

Heat-induced Signal Transduction Pathways Leading to Cell Death and Cell Survival in Cancer Cells

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Abstract : In recent years, cancer therapy research has focused on molecular targets. For efficient hyperthermic cancer therapy, potential targets of interest are molecules which respond to heat and selectively activate signal transduction factors which can inhibit cancer cell proliferation. Signal transduction pathways affected by heat include *p53* mediated pathways, *JNK* (Jun N-terminal kinase) mediated pathways, *Akt* (protein kinase B) mediated pathways, *NBS1* (Nijmegen breakage syndrome 1) mediated pathways, classic *MAP* (mitogen activated protein) *kinase* mediated pathways, and *p38 MAP kinase* mediated pathways. Events such as cell death, cell survival, cell proliferation, and/or cell cycle arrest can be affected by these pathways. To learn more about heat-induced gene and protein expression, cDNA arrays and protein microarrays were used to study cellular responses to heat. This paper briefly reviews interactions of pro- and anti-apoptotic genes and proteins which are induced by heat shock and are components of signal transduction pathways.

Key Words : signal transduction, cell death, cell survival, protein microarray, heat

Introduction

Hyperthermia now plays an important role as a therapy for human malignant tumors, particularly when used in conjunction with recombinant adenovirus-*p53*¹⁾ therapy, radiotherapy²⁻⁵⁾, or chemo-radiotherapy⁶⁻¹⁰⁾. Responses of cells to heat shock or heat stress include cell death, cell survival, cell proliferation, cell cycle arrest, *etc.*, and these responses are induced through specific signal transduction pathways. There are cellular adaptive responses, which help to maintain cellular homeostasis under stress. Apoptosis, a programmed cell death pathway, is a natural process which is essential during development and eliminates redundant or superfluous cells to allow normal patterning and development of tissues and organs. Stressed and damaged cells, if they cannot be repaired, are eliminated through this mechanism.

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Recently, attention has been focused on cancer therapies which could target specific molecules, and theoretically be designed to selectively affect heat-related signal transduction factors. In view of this, it would be very helpful to understand which genes and what classes of genes are induced by heat shock. It has already been reported that *p53* gene status affects heat sensitivity in human cancer cells. In particular, wild-type *p53* (*wtp53*) cells are heat sensitive when compared to mutated *p53* (*mp53*) cells. This was demonstrated by using two cell lines with identical genetic backgrounds except for their *p53* gene status¹¹. In addition, studies have described the expression of apoptosis related genes and proteins after hyperthermia treatments by using cDNA array analysis and protein microarray analysis^{11,12}.

This paper reviews recent work focused on interactions between pro- and anti-apoptotic genes and proteins with components of heat induced signal transduction pathways.

Cell death pathways

p53-mediated pathways leading to apoptosis induction

The p53 protein, a tumor suppressor gene product, plays a crucial role in cellular responses during apoptosis induction¹³. It is evident that p53 is activated, not only by irradiation, but also by heat shock, and this is drawing more attention to hyperthermia. Heat shock leads to the phosphorylation of p53 by ATM (ataxia telangiectasia-mutated protein kinase), and its subsequent activation (Fig. 1). Activated p53 promotes the expression of *Bax* (Bcl-2 associated x protein), a pro-apoptotic gene¹⁴. This sequence of events then triggers the translocation of Bax from the cytoplasm to the mitochondria, and to the release

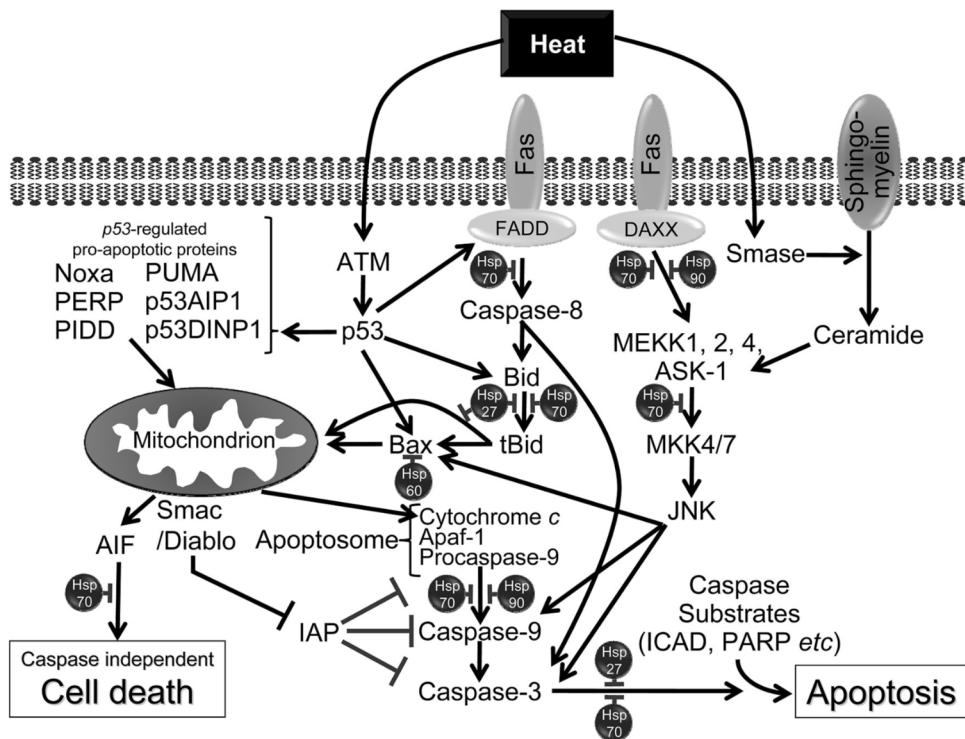


Fig. 1. Heat-induced signal transduction pathways for cell death, and the anti-apoptotic roles of HSPs.

of pro-death molecules such as cytochrome *c* and Smac/Diablo (second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with low pI) into the cytoplasm. Cytochrome *c* binds to Apaf-1 (apoptotic protease activating factor-1) leading to its oligomerization and to the recruitment of procaspase-9 to form a functional apoptosome. At the same time, Smac/Diablo inhibits IAPs (*i.e.* inhibitor of apoptosis proteins). The apoptosome complex proteolytically processes procaspase-9 into an active form, which ultimately leads to cell death by activating the effector Caspase-3^{15–18}. Activated p53 also contributes to the Caspase-8 mediated activation of Bid (or the truncated or tBid). tBid allows Bax to migrate to the mitochondrial membrane and trigger the release of various death factors^{19,20}. p53 also activates Caspase-3 *via* another pathway, the Fas (FS-7-associated surface antigen) mediated pathway. In this pathway, FADD (Fas-receptor) and its adaptor molecule bind together^{18,21,22}. Eventually, a DISC (death inducing signal complex) is formed which activates procaspase-8, which in turn triggers Caspase-3 mediated cell death events. The Fas-induced apoptosis pathway can also recruit the adaptor protein DAXX (death domain-associated protein) instead of FADD, to activate ASK-1 (apoptosis-signal-regulated kinase-1)²³, which activates SAPK/JNK (stress-activated protein kinase/c-Jun N-terminal kinase) and thereby, triggers apoptosis²⁴. In p53-dependent pathways which induce apoptosis, other p53-regulated genes have been identified including *Noxa* (noxious stresses inducible pro-apoptotic gene)²⁵, *PERP* (p53 apoptosis effector related to PMP-22)²⁶, *PIDD* (p53-induced death-domain-containing protein)²⁷, *PUMA* (p53-upregulated modulator of apoptosis)²⁸, *p53AIP1* (p53-regulated apoptosis induced-protein 1)²⁹ and *p53DINP1* (p53-dependent damage-inducible nuclear protein 1)³⁰.

DNA microarray analysis of apoptosis-related gene expression following a heat shock shows that gene expression of anti-apoptotic genes such as *IL* (interleukin)-*12 p35* decreased in *wtp53* cells, and *IL-12Rβ1* increased in *mp53* cells, although the pro-apoptotic genes Caspase-9, CD30 and CD40 were induced independently of p53 gene status by hyperthermia¹¹. The protein microarrays used here indicated that anti-apoptotic proteins such as Bcl-2, Bcl-xL and IL6 were highly induced in *mp53* cells after heat treatment when compared to their induction in *wtp53* cells. However, the pro-apoptotic proteins Bax α and cytochrome *c* were also induced by heat shock in the presence of *mp53*¹².

JNK-mediated pathways for apoptosis induction

In the phosphorylation pathway going from ceramide to MEKKs (MAP (mitogen activated protein) kinase-ERK kinase kinases), and ASK-1/MKKs (MAP kinase kinases), phosphorylated JNK ultimately triggers Caspase-3 dependent or independent apoptosis (Fig. 1). When cells are heat-treated, sphingomyelin undergoes hydrolysis by Smase (sphingomyelinase), and as a result, ceramide levels increase. Ceramide plays a role as a second messenger in the activation of JNK³¹.

Cell survival pathways

Akt-mediated pathways for cell survival

One of the signal cascades which promotes cell survival utilizes a serine/threonine kinase, Akt (protein kinase B), which is activated *via* PI(3)K (phosphoinositide 3 kinase) and PDK1 (3-phosphoinositide-dependent kinase-1) in the presence of various growth factors^{32–35} (Fig. 2). In normal cells, PTEN (phosphatase and tensin homologue deleted on chromosome 10) inhibits the activity

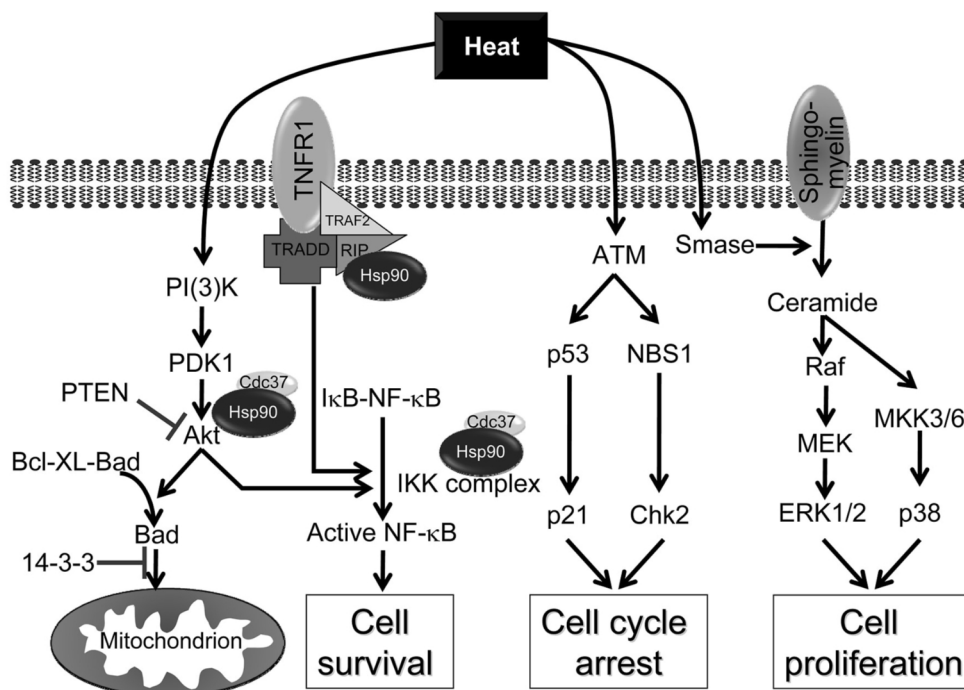


Fig. 2. Heat-induced signal transduction pathways for cell survival and for cell growth inhibition.

of Akt. Many cancer cells have a PTEN deficiency, and show a tendency to have a high level of activation of Akt. Because excessive activation of Akt can lead to tumor formation and abnormal cell proliferation, a cancer cell specific cytotoxic reaction could result from the specific inhibition of Akt. Phosphorylation stabilizes Akt³⁶, which activates NF- κ B (nuclear factor- κ B) and induces the phosphorylation of Bad (Bcl-2-antagonist of cell death protein), which results in the latter's dissociation from Bcl-xL. Phosphorylated Bad is sequestered by the cytosolic 14-3-3 protein, and this prevents its translocation into the mitochondria. Consequently, downstream apoptotic events are not triggered³⁷.

Under normal conditions, NF- κ B remains bound and sequestered in the cytosol by its inhibitor I κ B kinase^{38,39}. However, upon exposure to stimuli, including TNF (tumor necrosis factor), I κ B is degraded, resulting in release of NF- κ B, which can then translocate into the nucleus and activate the transcription of cell survival genes^{40,41}. Although TNF is a crucial inducer of apoptosis, it may also stimulate cell survival through NF- κ B, an important link between various biological processes which include stress response, cell growth or cell death⁴². Phosphorylation and inactivation are mediated by a protein kinase complex, IKK (I κ B kinase). TNF induces the activation of IKK through its association with signal transducing molecules like RIP (receptor interacting protein), and TRAF2 (TNF receptor-associated factor 2)^{43,44}. Activated Akt up-regulates kinase activity of the IKK complex triggering NF- κ B mediated cell survival^{45,46}.

Hsp90 enhances cell survival through its involvement in different steps which occur in the formation of active NF- κ B (Fig. 2). Hsp90 is an important factor in the generation of a stable state for RIP, which is then recruited by activated TNFR-1 (TNF receptor 1) after that binds with its ligand, TNF, and this

leads to sustained NF- κ B activity^{47,48}. Hsp90 also directly interacts with and preserves the activity of Akt by preventing its dephosphorylation^{49,50}. In addition, Hsp90 and its co-chaperone Cdc37 participate in the formation of active IKK and Akt complexes, each of which can phosphorylate I κ B and trigger dissociation of NF- κ B from its inhibitor⁴⁷. The Hsp90-Akt complex can also indirectly promote cell survival through the suppression of JNK-mediated apoptosis. It does this through the phosphorylation and resulting inactivation of ASK-1, which is one of the activators of JNK⁵¹. Hsp90 also prevents the formation of an active apoptosome complex by suppressing the oligomerization of Apaf-1, and plays a role in modulating apoptosis⁵². Hsp27 regulates apoptosis in neutrophils through an interaction with Akt: Hsp27 is phosphorylated by Akt which results in dissociation of Hsp27 and the stabilization of Akt. Disruption of interactions between Akt and Hsp27 depresses Akt activation, which triggers enhanced constitutive apoptosis in neutrophils⁵³.

Protein microarray analysis showed an over-expression of PI(3)K, TRAF-2, NF- κ B and Hsp90 in heat-treated *mp53* cells in this pathway¹². This indicates that the Akt-mediated pathway may work effectively after heat shock, if *p53* does not function normally.

p53 mediated pathways and cell cycle arrest

p53 plays a crucial role in cellular responses towards DNA repair and the regulation of the cell cycle¹³. *p21* is the major gene which responds downstream of *p53* and is the principle mediator of cell cycle arrest in response to DNA damage (Fig. 2). *p21* primarily mediates G₁ cell cycle arrest by inactivating G₁-associated cyclin A- and cyclin E-containing cyclin/cdk complexes. It was reported that *p21* was induced by heat as well as other genotoxic stresses (ionizing radiation and DNA-damaging agents)⁵⁴. The possibility that heat can also induce DNA DSB (double-strand break) formation has been supported by previous studies of γ -H2AX (histone H2AX phosphorylated at serine 139) foci formation^{55–57}.

NBS1-mediated pathways for cell cycle arrest

Homologous recombination and non-homologous end joining both functions as DNA DSB repair systems. If DNA damage occurs, ATM phosphorylates histone H2AX in the neighborhood of the damaged sites resulting in the foci formation of γ H2AX. The MRN complex which consists of MRE11 (meiotic recombination 11), Rad50, and NBS1 (Nijmegen breakage syndrome 1) then binds with γ H2AX, and foci are formed. NBS1 plays an important role in the formation of the MRN complex. In addition, NBS1 is associated with the G₂ check point through the phosphorylation of Chk2 (checkpoint kinase 2). NBS1 is also phosphorylated by ATM following heat shock⁵⁸ (Fig. 2). Although heat treatment causes early the translocation of Nbs1, Mre11 and Rad50 proteins from the nucleus to the cytoplasm as previously reported⁵⁹, both phospho-Nbs1 and Mre11 co-localized with γ H2AX foci, which were already present, in the nucleus at 8 h after heat treatment⁶⁰. It is possible that the biological responses which lead to the formation of DSBs are different after exposure to X-rays or heat. For example, heat induces γ -H2AX and MDC1 foci but not 53BP1 or SMC1 foci⁶¹.

Other anti-apoptotic proteins

Hsp70 acts primarily as an anti-apoptotic factor. It inhibits apoptosis through chaperone dependent as well as chaperone-independent activities⁶². Hsp70 protects cells from cytotoxicity which is induced by TNF, monocytes, oxidative stress, chemotherapeutic agents, ceramide and radiation^{31,63–66}. The

apoptosis cascade initiated by heat stress triggers the translocation of Bax from the cytoplasm to the mitochondria, and this cascade is suppressed by an increased expression of Hsp70^{67,68}). Moreover, downstream, Hsp70 inhibits the formation of a functional apoptosome complex through a direct interaction with Apaf-1^{69,70}). Hsp70 inhibits late Caspase dependent events such as the activation of cPLA2 (cytosolic phospholipase A2) and changes in nuclear morphology; it can rescue cells from apoptosis which is induced by the enforced expression of Caspase-3⁷¹). Hsp70 can suppress JNK mediated apoptosis by inhibiting JNK phosphorylation either directly, and/or through the upstream SEK kinase (also known as MKK4 and JNK kinase), independently of its chaperone activity⁷²⁻⁷⁴). In addition, independently of its chaperone function⁷⁵), Hsp70 can also regulate the activation of Bid. Furthermore, various death-inducing stimuli, TNF- α , Fas and many others are known to cause apoptosis *via* ASK-1 activation. Hsp70 prevents TNF mediated cell death by suppressing ASK-1⁷⁶). Hsp70 binds with AIF (apoptosis inducing factor) released by the mitochondria following death-inducing stimuli, and restricts its translocation into the nucleus, thereby inhibiting apoptosis⁷⁷). Cytosolic Hsp60 in rat cardiac myocytes inhibits the triggering of the apoptotic machinery⁷⁸). Increases in Hsp60 act primarily in anti-apoptotic roles because it binds with pro-apoptotic Bax and Bak proteins. Increased expression levels of Hsp27 during stress responses correlate with improved survival rates after exposure to cytotoxic stresses. Hsp27 negatively regulates the activation of procaspase-9 by blocking cytosolic cytochrome c from Apaf-1, after its release from mitochondria and, suppresses the assembly of apoptosomes^{79,80}). Hsp27 can prevent the release of cytochrome *c* from mitochondria in cells exposed to staurosporine, etoposide or cytochalasin D⁸¹). It also mediates the suppression of procaspase-3⁸²). In addition, Hsp27 maintains the integrity of the actin network and inhibits tBid (*i.e.* translocation of pro-apoptotic factors like activated Bid) from reaching the mitochondrial membrane⁸¹).

Classic MAP kinase pathways

The cascade from ceramide to Ras/Raf/MEK (MAP kinase-ERK kinase) /ERKs (extracellular signal-regulated kinases) is considered to exemplify a classic pathway for MAP kinases. This pathway is influenced by heat shock and transcription factors such as Ets-1 (Ets-like protein 1), c-Jun, and ATF2 (activating transcription factor 2)⁸³). Heat shock produced a rapid activation of ERK1/2 kinases⁸⁴) (Fig. 2).

p38 MAP kinases mediated pathway

The pathway from ceramide to MEKKs/ASK-1/MKKs/p38 is also activated by heat shock⁸⁵) (Fig. 2). This pathway is being studied as a potential molecular target for thermal sensitization because p38 is related to cell proliferation. Protein microarray data indicated up-regulated p38 activity was present in heat-treated *mp53* cells¹²).

Closing comments

As previously indicated, a number of heat-induced genes or/and proteins can serve two distinct and seemingly opposite functions : the promotion of cell survival and of cell death. However, living systems are constructed to ensure an optimum homeostasis, and many complex aspects are present in these systems.

Ideally, it will be possible to use hyperthermia to induce signal transduction activity leading to the

efficient induction of apoptosis and cell death in cancer cells, and to the inhibition of cell proliferation. Any such mechanism-based drug or therapy which can target appropriate signal transduction pathways would also be expected to have minimal side effects, and produce an efficient mode of therapy.

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