The Journal Of The International Federation Of Clinical Chemistry And Laboratory Medicine

eJIFCC

INTERACTION BETWEEN CELL DEATH AND CELL PROLIFERATION IN CANCER

Corresponding author's address:
Prof. Tihana Žanic Grubišic, PhD
Instutute for Medical Biochemistry,
Faculty of Farmacy and Biochemistry,
University of Zagreb,
Domagojeva 2,
10 000 Zagreb
CROATIA

Apoptosis is molecularly regulated and genetically programmed cell death. A range of environmental, physical or chemical stresses can induce it. It is characterised by a sequence of precisely regulated events that culminate in self-destruction of a cell. Apoptosis is a common phenomenon in developmental processes and in normal physiological conditions when unnecessary cells have to be eliminated. Apoptosis is also the predominant form of cell death triggered by cytotoxic drugs in tumour cells. There are many biochemical and genetic parallels between cell death pathways in different animal species.

Methods of cellular survival under the stressful environmental conditions are also genetically programmed and mediated by the activity of physiological defence mechanisms. That is another, even more conserved and evolutionarily ancient cellular response. This response is mediated by the **heat shock proteins (Hsp).**

Hsp is a highly conserved family of proteins that play a major role in cytoprotection. However, apoptosis that is induced to eliminate unwanted, damaged or old cells may be understood as another way of protecting tissues, from the great changes in the environment. Consequently, there are many functional interactions between these two, mechanistically opposing, mechanisms that regulate cellular decision to live or to die. Recent studies have established that the survival-promoting effects of Hsp can be partially attributed to the suppression of apoptosis. Therefore there is a great potential in pharmacological applications of Hsp inhibitors that may help inducing apoptosis when that may be beneficial, as in various tumours.

2.1 Heat shock proteins

The eukaryotic stress response is highly conserved and involves the induction of **Hsp**. Cellular protection against harmful insults relies on transient increase in **Hsp** production. Many vital functions of the cell, such as maintenance of cell cycle and proliferation are under regulatory control of **Hsp**. In mammalian cells, the stress response involves the induction of 5 major classes of **Hsp** families, the small **Hsp** 27, **Hsp** 60, **Hsp**70, **Hsp**90, and **Hsp**104. **Hsp** synthesis is tightly regulated at the transcriptional level by heat shock factors, HSF1 and HSF2. In resting cells, HSF1 is a monomer but active HSF1 exists as a trimer and binds to the heat shock elements, the consensus sequence at DNA.

Hsp function as molecular chaperones in regulating cellular homeostasis and promoting cell survival. The main function of Hsp is helping in folding of nascent proteins, refolding of denatured proteins, inter-organellar transport of proteins and prevention of illegitimate aggregations. Cells failing to respond to stress are sensitive to induced cell death via apoptosis.

FAMILY	CHAPERONS	LOCATION	FUNCTION
sHsp	Hsp27, α- β-crystallin	cytosol	stabilisation against aggregation
Hsp60	chaperonin	mitochondria	prevents aggregation of denatured proteins
Hsp70	Hsp70	cyt/nucleus	folding of nascent proteins, interorganellar transport, refolding of denatured proteins
	Hsc70	cyt/nucleus	
	mHsp70 GRP78	mitochondria ER	
Hsp90	Hsp90α	cytosol	conformational maturation of steroid hormone receptors and signal transducing kinases
	Hsp90β	cytosol	
	Grp94	ER	

Table 1. Main families of mammalian Hsps

2.2 Cellular senescence, apoptosis, and necrosis: chaperon overload as a potential regulator

Cells typically die either by **apoptosis or necrosis**. During necrosis, cell membrane looses its integrity and cell content is released causing an inflammatory response. In apoptosis, however, cell content remains "well-packed" in the **apoptotic bodies** and inflammation does not develop. Nevertheless, these two forms of cell death share some common features. Both processes could be:

- caused by the same pathophysiological conditions,
- prevented by antiapoptotic mechanisms and
- transformed from one form to another.

There is another cellular state that is seen in some cell types - a **nondividing-senescent–state**. These cells exhibit only a limited number of replications in cell culture. Morphological and functional properties of a cell change until it reaches a senescent-

state. These cells are unable to undergo apoptosis and are shifted to necrosis upon DNA damage.

The **Hsp** play an extremely complex role in the regulation of apoptosis. The principal role is maintaining the physiological homeostasis needed for the cell survival. However, by chaperoning the active structure of key apoptotic signalling proteins**Hsp may directly promote apoptosis** and act as chaperones of the death.

On the other hand, the**protein folding capacities of Hsp may be exhausted** due to massive stress, during ageing, or in chronic diseases. In these conditions protein misfolding and aggregation are prevailing. Various levels of **chaperone overload** may have an important contribution to the signals directing the cell to senescence, necrosis or apoptosis.

2.3 Major elements in the mechanism of apoptosis

Apoptosis is an **energy-dependent, ubiquitous and genetically controlled physiological process.**

• It is **morphologically** well characterised with nuclear condensation, cell shrinkage, and membrane blebbing.

• The **physiological changes** involve fragmentation of nuclear DNA into 80-200 oligo-nucleosomal fragments. The DNA fragments are produced by the specific caspase-activated endonucleases.
This highly regulated process develops as the response to some initial stimulus followed by a specific cascade of events. Apoptosis proceeds in three phases:

• **The initiation - signalling phase**, which involves the activation of surface death receptors, or the mitochondrial pathway;

• **The signal transduction - preparation phase** where activation of initiator caspases and certain kinases/phosphatases takes place and

• **The execution – death phase** involving activation of effector caspases (Table 2).

	Mediators
SIGNALLING PHASE	
Death receptors	TNF-α, Fas (Apo1/CD95), FADD
Physiological inducers	ROS, Ca2+, JNK/SAPK activation
Protease activators	granzymes, calpains, cathepsins, proteasome
PREPARATION PHASE	
Initiator caspases	caspase-8, -9, -10, -12
Physiological inducers	Bax, ROS, MMPT, cytochrome c, apoptosome
Nucleases	AIF, endonuclease G, Parp
EXECUTION PHASE	
Effector caspases	caspase-3, -6, and -7
Physiological factors	membrane blebbing, apoptotic body formation, DNA fragmentation

Table 2. The most important members of the apoptotic machinery

2.4 Sites of initial signalling events

2.4.1 Plasma membrane

Activation of the **TNF** and the **Fas** receptor, the so-called death receptors on the plasma membrane activates factors that promote cell death. The superfamily of TNF receptors is implicated in the inflammatory and immune response. **Death receptors** contain an intra-cytoplasmic domain called death domain. Through this domain receptors interact with the cytosolic proteins and propagate the death signal by activating caspases. They are the final executioners in a stereotyped cascade leading to cell death.

In the late execution phase, apoptosis is characterised by marked changes in cell morphology, including **membrane blebbing**, loss of the membrane phospholipid asymmetry and **exposure of phosphatidylserine** on the surface. The phosphatidylserine can be recognised by the immune system. **Hsp** can help translocate phosphatidylserine to the cell surface making cells more vulnerable to immune lysis.

The role of **Hsp70** is pleiotropic to cellular life and in some cases over-expression of **Hsp70** is protecting from apoptosis, but in other cases it may promote the cell death. **Hsp90** is helping in propagation of the apoptotic signal from plasma membrane.

2.4.2 Cytosol

In the cytosol **stress kinases** are important elements of signal transduction pathway in inducing and/or modulating the apoptotic response. Among the **mitogen-activated protein kinases**, the activation of the signal-regulated protein kinase **ERK** is associated with mitogenic stimulus, whereas the **JNK** and **p38** kinases are stress responsive.

The small **Hsp27** is activated by **p38**-activated phosphorylation. The phosphorylated dimers of **Hsp27** interact with **Daxx**, a protein that contains the death domain. Association with **Hsp27** prevents Daxx from interaction with another serine/threonine kinase. That is the way of inhibiting the Fas–mediated apoptosis.

Hsp70 has a general inhibitory role in stress kinase pathways. **Hsp72** also interacts with peptide binding domain of **JNK** and is necessary for **JNK** down-regulation.

2.4.3 Nucleus

The biochemical signature of apoptosis is **DNA damage** and **nucleosomal fragmentation of DNA** that is resulting from activation of specific **endonucleases**. These enzymes cleave the chromatin to shorter, oligo-nucleosomal DNA fragments.

Hsp play a major role in protecting the cells from DNA damage induced by various agents. Members of the **Hsp**27 and **Hsp**70 families have a protective role for the DNA integrity against oxidative stress. Nuclear **Hsp**72 suppresses the appearance of apoptosis after DNA damage.

The specific form of DNA damage occurs with **telomere** shortening. At the critical length of the telomere regions, around 7kb, cells go to the state of senescence, which may further proceed to apoptosis. Telomere regions are maintained by enzyme telomerase and **Hsp90** is necessary for the enzyme activity.

2.4.4 Mitochondria and reactive oxygen species

The mitochondrion appears to be the central coordinator of apoptotic events. Many proapoptotic and signal transduction pathways converge on the mitochondria to induce the membrane permeabilisation. Rupture of the outer membrane and formation of the **mega-channel, permeability transition pore** (PTP) is the starting event. The **adenine nucleotide translocator** present in the inner mitochondrial membrane and the voltage-dependent anion channel at the outer membrane are the major components of PTP. These proteins are responsible for the lethal changes in mitochondrial

membrane potential and release of certain molecules from intermembrane space to cytosol. The reaction is controlled by **Bcl-2** and Bcl-2 related proteins. The PTP formation is connected with the **Bax** protein and physical disruption of the outer membrane. Changes in membrane permeability lead to matrix swelling and finally to leakage of **cytochrome c**, and this is a starting signal for the execution phase of apoptosis.
The second mitochondrial protein involved in apoptosis is **activator of caspases** (**Smac**/**DIABOLO**). This protein inhibits inhibitor of apoptosis (**IAP**), that blocks processing of effector caspases –3 and –9. The release of cytochrome c from mitochondria drives the assembly of the high molecular weight caspase-activating complex – **apoptosome**. The apoptosome contains oligomerised **Apaf-1**, which in the presence of **dATP** and **caspase-9** helps auto-activating cleavage of caspase –3, an executioner of apoptosis.

Hsp27 may decrease caspase activity by binding to cytochrome c and down-regulate mitochondria pathway of caspase dependent cell death. **Hsp70** and **Hsp90** suppress apoptosis by directly associating with Apaf-1 and blocking formation of apoptosome.

There is another role of mitochondria in the development of apoptosis. Mitochondria are primary sites of **reactive oxygen species (ROS) formation.** ROS have a major role in mediation of cellular damage. ROS can be generated in the electron transport chain, xanthine and other flavoprotein oxidases, auto-oxidation of catecholamines, thiols, intracellular xenobiotics, haemoglobin and NADP(H) oxidase. In normal cells there is a balance between pro- and anti-oxidant pathways. Upon stress stimuli an imbalance in redox system develops that leads to accumulation of ROS. ROS may induce damage to cell by **oxidizing the membrane lipids, proteins and DNA.** The overproduction of ROS is associated with many forms of apoptosis and necrosis. ROS-induced apoptosis is associated with up-regulation of **Fas death receptor**. Anti-apoptotic protein Bcl-2 prevents generation of ROS.

Small **Hsp**27 and **Hsp** 70 appear to be protective agents against oxidative stimuli, by **elevating reduced gluthatione** level, or stimulating glucose-6-phosphate dehydrogenase activity, or inhibiting lipid peroxidation.

Nitric oxide (NO) is an important signalling molecule regulating a number of diverse physiological processes and is produced by **nitric oxide synthases (NOS).** There are three types of NOS in the cell: **neuronal, endothelial and inducible**. NO inhibits apoptosis, through up-regulation of survival kinases, and inhibition of caspase-3.

2.4.5 Endoplasmic reticulum

The ER plays important function in intracellular calcium homeostasis. Conditions leading to alteration of ER intraluminal oxidative status can also induce stress. Participation of ER in induction and progression of apoptosis involve the disturbed Ca++ signalling and accumulation of unfolded proteins. **Glucose regulated proteins (Grp)** belong to the **Hsp**70 family and could be induced by ER stress. After translocation across ER membrane they act as apoptotic regulators by protecting the host cell against stress-induced death.

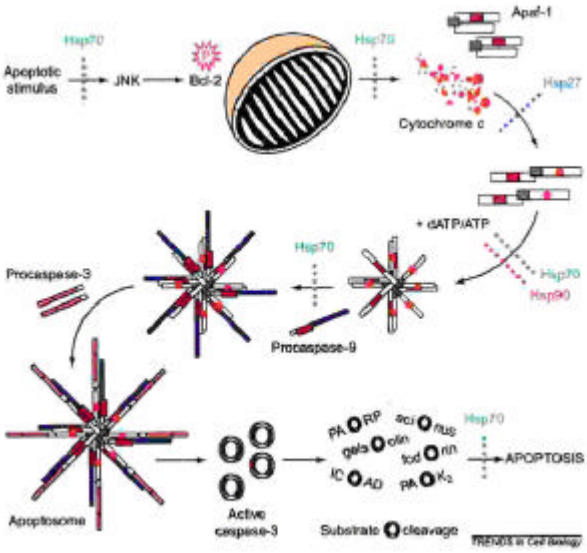

Figure 1. Figure represents the summary of the proposed mechanisms of the Hsp-mediated regulation of the apoptotic pathway. **Hsp** 70 and **Hsp**27 may block the apoptotic signalling at different stages.

2.5 Effector molecules

2.5.1 Caspases

Caspases represent the family of **proteases** that hydrolyse proteins at **aspartate residues**. There are 14 types of caspases that are classified into 3 major groups, which are:

- **initiator**,
inflammatory, and
- **effector caspases**

The activation of caspases is organised through an apoptotic cascade pathway. The **TNF-induced apoptosis** involves activation of initiator **caspases –8 and –10**. The **mitochondrial pathway** involves **initiator caspase –9** and **effector caspases –3, -6.**

Hsp27 inhibits mitochondrion – dependent caspase activation. The small **Hsp** α- and β- crystallines, inhibit both mitochondrial and death receptor pathways. **Hsp**-70 binds to caspase-3 and inhibits its activity.

2.5.2 Nucleases

There are various endonucleases expressed in the cell. The deoxyribonuclease (DNase) implicated in apoptosis is an Mg++ endonuclease called **caspase–activated DNase (CAD). Hsc**70, with its cofactor **Hsp**40 is involved in folding of CAD.

2.5.3 Transglutaminases

Tissue transglutaminase (TGase) is a member of a family of enzymes that catalyse protein cross-linking by **transdamidation.** Transamidation has an important role in packing the cells in the tissue. At the late phase of apoptosis this protein cross-linking is important for preventing the massive inflammatory processes. TGase binds and hydrolyses ATP and GTP. The enzyme is inhibited by NO and is activated by increased intracellular Ca++ concentration. TGase expression is inversely correlated with the expression of

antiapoptotic protein Bcl-2, and inhibition of TGases confers protection against apoptosis.

2.6 Heat shock proteins and caspase independent apoptosis

It has been shown that the signalling pathways are interrelated and that **caspase-independent pathways** may interconverge with caspase-dependent pathways in induction of apoptosis. The therapeutic use of **Hsp** modulation in anti-cancer protocols points to the importance of caspase-independent apoptotic pathways, which are predominant pathways of apoptosis in tumour cells. A number of enzymes and lipid molecules participate in the development of caspase-independent apoptosis.

Serine proteases are participating in amplification of apoptosis. **Granzyme**, which is a serine protease, is an activator of caspases, and activator of cytochrome c release. The surface–expressed **Hsp**70 mediates the apoptosis of tumour cells by binding of granzyme B. **Cathepsins** are a class of proteolytic enzymes involving 3 major groups: **cysteine proteases**, **aspartyl proteases and serine proteases** (B, C, L, H, K, S, and O). The enzymes are of lysosomal origin and are involved in peptide formation and protein degradation. They are involved in autophagy-associated apoptosis and in oxidative stress-induced apoptosis. In tumour cells cathepsin-B is the most important mediator of cell death.

The **Hsp**70/**Hsp**90 chaperone plays an important role in lysosomal proteolytic pathways. Hsc70 is involved in uptake of cytosolic proteins into the lysosomal lumen.

2.6.1 Calpains

Calpains are calcium-dependent proteases involved in cytoskeletal reorganisation and muscle protein degradation. The enzymes are heterodimers composed of small regulatory and large catalytic subunit. Calpains and caspases often act in a synergistic way in promotion of apoptosis.

2.6.2 Ceramide induced apoptosis

Ceramide is a lipid mediator in induction of apoptosis. It activates stress activated protein kinase signalling pathway. **Hsp**70 protects cells from ceramide-induced apoptosis

2.6.3 Apoptosis inducing factor

Apoptosis inducing factor (AIF) is a mediator of caspase-independent apoptosis. AIF translocates from mitochondria to both cytosol and nucleus. Bcl-2 and **Hsp**P70 can inhibit AIF translocation.

2.6.4 Anoikis

Anoikis is a type of cell death where cells fail to find substratum and connection with other cells or extracellular matrix. The lack of **integrin**-mediated interactions with extracellular matrix induces apoptosis. It mainly occurs at epithelial cells and it assures proper opmental positioning in specialized structures. Failure of anoikis contributes substantially to tumour progression and facilitates metastasis. It is possible that cytoskeletal alterations and cell-matrix detachment could release death receptors leading to death domain induced apoptosis.

The phosphorylated form of **Hsp**27 helps the stability of integrin. It was shown that **Hsp**27 inhibit metastatic potential in melanoma cells.

2.6.5 Heat shock proteins and antiapoptotic mediators

Hsp are involved in negative regulation of pro-apoptotic pathways, but also in activation of anti-apoptotic mediators. **Hsp** 70 acts in helping Bcl-2 activation.

Apoptosis itself inhibits Hsp synthesis by down-regulating the respective transcription factor HSF-1. In that manner apoptosis stops one of the important surviving signals.

2.6.6 Molecular mechanisms of Hsp action

Hsp act as molecular chaperones preventing protein aggregation and promoting protein folding. **Hsp** function as oligomers and often form chaperone complexes with each other. The biological role of Hsp is mediated by their ability to interact with protein or polypeptide substrates. The peptide binding activity of Hsp70 is mediated through interactions between its C-terminal peptide binding domain and hydrophobic residues exposed in unfolded substrates. Association of Hsp70 with its target peptides is further regulated by the activity of its N-terminal ATPase domain (Figure 2.)

Hsp may function in "**passive mode**" when they behave as ATP-independent "**holders**" of damaged proteins, sequestering them and preventing their fatal aggregation. In ATP dependent "**active mode**" chaperones are working as "**folders**" helping in the folding, transport and ATP-dependent degradation of unfolded or misfolded proteins. The passive mode is typical during stress when cellular ATP level is low. The active mode prevails when cells have recovered and the ATP level is increased again. Many proteins, such as protein kinases and nuclear hormone receptors, require the continuous help of **Hsp** chaperone complex to keep them in activation competent state. However, **Hsp** have no priority or selection between substrates and hence the chaperone function is extended to pro-apoptotic factors, too. **Hsp** 60 promotes apoptosis by helping in the maturation of procaspase-3.

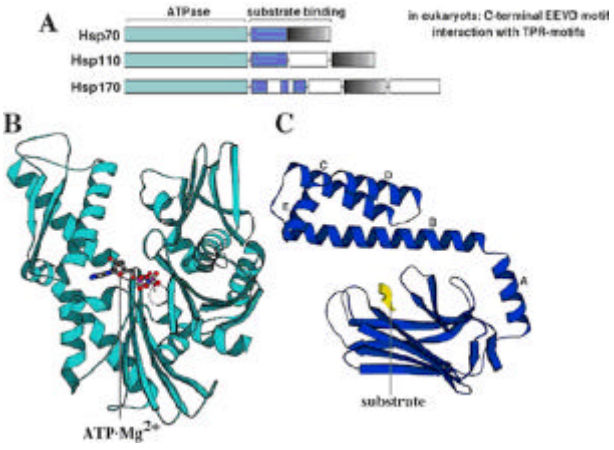

Figure 2. Molecular organisation and structure of Hsp70

2.6.7 Heat shock proteins and cellular homeostasis

Hsp have essentially a dual function in the cell. They are **eliminating misfolded** and damaged proteins produced by stress and other

insults. However, they also play a critical role in the maintenance of cellular homeostasis by **continuously chaperoning** of a number of cellular proteins.

• Redox homeostasis

Hsp act as antioxidants, and **haem oxygenase** is one of the **Hsp** members that are responsible for the production of antioxidants biliverdin and bilirubin. The redox state of the cell influences **Hsp** synthesis and a decreased gluthatione level may lead to direct activation of HSF-1. On the contrary, strong oxidative agents block activation of HSF-1 and its binding to DNA. It has been shown that mild changes of redox homeostasis lead to activation of HSF-1. However, large changes cause HSF-1 inhibition.

• Cell organisation

Hsp help in stabilising the cytoskeleton and cytoarchitecture by direct interactions with cytoskeletal proteins. Inhibition of major cytoplasmic **Hsp**, **Hsp90** leads to increased cellular lysis and disruption of cytoplasmic organisation. Small **Hsp** protect actin filaments and help cell survival in apoptosis.

2.7 Heat shock proteins as pharmacological targets in apoptosis modulation

2.7.1 Heat shock protein inhibition – an efficient way to induce apoptosis of tumour cells

Apoptosis in tumour cells
From the various mouse models and cultured cells it becomes evident that acquired resistance to apoptosis is hallmark of most, if not all, types of cancers. Although tumour cells are resistant to apoptosis, they are not completely devoid of death. Cell death in tumour cells is mostly associated with cellular senescence and mostly involves **caspase-independent routes** of apoptosis or necrosis. A tumour cell may escape from caspase-mediated apoptosis either by **over expressing antiapoptotic** proteins or by severe **mutations** in **proapoptotic factors**. The antiapoptotic Bcl-2 is known to be over-expressed in many tumours. In Hodgkin's lymphoma, mutations of Fas receptor were found and caspase-8 is frequently mutated in neuroblastoma. Tumour cells also have ways to escape caspase-dependent apoptosis, by expressing **survivin**, an inhibitor of apoptosis. The survivin expression is associated with poor prognosis. Mutations in the tumour-suppressor p53 gene are one of the major mechanisms of the tumour escape from apoptosis. **Hsp** regulates the function of p53.

2.7.2 Heat shock proteins in tumour cells

It was found that members of the **Hsp** family, such as **Hsp**70, **Hsp**27, and **Hsp**90 are over- expressed in several tumour cells. It has been shown that **Hsp**90 can inhibit apoptosis by direct physical interaction with apoptotic molecules. There are numerous examples of **Hsp** involvement in tumourigenicity; **Hsp**27 is over expressed in colon carcinoma cells, **Hsp**90 in prostate cancer and **Hsp**70 in breast tumours where it is found to be necessary for the progression.

Hsp bind to caspases inhibiting their activation, but they are also efficient in blocking caspase independent apoptosis. These characteristics make inhibition of **Hsp** an efficient tool in inducing a cell-specific apoptosis. Depletion of **Hsp**70 and **Hsp**90 in tumour cells induces their apoptosis.

Aging and various degenerative diseases induce accumulation of damaged/misfolded proteins that are produced by the oxidative stress and proteotoxic insults. At the same time the essential chaperone functions of Hsp are also impaired. Increased demands of chaperone function may exceed the available chaperone capacity leading to imbalance of cellular homeostasis.

On the other hand tumours undergo facilitated evolution due to increased proliferation. Conventional antitumour therapies (chemotherapy, radiotherapy, hyperthermia) all induce **Hsp** in surviving cells. The over-expression of **Hsp** may help the accumulation of mutations in tumours, which can help their further progression to more aggressive types of malignant cells. Use of **Hsp** inhibitors may affect this process and release some of mutations that have been rescued by **Hsp** before.

2.7.3 Enforced apoptosis of tumour cells

Inhibitors of **Hsp** can suspend the **Hsp**-dependent block of both caspase-mediated and caspase-independent apoptosis of tumour cells. It is well known that **Hsp** are not selective in their chaperoning function. They assist in the folding of a variety of cellular proteins. Consequently, **Hsp** inhibitors will target a number of different molecules. That makes inhibition of **Hsp** potentially very effective in induction of tumour cells apoptosis.

Although there are efficient inhibitors of **Hsp**60 and **Hsp**70, targeting of **Hsp**90 represents a central attraction in **Hsp**-related tumour inhibition. Inhibition of **Hsp**90 induces apoptosis in various tumour cells and also leads to a defect in number of proliferative signals. The most important **Hsp**90 inhibitor is *geldanamycin* and its derivatives. **Hsp**90 inhibition leads to dissociation of various **Hsp**90 client proteins from chaperone complex and to their consecutive **degradation by the proteasome**. Some drugs interact with **Hsp**90, like cisplatin, taxol and the antibactericide, novobiocin, and influence its function.

It appears that applying low doses of **Hsp**90 inhibitors together with conventional chemotherapeutic represents an effective way to target various cancers. Cytoprotective effects of **Hsp** come from the inhibition of stress-induced apoptosis. Rescue from apoptosis may also be helpful in anticancer protocols, where by-stander non-malignant cells are also damaged by the therapy.

At some point **Hsp** inhibitors may act as **Hsp** inducers. **Hsp** synthesis is regulated at transcriptional level by HSF-1. **Hsp**90 and **Hsp**70 have been shown to bind to HSF-1 and keep it repressed in the absence of stress. During stress, misfolded proteins occupy both chaperones, which results in dissociation, nuclear translocation and activation of HSF-1. Pharmacological **Hsp** inhibition may therefore paradoxically lead to an increase in their amount.

Increased **Hsp** may lead to tumour cell sensitisation against immune attacks, providing a simultaneous protection of bystander cells in various cancer therapies, such as chemotherapy, radiotherapy, hyperthermia etc. Tumour cells may express **Hsp** on their surface, which leads to their enhanced recognition by the natural killer cells of the native immune system, and a specific anti-tumour immunity may develop. Extracellular **Hsp**, released as a result of cell death and taken up by antigen-presenting cells

through **Hsp** receptors, are involved in the cross presentation of chaperoned peptides.

Proteasome inhibitors up-regulate **Hsp** synthesis by increasing amounts of misfolded proteins that compete for **Hsp** with HSF-1. The level of various **Hsp**, as well as, the amount of **Hsp**, which are not occupied by damaged, misfolded proteins, can be critical in cytoprotection and cell survival.

2.7.4 Therapeutic use of heat shock protein up regulation

A number of clinical applications can be derived from the general cytoprotective / antiapoptotic role of **Hsp**. It could be applied in cardioprotection, in cellular defence against stroke and in various neurodegenerative diseases, as well as, for the improvement of efficacy in tissue transplantation. **Hsp** induction eases the deleterious consequences of chronic diseases, such as diabetes, and conditions like Alzheimer's, Parkinson's or prion disease, where the accumulation of misfolded proteins is the major cause of neurodegeneration. These conditions may gain beneficial effects from the Hsp over expression.

Cell life and proliferation, as well as, cell death involves regulation through the dynamic conformational changes of a number of apoptotic molecules, involving various oligomerisation and autoactivation steps. These suggest an extensive need for molecular chaperones. **Hsp** are capable of assisting in all these processes. Their proapoptotic role is usually balanced, and very often overwhelm by their participation in cytoprotection. Therefore, finely tuned balance of **Hsp** function is a key point for regulation of cell death, or survival, and also for making switch between two forms of cell death, apoptosis and necrosis.

References

1. P. Workman.: Combinatorial attack on multistep oncogeniesis by inhibiting the Hsp90 molecular chaperone. Cancer Letters, 206 (2004) 149-157.

2. C. Jolly, R.I. Morimoto, Role of heat shock response and molecular chaperones in oncogenesis and cell death. J.Natl. Cancer.Inst. 92 (2000) 1564-1572.

3. Maloney and P. Workman: Hsp90 as a new therapeutic target for cancer therapy: the story unfolds. Exp. Opinion Biol. Ther. 2 (2002) 3-24.

4. A.S. Sreedhar, P. Csermely: Heat shock proteins in the regulation of apoptosis: new strategies in tumour therapy, A comrehensive review. Pharmacology& Therapeutics 101 (2004) 227-257.

5. H.M. Beere and D.R. Green: stress management – heat shock protein-70 and the regulation of apoptosis. Trends in Cell Biology, 11 (2001) 6-10.

6. Garrido, S. C. Gurbuxani, L. Ravagnan and G. Kroemer: Heat shock proteins: Endogenous Modulators of apoptotic cell death. Biochem. biophys res. Commun. 286 (2001) 433-442.