression of Hsp72 which correlated with increased survival (Gabai et al., manuscript in preparation). Therefore protective action of Hsp72 could be associated with inhibition of JNK and p38 after ischemia/reperfusion.

4. Hsp72 and aging

The discovered phenomenon of suppression of the JNK signalling pathway by Hsp72 has an important implication in aging. Aged organisms exhibit a greatly decreased ability to induce Hsp72 and other heat shock proteins in response to stresses [43], which correlates with the increase in morbidity and mortality of aged organisms exposed to hyperthermia [43,44]. Diminishing of inducibility of Hsps and increasing of sensitivity to stresses with aging could also be observed in cell cultures. Indeed, cells isolated from aged organisms, as well as cells isolated from young organisms and aged in culture, show reduced ability to induce Hsps and reduced rate of survival upon exposure to stresses [43]. We suggested that, owing to the diminished inducibility of Hsp72 in aged cells, Hsp72-mediated control of JNK in response to stresses is compromised, thus resulting in a higher rate of apoptosis. In fact, aged human primary fibroblasts IMR90 which passed 62–67 population doublings could not induce Hsp72 after pretreatment with mild heat shock, and subjecting of such cells to severe heat shock led to strong activation of JNK and massive apoptosis [33]. By contrast, young cells which passed only 12–17 population doublings accumulated Hsp72 after mild heat shock pretreatment. This led to suppression of JNK and greatly reduced apoptosis following a severe heat shock [33]. It is important that the loss of control of JNK in aged cells can be attributed exclusively to the diminished Hsp72 inducibility. Indeed, forced expression of recombinant Hsp72 from an adenovirus-based vector suppressed activation of JNK by heat shock and consequently protected from heat-induced apoptosis [33].

We suggest that the compromised induction of heat shock proteins in aged cells could be a special adaptive cellular response rather than just a non-specific damage of transcription system. In other words, it is possible that induction of Hsps in aged cells is specifically inhibited in order to preserve ability to activate JNK and probably other homologous stress kinases in response to stimuli. It has been shown that the primary signal for induction of Hsps after heat shock and other stresses in accumulation of damaged proteins; under these conditions a transcription factor HSF1 is activated and initiates transcription of *Hsp72* and other *Hsp* genes [45,46]. It is important to note that cells aged in culture or isolated from aged organism have an increased level of abnormal proteins. Indeed, it has been reported that such cells have high levels of oxidatively damaged polypeptides (see [47,48] for review). Furthermore, both normal aged cells and cells affected by age-related diseases (e.g. Alzheimer's, Huntington's, and others) have increased amounts of polyubiquitinated proteins [49], suggesting that although the ubiquitinating machinery is functional, 26S proteasome in aged cells for some reason cannot degrade certain ubiquitinated polypeptides. Therefore, if HSF1 were functional in aged cells, constantly present abnormal polypeptides would ultimately activate it, and heat shock proteins including Hsp72 would be permanently expressed at high levels. Such elevated expression of Hsp72 would lead to suppression of the JNK signalling pathway and apoptosis in response to stresses. Consequently, aged cells would become resistant to many apoptotic inducers. This, in turn, may potentially have disastrous consequences for the whole organism, for instance, increased incidence of oncogenic transformation. Aged cells, therefore, may overcome this problem by repressing HSF1, and thus reducing the ability to induce Hsps. It should be noted that prolonged presence of abnormal proteins in a cell (not necessarily an aged cell) was suggested by Welch and Gambetti to trigger the repression of Hsps inducibility [50].

In summary, the new finding of suppression of stress kinase JNK by Hsp72 not only delineates the protective function of

this heat shock protein but also explains increased sensitivity to stresses in aged cells, and has significant implications in treatment of ischemia, and probably other pathological conditions.

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