Mitochondrial chaperones in cancer – From molecular biology to clinical diagnostics

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Review

Mitochondrial Chaperones in Cancer

From Molecular Biology to Clinical Diagnostics

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Hsp60, Hsp10, mtHsp70, mortalin, prohibitin, cancer, mitochodnria

ABSTRACT

Mitochondria are cell organelles involved in processes of cell life and death, and therefore also in tumoral transformation. Indeed, mitochondria dysfunction is a prominent feature of cancer cells. Mitochondrial proteins and DNA have also been previously studied as markers of tumorigenesis.

Heat shock proteins (HSPs) are ubiquitous evolutionary conserved proteins. HSPs enhance their expression in stressed cells and they are involved in gene expression regulation, DNA replication, signal transduction, differentiation, apoptosis, cellular senescence or immortalization.

This review reflects recent views on the role of some mitochondrial molecular chaperones as prohibitin, mortalin and HSP60/HSP10 complex and their modifications leading to cell transformation and cancer development. These molecules could represent modern molecular biomarkers for oncological management.

INTRODUCTION

A Neoplasm is a cell mass consisting of abnormal cells that have evolved in a process of cell transformation; it may also be defined as a heritably altered, relatively autonomous growth of tissue with abnormal regulation of gene expression. Factors of transformation are genetic and epigenetic changes leading to deregulation of cell proliferation, differentiation and adhesion.[1] Although human cells spontaneously immortalize in vitro with very limited probability, transformation with SV40 (large T antigen), papillomavirouses (E6 and E7 genes) or adenoviruses (E1A and E1B genes) are common triggers of immortalization. Until now, many studies on immortalization have been focused on telomere shortening, p53, pRb/p16^{INK-4} pathways, so it would seem that immortalization is achieved by p53 or pRb function abrogation on one hand or activation of telomerase on the other.[2,3] One of the cell organelle involved in the process of cell life and death, and therefore also in cell transformation and clonal evolution is the mitochondrium.[4-6]

Mitochondria (from Greek *mitos* thread + *khondrion* granule) are semiautonomous rod-shaped, round organelles of Eucaryotic cells carrying their own genome and protein translation machinery. Mitochondria, like nuclei, are bounded by a double membrane. This is one of the relicts of endosymbiosis of Purple NonSulfur Photosynthetic Bacteria (*Rhodospirillaceae*) within pro-Eucaryotic cells. Human mitochondrial DNA contains 16,569 base pairs organized in a closed circle[7] and encodes for 13 polypeptides, 22tRNAs and 2 rRNAs.[5,6,8] Mitochondria are also called "cellular power plants," as the primary function of these organelles is to produce ATP in the process of oxidative phosphorylation (OXPHOS). At the same time, many housekeeping metabolic reactions take place in this cell compartment, including production of intermediates of carbohydrates, nucleotides, fatty acids and aminoacids metabolism. Mitochondria are irrelevant proto folio because of their key role in homeostasis, which is probably the reason of their maintenance within cells lacking mtDNA (ρ^o).[6,8,9] Climatic adaptation, ageing, longevity, degenerative disease and cancer are only few of the processes which mitochondria are assigned to.[8,10]

Today we know that mitochondria dysfunction is one of the most prominent features of cancer cells. Mitochondria failure, damage or dysfunction has been reported on all levels of their biogenesis, structure and physiology (Fig. 1). The situation is further complicated by the mitochondria-nucleus interaction, and homo- and heteroplasmy of cells.[5,11] Some of the processes that mitochondria are involved in or that take place within mitochondria have been widely studied for many years; these include OXPHOS and apoptosis.[12-14] Other processes, such as protein import, and complex assembly, have not yet been adequately described and further experiments need to be performed to put all

the fragmentary data into one functional model.[15,16] Mitochondria, mtDNA and mitochondrial proteins have been already recognized as important in the process of establishment of new markers of tumorigenesis in the era of molecular medicine also by clinicians, and therapies have been individually adjusted for specific patients. In many medical reports, a strong correlation between mitochondria function and cancer development has already been pointed out. A vast amount of clinical data provides evidence of mtDNA as cancer markers.[17-19] For some types of cancer, a specific pattern of mtDNA mutations has been assigned.[17,20] This review represents the current knowledge on mitochondrial molecular chaperones such as prohibitin, mortalin and Hsp60/Hsp10 and their role in mitochondria function, focusing on the alterations in expression and function modifications that may lead to cell transformation and cancer development.

HEAT SHOCK PROTEINS

Heat shock proteins (HSPs) are ubiquitous and evolutionary conserved proteins, that were first discovered as heat shock triggers over 40 years ago.[21] Their fundamental role in cellular homeostasis and cell viability was recognized in 1962 when F. Ritossa exposed *Drosophila* to 37°C for 30 min. and proteins of 70 and 26 kDa were highly expressed, suggesting they are indispensable to overcome heat stress, which was later confirmed.[21] HSPs are functionally related proteins classified into families according to molecular weight. In most organisms stress proteins are represented by families of HSP100, HSP90, HSP70, HSP60 and small HSPs, with several members in each class. Proteins are affiliated to these diverse groups of molecular chaperones by their capacity to recognize and bind substrate proteins that are in an unstable or inactive state.[22]

Initially, chaperones were associated only with protein folding,[22] but they are now recognized as key players in a wide spectrum of processes. HSPs act as molecular chaperones assisting in protein transport, oligomeric proteins and protein complexes assembly, refolding of misfolded proteins[20] and triggers of degradation by proteosome.[23] Chaperone proteins adhere to hydrophobic sites on newly synthesized or unfolded proteins, thus preventing the formation of functionless aggregates by random adhesion to hydrophobic sites on other proteins. Some of the HSPs also function as unfoldases of aggregated polypeptides, which means that they reactivate heat-inactivated proteins that can subsequently assume their proper configuration after disengagement, as they are given a second chance for folding. Chaperonin-mediated protein folding is explained by two models—the "Anfinsen cage" and the "iterative annealing". The first model proposes that chaperonins provide a passive box, where folding proceeds because no intermolecular interactions disrupt the process and off-pathway aggregation reactions are prevented. The second assumes that ATP - dependent cycles of forced unfolding of energetically trapped intermediates accelerates native conformation acquisition.[22]

HSPs are present in all cell compartments—cytosol, mitochondria, ER and nucleus, and typically they have a long half-life. Different chaperone proteins cooperate in building a network to assist polypeptides in the maintenance of native conformation. HSPs seem to accumulate in a dosage-dependent manner to amounts that are sufficient to protect cells against imbalance in the protein folding status of the cellular proteome.[24,25] Although HSPs are highly expressed in stressed cells and could be considered only as cell stress "buffers", they are also involved in gene expression regulation, DNA replication, signal transduction,[26] differentiation, apoptosis, cellular

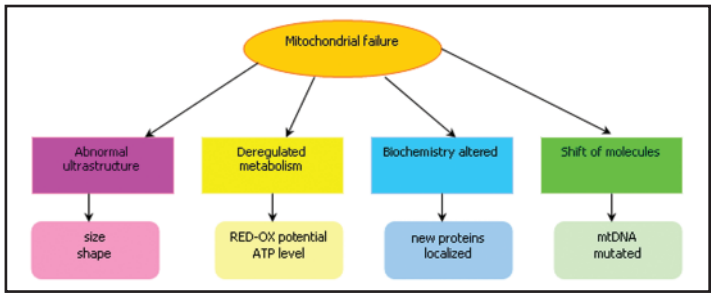

Figure 1. Levels of mitochondria dysfunctions found in cancer cells.

senescence or immortalization.[27] In the last few years numerous experiments have shown chaperones involvement in cell transformation, metastasis formation and multidrug-resistence development.[26-28] At the same time, they have been shown to be involved in immune response stimulation, leading to the development of the symptoms of infectious and autoimmune diseases.[29]

HSP60: WHEN THE DEVIL SITS BEHIND THE CROSS

Human heat shock protein 60—Hsp60 (CPN60, GROEL, HSP60, HSP65, HuCHA60 or SPG13) is encoded by a nuclear gene HSPD1 (GeneID: 3329) localized on chromosome 2q33.1. It is also designated as P60 lymphocyte protein, chaperonin, heat shock 60 kD protein 1, heat shock protein 65, mitochondrial matrix protein P1 or short heat shock protein 60 Hsp60s1. Two pseudogenes, located on chromosome 8, have been associated with this gene.[30] In normal cells, Hsp60 is mostly localized in the mitochondrial matrix, in the outer mitochondrial membrane and, less frequently, in extra-mitochondrial sites.[31] Hsp60 is constitutively expressed under normal conditions, and induced by heat shock, mitochondria damage and mtDNA depletion. Its transcription is regulated by a HSE (heat shock element) acting on a bidirectional promoter.[32] Chaperonin primary function is the assistance in mitochondrial protein folding, unfolding and degradation.[22,33,34] The involvement of Hsp60 in the process of apoptosis and tumorigenesis is now under investigation.

First report suggesting that Hsp60 was involved in apoptosis was published by Samali et. al. These authors showed that activation of caspase-3 by staurosporine occurs simultaneously with Hsp60 and Hsp10 release from mitochondria in Hela and Jurkat T cells. Furthermore, in vitro and in vivo, Hsp60 and Hsp10 have been shown to associate with pro-caspase3 and possibly accelerate its activation.[35] On the other hand, Kirchhoff et al have argued an anti-apoptotic role of cytosolic Hsp60 in cardiac myocytes.[36] The authors demonstrated that Hsp60 interacts with pro-apoptotic Bax and Bak proteins thus preventing apoptosis onset. In their experiments, reduced Hsp60 expression led to an increase in the number of Bax protein associated with a mitochondrial membrane fraction. Since Bax alone is sufficient to induce cytochrome *c* release and subsequently induce apoptosis, Hsp60-Bax interaction may be considered as critical in preventing apoptosis in normal cells.[36]

The exact molecular role of Hsp60 is still far from being completely understood. Interactors reported to date include basic metabolism proteins that are probably substrates of Hsp60 such as a chaperone like aldehyde dehydrogenase 2, carbonic anhydrase II, dihydrofolate reductase, and its partner in mitochondrial protein folding mtHsp70 and heat shock 10kDa protein 1, but also proteins

involved in apoptosis and cell cycle regulation, like BCL2 antagonist killer 1, caspase 3 preproprotein, caspase 6 isoform alpha preproprotein, caspase 9 isoform alpha preproprotein, p21ras, H2B histone family, member Q, Alpha 3 beta 1 integrin and PKA C.[22,36-39] Indeed, the actions of HSP60 could lead to the modification of those proteins and, due to their role in cell cycle regulation, influence the carcinogenesis, and the mitochondrial regeneration during normal cell proliferation.

In recent studies, we have focused on the presence and the expression of Hsp60 in carcinogenic models: the adenoma-carcinoma sequence of colorectum and the dysplasia-carcinoma sequence of uterine exocervix and prostate. Using immunohistochemical and western blotting analyses, we found that Hsp60 expression gradually increases from normal through dysplastic to neoplastic tissues, arguing that its overexpression during carcinogenesis may be functionally correlated to tumoral growth. Our attention is now focused on the overexpression of Hsp60 which can be used as a molecular marker of clinical stage and patient prognosis in a variety of tumors and pretumoral lesions, i.e., adrenal Cushing tumors,[40] human breast ductal carcinoma,[41] large bowel,[42,43] bronchial,[44] exocervical[45] and prostate[46] carcinogenesis. Hsp60 expression has also been suggested as a good prognostic marker in human oesophageal squamous cell carcinoma (ESCC).[47] In this study the Authors have hypothesised that Hsp60 expression correlates with the apoptotic index and patient outcome in ESCC.

The literature reported thus far has led us to consider Hsp60 as a carcinogenesis marker, with a potential clinical significance. Although the exact molecular details of Hsp60 actions have not been defined to date, it is reasonable to think that its expression may serve as a prognostic tool in clinics and histopathologic diagnostics. In summary, these studies demonstrate that hsp60 might significantly contribute to tumorigenesis, making it a good candidate target for cancer therapy.

HSP10: WHEN SMALL IS NOT ALWAYS BEAUTIFUL

Heat shock 10 kDa protein 1 (Hsp10, CPN10, GROES) is encoded by a nuclear gene HSPE1 (GeneID: 3336) localized on chromosome 2q33.1. It is also designated as chaperonin 10, and it is translated in the cytoplasm and transported into mitochondria. Although in normal cells Hsp10 is localized mostly in the mitochondrial matrix, it has also been found in other sub-cellular localizations, such as zymogene granules, hormone granules, secretory granules and mature red blood cells.[48] For many years Hsp10 has been mostly considered a partner of Hsp60 in the Hsp60/10 protein folding machine. Chaperonin complex structure was resolved at high resolution, and detailed descriptions of the chaperonin mediated folding cycle have been proposed by different research groups.[22,33,49]

The Hsp60/10 complex is believed to be responsible for accelerating the folding of polypeptides imported into, and translated within, mitochondria, reactivation of denaturated proteins, diminishing aggregation of nonnative polypeptides and partially unfolded kinetically trapped intermediates. It is believed that it has the power to smooth the 'energy landscape' of protein folding as it prevents intermolecular interactions between nonnative polypeptides.[22,33,49] As a partner of Hsp60, Hsp10 participates in protein complex assembly, intra-mitochondrial sorting and biogenesis of mitochondria. Hsp10 binds Hsp60 in the presence of Mg-ATP suppressing the ATPase activity of the latter and participating in the encapsulation of the substrate.[50]

So far, little is known either about the physical interactions of human Hsp10 with other proteins within the cell, or its involvement in signal transduction pathways. Most of the data available describe a well known interaction of Hsp10 with Hsp60 in a barrel-like structure of multi-subunit chaperon machine involved in protein folding.[22] This interaction has been widely studied in *E. coli*, *S. cerevisiae* and human cells.[51] A recent report by Lin et al suggested an interaction of human Hsp10 with Ras GTP-ase pathway in myocyte protection after ischemia/reoxygenation insult.[52] Other proteins that have been reported to interact with HSP10 are mitochondrial aldehyde dehydrogenase 2 protein family member, and caspase 3 preproprotein.[35] It is not possible to exclude that interference of Hsp10 with the signalling pathway might play a specific role in anti-apoptotic protection, which is supported by the involvement of Hsp10 in post-ischemic cell viability.[52] It has only been proven that individual overexpression of chaperonins may protect cells from ischemia-reoxygenation induced cell death. It has also been shown that Hsp10/60 accumulation is caused by increased transcription and translation of the protein, as Hsp10 mRNA is induced following global brain ischemia.[53] In the myocardium of patients with chronic atrial fibrillation, the expression of the mitochondrial heat shock proteins HSP60 and HSP10 is increased, as well as in brain stem after subarachnoid haemorrhage.[54,55] It has been proposed that myocyte protection by Hsp10 involves the mobile loop and attenuation of the Ras GTP-ase pathway.[38] Hsp10 could also influence the function of signaling proteins, according to Shan et al.[56] In this study, overexpression of Hsp10 was shown to increase the abundance of IGF-1R and IGF-1-stimulated receptor auto-phosphorylation, the number of functioning receptors and to amplify activation of IGF-1R signaling. Hsp10 has also been suggested to be able to suppress poly-ubiquitination of IGF-1 receptor.[56] Moreover, Hsp10 has been shown to modulate the Bcl-2 family and mitochondria apoptosis signalling in cardiac muscle cells. Overexpression of Hsp10 increases the abundance of anti-apoptotic Bcl-xl and Bcl-2, reduces the protein content of the pro-apoptotic Bax, but does not alter the expression of Bad. Finally, HSP10 stabilizes mitochondrial cross-membrane potential, inhibits caspase-3, and suppresses PARP.[56]

In the human body, Hsp10 is upregulated by neuronal vesicular cell trafficking and synaptic plasticity,[57] and it is able to stimulate the synthesis of type I collagen.[58] Concerning cell proliferation and maturation, HSP10 plays a role in bone marrow cell differentiation, and is selectively overexpressed in myeloid and megakaryocytic precursors in normal human bone marrow.[59]

The overexpression of Hsp10 has been reported in a variety of tumours and pretumoral lesions, such as large bowel, exocervical, and prostate cancer.[43,46,60] Again, it may be postulated that Hsp10 expression may serve as a marker in tumor grading and staging.

MORTALIN: WHEN A CLOSE FRIEND BECOMES A CLOSE ENEMY

Mitochondrial heat shock 70kDa-mtHsp70 (CSA, GRP75, HSPA9, MGC4500, MOT, MOT2, MTHSP75, PBP74, mot-2) is encoded by the nuclear gene HSPA9B (GeneID: 3313) localized on chromosome 5q31.1.1. It is also designated as 75 kDa glucose regulated protein, heat shock 70kD protein 9, heat shock 70kD protein 9B, mortalin-2, heat shock 70kDa protein 9B, mortalin, perinuclear protein, p66-mortalin, peptide-binding protein 74 and stress-70 protein. It is translated in the cytoplasm and transported into mitochondria.[61] Mammalian mitochondrial Hsp70-mortalin- mot-1 was cloned in mice due to its

detection in the cytoplasmatic fraction of immortal cells, and it was then referred as an anti-proliferative protein. Human cells express only one type of mortalin protein, hmot-2.[62] Mortalin is distributed in a pancytoplasmatic manner in normal cells, but in immortal cells this localization changes into the perinuclear zone and it may reverse to normal cell pattern after induction of senescence.[63]

Known interactors of mortalin include metabolic enzymes, such as diphosphomevalonate decarboxylase, structural mitochondrial proteins, such as the voltage-dependent anion channel 1, and proteins involved in cell survival and differentiation, like the fibroblast growth factor 1, mitogen-activated protein kinase kinase kinase 7 isoform B, mitogen-activated protein kinase kinase kinase 7 interacting protein 2 isoform 1, tumor rejection antigen 1, and above all p53.[62,64,65]

Colocalization of p53 and mortalin in the perinuclear region has been shown in many types of cancer cells such as NIH 3T3 (murine fibroblasts, wt p53), Balb/3T3 (immortalized cell line), HeLa (cervical carcinoma, wt p53), A2182 (bladder carcinoma, wt p53), U2OS (osteosarcoma, wt p53), A172 (glioblastoma, wt p53), NT-2 (teratocarcinoma, wt p53), SY-5Y and YKG-1 (neuroblastoma, wt p53), COS7 (monkey kidney), and MCF7 (breast carcinoma).[61,65] Available data suggest that mot-2 is somehow also responsible for the reduction of the stable pool of p53, as it might be involved in repression of PT53 transcription or p53 degradation.[65] Mot-2 operates as an inhibitor of p53 function, by sequestering it in the cytoplasm and decreasing p53-target genes expression (e.g., $p21^{SD11/WAF}$). It is highly probable that mortalin functions in the same manner as its cytoplasmatic homolog, hsp70, binding p53 in the cytoplasm and sequestering it, which leads to abrogation of p53 function.[66] An even more pronounced impact of mot-2 overexpression and interaction with p53 is observed in immortal cells as NIH 3T3, where it changes the cell phenotype from immortal to malignant. Overexpressed mot-2 may also cause a temporary escape of human fibroblast (MRC-5) from senescence, leading to increased replicative potential (9 to 17 PD population doublings extra), maintenance of young cell morphology, and reduced expression of β-galactosidase (senescent cells marker).[67]

The role of mtHsp70 was likewise widely explored in the context of cell proliferation and differentiation; an example is the acute myeloid leukemia HL-60 cell line: when these cells undergo differentiation, mtHSP70 is downregulated, whereas Hsp70, Hsc70 and Grp78 expression is not changed.[68] The overexpression of mtHsp70 in HL-60 cells attenuates the differentiation process induced by RA, $1,25(OH)_2D_3$ or NMF. On the other hand, the constitutive expression of inducible hsp70 has been shown to prevent the apoptosis that occurs after terminal differentiation or after apoptotic agent treatment.[68] Overexpression of mortalin is also sufficient to increase the malignancy of breast carcinoma cells. Moreover, cells with an increased expression of mortalin may be anchorage-independent and acquire the ability to form tumours in nude mice.[69]

Mortalin expression has been reported in all the 60 tissues checked so far.[63,69] Highest level of mortalin has been shown in brain, heart muscle tissue, and skeletal muscles; in contrast, lowest levels were detected in testis and spleen. Higher levels of mortalin are reported in tissues characterized by high mitotic index, which confirms the anti-proliferative role of this protein.[61,65] To date, mortalin overexpression has been proven in many cancers types including brain tumours and B cell lymphoma. It is reasonable to consider its overexpression as a universal marker of cell transformation.[65] All data presented so far, reveal that mortalin contributes to human carcinogenesis and may be a candidate for gene therapy.[69]

PROHIBITIN NOT ALWAYS PROHIBITS CANCER DEVELOPMENT

Human prohibitins (hPhb1p and hPhb2p) are encoded by two nuclear genes PHB and PHB2 (GeneID: 5245 and 1331, respectively) localized on chromosome 17q21 and 12p13. PHB is expressed as two transcripts with varying lengths of the 3' untranslated region. Both PHBs are translated in the cytoplasm and transported into mitochondria.[70] Prohibitins primary function is to stabilize new synthesized polypeptides in mitochondria, as they serve as foldase-unfoldase molecular chaperones.[71,72] hPhb1p and hPhb2p localize in the inner mitochondrial membrane (IM) forming a 1MD complex, composed of 14 subunits (hPhb1p-32 kDa, hPhb2p-34 kDa) in a 1:1 ratio.[70,72-74] The name of the protein refers to its chaperone function—proteins that hold badly formed subunits.[72,73] The substrates of the PHB complex are not known in details yet, but the most important seem to be electron chain transport subunits.[72,74] The Palisade shaped model suggests that the prohibitin complex forms a barrel like structure, similar to Hsp60.[74] In the regulation of mitochondrial protein metabolism the most important seems to be PHB AAA-proteases interaction. AAA-proteases are evolutionary conserved ATP-dependent proteases of the inner mitochondrial membrane with a catalytic site in the mitochondrial matrix (*m*-AAA) and intermembranous space (*i*-AAA).[15,75,76]

It seems that PHBs are negative regulators of *m-AAA-proteases and PHBs stabilize m*-AAA proteases in low activity conformation. PHBs are also able to modulate accessibility and conformation potential substrates of proteases.[15,72,75,76] PHBp have also been recognized as negative regulators of cell cycle.[68,71] It was established that PHBS are overexpressed in metabolic stress, when the mtDNA/nDNA balance is altered, after heat shock or oxidative stress.[73] To date, prohibitins have been proposed to be active players in cell growth, ageing and transformation.[72]

Clinical data suggest a possible role of prohibitin during immortalization, ageing and cell cycle regulation. PHBs have been reported to have an anti-proliferative potential and its function have been proven to be deregulated in breast cancer patients.[71] Wide investigation of PHB expression revealed that it is constitutively expressed in normal mammalian cells as hepatocytes, smooth muscle cells, chondrocytes, spermatocytes or oocytes. Higher levels of prohibitin expression were found in regenerating liver cells, chemically induced carcinoma, hyperplastic hepatic nodules and hepatocellular carcinomas, and in cancer cells lines and primary tumour samples.[72,73] In detail, PHBs have been reported to be overexpressed in hepatocellular carcinoma cell line (HCC-M),[77] human endometrial hyperplasia and adenocarcinoma,[72] gastric cancer,[78] 13 breast cancer cell lines,[72] and bladder cancer (86). The etiology of PBH overexpression seems to originate from myc-binding elements found in the promoter region of PHB, whereas c-*myc* oncogene is overexpressed in many cancer cells and may facilitate PBH expression.[72,73] Decline of PHB expression seems to induce cell ageing, that has also been confirmed by studies on fibroblasts.[79]

Although many cancer cell lines and tumour samples, including breast, ovarian, hepatic and lung cancer, have been tested for *phb* gene mutations, so far only sporadic breast cancer samples seem to be positive, whereas loss of heterozygosity (LOH) has been reported, although it needs to be taken into account that not all the gene sequence has been tested.[80,81] It seems that genotyping prohibitin

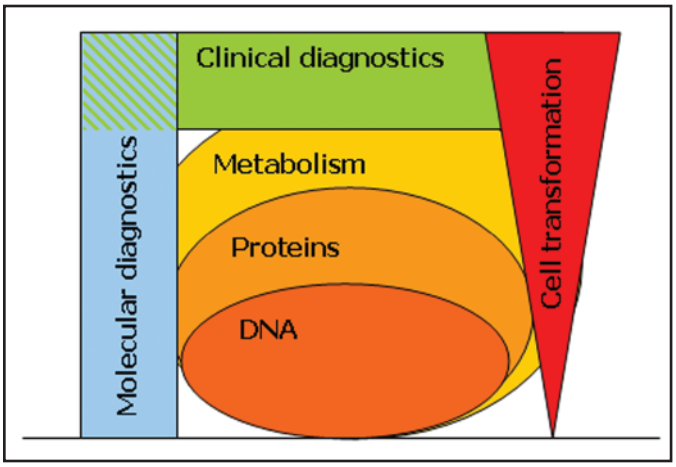

Figure 2. Co-dependence between cell transformation and clinical and molecular diagnostics.

may be a prognostic marker in breast cancer in women before 50 years of age.[82]

Besides its chaperone activity prohibitin has to be classified as an antiapoptotic protein.[73] Its interaction with pRb protein has already been widely explored.[83] Transcription factor E2F (regulated by pRb) interacts with PHB leading to cell cycle block,[83] due to E2F inactivation mediated by pRb, p107 and p130.[84] PHB inhibits transcription induced by E2F1,2,3,4 and 5 and it is thought to be an active player in cell signalling cascades.[83] Moreover, PHB interaction with SV40 T antigen has been reported,[84] and PHB localized in the nucleus was reported to mediate histone deacethylation.[83] PHB is also able to inhibit DNA synthesis in fibroblasts and HeLa cells. Overexpression of PHB protects B lymphocytes against apoptosis mediated by topoisomerase inhibitors.[81] It has been proven that PHB mRNA can regulate genes expression.[85] Microinjection of PHB mRNA blocks S phase entry in HeLa cells and fibroblasts. Further investigations have proven that PHB 3'UTR encodes for an RNA molecule that inhibits the G_1/S transition and that mutations in that region can abolish anti-proliferative action. C to T transition in PHB mRNA 3'UTR modulates its function and increases the risk of breast cancer development in women under 50 years if age.[86]

Plenty of fragmentary data on PHB action do not seem to give a relevant answer to its role in cancer development and progression. PHB mitochondrial localization and its chaperon function supports the hypothesis that it is actively involved in cell transformation, but further experiments might reveal its secrets and confirm it as a good candidate for molecular medicine and gene therapy.[15,66,74]

CONCLUSIONS

Molecular biology data suggest that cell transformation and immortalization are not controlled by a simple, single mechanism. Those processes are driven by the complex interplay of many genes functioning in multiple pathways. The best known cell cycle regulators, such as p53, pRb or telomerase must be considered as the contributors of clonal evolution and un-differentiation.[2,87] It seems that we are just beginning to uncover the molecular background of the tumorigenesis process, its constituents, their role and mode of action. A large body of evidences accumulated during the last few years stresses out that mitochondrial molecular chaperones have to be considered as an indispensable kink of this regulatory network. Many laboratories consistently indicate that mitochondrial heat shock proteins may play a unique role in cell transformation and cancer development (Fig. 2). Chaperones, through their ability to renaturate or disassemble protein aggregates, interact with other stress-response mechanisms such as superoxide dismutases or transcription factors, inducing reciprocal effects on cell life.[69] As repeatedly demonstrated, HSPs function as molecular chaperones in regulating cellular homeostasis, promote cell survival and may influence apoptosis.[27,88] Various studies demonstrate that HSP-induced cytoprotection is partly due to the suppression of apoptosis.[89] Since apoptosis represents the negative counterpart of proliferation, defects in apoptosis are associated with maintenance of a transformed state and cancer. Therefore, chaperone action may represent continuous transition from stress protection to cell transformation. Even though cell stress and cell death are obviously linked, molecular chaperones induced in response to stress appear to function at key regulatory points in the control of apoptosis. Considering the main role of molecular chaperones in the regulation of steroid receptors, kinases, caspases, transcription factors and other protein modelling events involved in replication, cell cycle and differentiation, it is not surprising that the overexpression of these molecules has been proven to trigger cancer development.[69] Because cell stress and cell death are likely to have multiple points of regulatory cross-talk, the balance between the two pathways depends on the specific nature and intensity of the stress and the level and activity of individual components of the pathway. Disruption of such a delicate balance may end up with dramatic effects as insoluble proteins association, onset of enzymatic activity at inappropriate times, incorrect localization within the cell, or any combination of the above mentioned situations.[27] Recently many interesting observations have been made. The contribution of HSPs to tumorigenesis has been attributed to their pleiotropic activities as molecular chaperones that provide the cell with an opportunity to alter protein activities, in particular components of the cell machinery as transcription factors (mortalin interacting with p53) or kinases (mortalin interacting with mitogen-activated protein kinase kinase kinase 7 isoform B and mitogen-activated protein kinase kinase kinase 7 interacting protein 2 isoform 1). Chaperones may alter cell function by associating with vital cellular proteins (mortalin-p53; prohibitin-pRB) and modulating the function of proteins that are involved in the elimination of malignant cells.[83] This could be possible since they participate in the folding of numerous proto-oncogene and oncogene products, as well as other proteins involved in signal transduction (e.g., fibroblast growth factor 1).

All the molecular data suggest that mitochondrial chaperones are involved in cell transformation processes. Although it is not possible to find a clear and uniform model for tumorigenesis, the role of Hsp60, Hsp10, mortalin and prohibitin should not be underestimated. At the same time, data collected during the last few years may justify the application of HSPs expression patterns in molecular pathologic diagnostics. The last 30 years have brought dynamic development of molecular biology methods, medicine and pharmacology. The last 10 years have proven that basic life sciences may have a tremendous impact on medicine, diagnostics and patient treatment. It is possible to develop efficient therapies and prognostic markers if the effort of many disciplines is put together.

References

1. Bertram JS. The molecular biology of cancer. Mol Aspects Med 2000; 21:167-223.
2. Guarente L, Kenyon C. Genetic pathways that regulate ageing in model organisms. Nature 2000; 408:255-62.
3. Morris M, Hepburn P, Wynford-Thomas D. Sequential extension of proliferative lifespan in human fibroblasts induced by overexpression of *CDK4* or 6 and loss of *p53* function. Oncogene 2002; 21:4277-88.
4. Augenlicht LH, Heerdt BG. Mitochondria: Integrators in tumorigenesis? Nat Genet 2001; 28:104-5.
5. Bartnik E, Lorenc A, Mroczek K. Human mitochondria in health, disease, ageing and cancer. J Appl Genet 2001; 42:65-71.
6. Carew JS, Huang P. Mitochondrial defects in cancer. Mol Cancer 2002; 1:9.
7. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG. Sequence and organization of the human mitochondrial genome. Nature 1981; 290:457-65.
8. Delsite R, Kachhap S, Anbazhagan R, Gabrielson E, Singh KK. Nuclear genes involved in mitochondria-to-nucleus communication in breast cancer cells. Mol Cancer 2002; 1:6.
9. Desagher S, Martinou JC. Mitochondria as the central control point of apoptosis. Trends Cell Biol 2000; 10:369-77.
10. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: A dawn for evolutionary medicine. Annu Rev Genet 2005; 39:359-407.
11. Hockenbery DM. A mitochondrial Achilles' heel in cancer? Cancer Cell 2002; 2:1-2.
12. Lightowlers RN, Chinnery PF, Turnbull DM, Howell N. Mammalian mitochondrial genetics: Heredity, heteroplasmy and disease. Trends Genet 1997; 13:450-5.
13. Meier P, Finch A, Evan G. Apoptosis in development. Nature 2000; 407:796-801.
14. Fernandez-Silva P, Enriquez JA, Montoya J. Replication and transcription of mammalian mitochondrial DNA. Exp Physiol 2003; 88:41-56.
15. Steglich G, Neupert W, Langer T. Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria. Mol Cell Biol 1999; 19:3435-42.
16. Vogel RO, Janssen RJ, Ugalde C, Grovenstein M, Huijbens RJ, Visch HJ, van den Heuvel LP, Willems PH, Zeviani M, Smeitink JA, Nijtmans LG. Human mitochondrial complex I assembly is mediated by NDUFAF1. FEBS J 2005; 272:5317-26.
17. Penta JS, Johnson FM, Wachsman JT, Copeland WC. Mitochondrial DNA in human malignancy. Mutat Res 2001; 488:119-33.
18. Schmitt E, Parcellier A, Gurbuxani S, Cande C, Hammann A, Morales MC, Hunt CR, Dix DJ, Kroemer RT, Giordanetto F, Jaattela M, Penninger JM, Pance A, Kroemer G, Garrido C. Chemosensitization by a nonapoptogenic heat shock protein 70-binding apoptosis-inducing factor mutant. Cancer Res 2003; 63:8233-40.
19. Simonnet H, Demont J, Pfeiffer K, Guenaneche L, Bouvier R, Brandt U, Schagger H, Godinot C. Mitochondrial complex I is deficient in renal oncocytomas. Carcinogenesis 2003; 24:1461-6.
20. Eng C, Kiuru M, Fernandez MJ, Aaltonen LA. A role for mitochondrial enzymes in inherited neoplasia and beyond. Nat Rev Cancer 2003; 3:193-202.
21. Ritossa F. Discovery of the heat shock response. Cell Stress Chaperones 1996; 1:97-8.
22. Bukau B, Horwich AL. The Hsp70 and Hsp60 chaperone machines. Cell 1998; 92:351-66.
23. Hohfeld J, Cyr DM, Patterson C. From the cradle to the grave: Molecular chaperones that may choose between folding and degradation. EMBO Rep 2001; 2:885-90.
24. Ellis RJ. The molecular chaperone concept. Semin Cell Biol 1990; 1:1-9.
25. Young JC, Agashe VR, Siegers K, Hartl FU. Pathways of chaperone-mediated protein folding in the cytosol. Nat Rev Mol Cell Biol 2004; 5:781-91.
26. Ellis RJ. Molecular chaperones: Pathways and networks. Curr Biol 1999; 9:R137-9.
27. Jolly C, Morimoto RI. Role of the heat shock response and molecular chaperones in oncogenesis and cell death. J Natl Cancer Inst 2000; 92:1564-72.
28. Macario AJ. Heat-shock proteins and molecular chaperones: Implications for pathogenesis, diagnostics, and therapeutics. Int J Clin Lab Res 1995; 25:59-70.
29. Calderwood SK, Theriault JR, Gong J. Message in a bottle: Role of the 70-kDa heat shock protein family in anti-tumor immunity. Eur J Immunol 2005; 35:2518-27.
30. Jindal S, Dudani AK, Singh B, Harley CB, Gupta RS. Primary structure of a human mitochondrial protein homologous to the bacterial and plant chaperonins and to the 65-kilodalton mycobacterial antigen. Mol Cell Biol 1989; 9:2279-83.
31. Soltys BJ, Gupta RS. Mitochondrial-matrix proteins at unexpected locations: Are they exported? Trends Biochem Sci 1999; 24:174-7.
32. Hansen JJ, Bross P, Westergaard M, Nielsen MN, Eiberg H, Borglum AD, Mogensen J, Kristiansen K, Bolund L, Gregersen N. Genomic structure of the human mitochondrial chaperonin genes: *HSP60* and *HSP10* are localised head to head on chromosome 2 separated by a bidirectional promoter. Hum Genet 2003; 112:71-7.
33. Fersht AR, Daggett V. Protein folding and unfolding at atomic resolution. Cell 2002; 108:573-82.
34. White GW, Gianni S, Grossmann JG, Jemth P, Fersht AR, Daggett V. Simulation and experiment conspire to reveal cryptic intermediates and a slide from the nucleation-condensation to framework mechanism of folding. J Mol Biol 2005; 350:757-75.
35. Samali A, Cai J, Zhivotovsky B, Jones DP, Orrenius S. Presence of a preapoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells. EMBO J 1999; 18:2040-8.
36. Kirchhoff SR, Gupta S, Knowlton AA. Cytosolic heat shock protein 60, apoptosis, and myocardial injury. Circulation 2002; 105:2899-904.
37. Hammarstrom P, Persson M, Owenius R, Lindgren M, Carlsson U. Protein substrate binding induces conformational changes in the chaperonin GroEL. A suggested mechanism for unfoldase activity. J Biol Chem 2000; 275:22832-8.
38. Wadhwa R, Takano S, Kaur K, Aida S, Yaguchi T, Kaul Z, Hirano T, Taira K, Kaul SC. Identification and characterization of molecular interactions between mortalin/mtHsp70 and HSP60. Biochem J 2005; 391:185-90.
39. Xanthoudakis S, Roy S, Rasper D, Hennessey T, Aubin Y, Cassady R, Tawa P, Ruel R, Rosen A, Nicholson DW. *Hsp60* accelerates the maturation of pro-caspase-3 by upstream activator proteases during apoptosis. EMBO J 1999; 18:2049-56.
40. Pignatelli D, Ferreira J, Soares P, Costa MJ, Magalhaes MC. Immunohistochemical study of heat shock proteins 27, 60 and 70 in the normal human adrenal and in adrenal tumors with suppressed ACTH production. Microsc Res Tech 2003; 61:315-23.
41. Bini L, Magi B, Marzocchi B, Arcuri F, Tripodi S, Cintorino M, Sanchez JC, Frutiger S, Hughes G, Pallini V, Hochstrasser DF, Tosi P. Protein expression profiles in human breast ductal carcinoma and histologically normal tissue. Electrophoresis 1997; 18:2832-41.
42. Cappello F, Bellafiore M, Palma A, David S, Marciano V, Bartolotta T, Sciume C, Modica G, Farina F, Zummo G, Bucchieri F. 60KDa chaperonin (HSP60) is overexpressed during colorectal carcinogenesis. Eur J Histochem 2003; 47:105-10.
43. Cappello F, David S, Rappa F, Bucchieri F, Marasa L, Bartolotta TE, Farina F, Zummo G. The expression of HSP60 and HSP10 in large bowel carcinomas with lymph node metastase. BMC Cancer 2005; 5:139.
44. Cappello F, Di Stefano A, D'Anna SE, Donner CF, Zummo G. Immunopositivity of heat shock protein 60 as a biomarker of bronchial carcinogenesis. Lancet Oncol 2005; 6:816.
45. Cappello F. HSP60 and HSP10 as diagnostic and prognostic tools in the management of exocervical carcinoma. Gynecol Oncol 2003; 91:661.
46. Cappello F, Rappa F, David S, Anzalone R, Zummo G. Immunohistochemical evaluation of PCNA, p53, HSP60, HSP10 and MUC-2 presence and expression in prostate carcinogenesis. Anticancer Res 2003; 23:1325-31.
47. Faried A, Sohda M, Nakajima M, Miyazaki T, Kato H, Kuwano H. Expression of heat-shock protein Hsp60 correlated with the apoptotic index and patient prognosis in human oesophageal squamous cell carcinoma. Eur J Cancer 2004; 40:2804-11.
48. Sadacharan SK, Cavanagh AC, Gupta RS. Immunoelectron microscopy provides evidence for the presence of mitochondrial heat shock 10-kDa protein (chaperonin 10) in red blood cells and a variety of secretory granules. Histochem Cell Biol 2001; 116:507-17.
49. Brinker A, Pfeifer G, Kerner MJ, Naylor DJ, Hartl FU, Hayer-Hartl M. Dual function of protein confinement in chaperonin-assisted protein folding. Cell 2001; 107:223-33.
50. Richardson A, Schwager F, Landry SJ, Georgopoulos C. The importance of a mobile loop in regulating chaperonin/ cochaperonin interaction: Humans versus *Escherichia coli*. J Biol Chem 2001; 276:4981-7.
51. Ranford JC, Coates AR, Henderson B. Chaperonins are cell-signalling proteins: The unfolding biology of molecular chaperones. Expert Rev Mol Med 2000; 2000:1-17.
52. Lin KM, Hollander JM, Kao VY, Lin B, Macpherson L, Dillmann WH. Myocyte protection by 10 kD heat shock protein (Hsp10) involves the mobile loop and attenuation of the Ras GTP-ase pathway. FASEB J 2004; 18:1004-6.
53. Ray PS, Martin JL, Swanson EA, Otani H, Dillmann WH, Das DK. Transgene overexpression of alphaB crystallin confers simultaneous protection against cardiomyocyte apoptosis and necrosis during myocardial ischemia and reperfusion. FASEB J 2001; 15:393-402.
54. Kirmanoglou K, Hannekum A, Schafler AE. Expression of mortalin in patients with chronic atrial fibrillation. Basic Res Cardiol 2004; 99:404-8.
55. Satoh M, Tang J, Nanda A, Zhang JH. Heat shock proteins expression in brain stem after subarachnoid hemorrhage in rats. Acta Neurochir Suppl 2003; 86:477-82.
56. Shan YX, Liu TJ, Su HF, Samsamshariat A, Mestril R, Wang PH. Hsp10 and Hsp60 modulate Bcl-2 family and mitochondria apoptosis signaling induced by doxorubicin in cardiac muscle cells. J Mol Cell Cardiol 2003; 35:1135-43.
57. Khawaja X, Xu J, Liang JJ, Barrett JE. Proteomic analysis of protein changes developing in rat hippocampus after chronic antidepressant treatment: Implications for depressive disorders and future therapies. J Neurosci Res 2004; 75:451-60.
58. Mansell JP, Yarram SJ, Brown NL, Sandy JR. Type I collagen synthesis by human osteoblasts in response to placental lactogen and chaperonin 10, a homolog of early-pregnancy factor. In Vitro Cell Dev Biol Anim 2002; 38:518-22.
59. Cappello F, Tripodo C, Farina F, Franco V, Zummo G. HSP10 selective preference for myeloid and megakaryocytic precursors in normal human bone marrow. Eur J Histochem 2004; 48:261-5.
60. Cappello F, Bellafiore M, David S, Anzalone R, Zummo G. Ten kilodalton heat shock protein (HSP10) is overexpressed during carcinogenesis of large bowel and uterine exocervix. Cancer Lett 2003; 196:35-41 63.
61. Wadhwa R, Taira K, Kaul SC. An Hsp70 family chaperone, mortalin/mthsp70/PBP74/Grp75: What, when, and where? Cell Stress Chaperones 2002; 7:309-16.
62. Wadhwa R, Yaguchi T, Hasan MK, Taira K, Kaul SC. Mortalin-MPD (mevalonate pyrophosphate decarboxylase) interactions and their role in control of cellular proliferation. Biochem Biophys Res Commun 2003; 302:735-42.
63. Wadhwa R, Pereira-Smith OM, Reddel RR, Sugimoto Y, Mitsui Y, Kaul SC. Correlation between complementation group for immortality and the cellular distribution of mortalin. Exp Cell Res 1995; 216:101-6.
64. Schwarzer C, Barnikol-Watanabe S, Thinnes FP, Hilschmann N. Voltage-dependent anion-selective channel (VDAC) interacts with the dynein light chain Tctex1 and the heat-shock protein PBP74. Int J Biochem Cell Biol 2002; 34:1059-70.

65. Wadhwa R, Takano S, Robert M, Yoshida A, Nomura H, Reddel RR, Inactivation of tumor suppressor *p53* by *mot-2*, a *hsp70* family member. J Biol Chem 1998; 273:29586-91.
66. Zylicz M, King FW, Wawrzynow A. Hsp70 interactions with the *p53* tumour suppressor protein. EMBO J 2001; 20:4634-8.
67. Kaula SC, Reddelb RR, Sugiharac T, Mitsuia Y, Wadhwac R. Inactivation of p53 and life span extension of human diploid fibroblasts by mot-2. FEBS Lett 2000; 474:159-64.
68. Xu J, Xiao HH, Sartorelli AC. Attenuation of the induced differentiation of HL-60 leukemia cells by mitochondrial chaperone HSP70. Oncol Res 1999; 11:429-35.
69. Wadhwa R, Takano S, Kaur K, Deocaris CC, Pereira-Smith OM, Reddel RR, Kaul SC. Upregulation of mortalin/mthsp70/Grp75 contributes to human carcinogenesis. Int J Cancer 2006; In press.
70. Ikonen E, Fiedler K, Parton RG, Simons K. Prohibitin, an antiproliferative protein, is localized to mitochondria. FEBS Lett 1995; 358:273-7.
71. McClung JK, Jupe ER, Liu XT, Dell'Orco RT. Prohibitin: Potential role in senescence, development, and tumor suppression. Exp Gerontol 1995; 30:99-124.
72. Nijtmans LG, Artal SM, Grivell LA, Coates PJ. The mitochondrial PHB complex: Roles in mitochondrial respiratory complex assembly, ageing and degenerative disease. Cellular and Mollecular Life Sciences Cell Mol Life Sci 2002; 59:143-55.
73. Coates PJ, Nenutil R, McGregor A, Picksley SM, Crouch DH, Hall PA, Wright EG. Mammalian prohibitin proteins respond to mitochondrial stress and decrease during cellular senescence. Exp Cell Res 2001; 265:262-73.
74. Back JW, Sanz MA, De Jong L, De Koning LJ, Nijtmans LG, De Koster CG, Grivell LA, Van Der Spek H, Muijsers AO. A structure for the yeast prohibitin complex: Structure prediction and evidence from chemical crosslinking and mass spectrometry. Protein Sci 2002; 11:2471-8.
75. Arlt H, Steglich G, Perryman R, Guiard B, Neupert W, Langer T. The formation of respiratory chain complexes in mitochondria is under the proteolytic control of the m-AAA protease. EMBO J 1998; 17:4837-47.
76. Langer T, Kaser M, Klanner C, Leonhard K. AAA proteases of mitochondria: Quality control of membrane proteins and regulatory functions during mitochondrial biogenesis. Biochem Soc Trans 2001; 29:431-6.
77. Seow TK, Ong SE, Liang RC, Ren EC, Chan L, Ou K, Chung MC. Two-dimensional electrophoresis map of the human hepatocellular carcinoma cell line, HCC-M, and identification of the separated proteins by mass spectrometry. Electrophoresis 2000; 21:1787-813.
78. Ryu JW, Kim HJ, Lee YS, Myong NH, Hwang CH, Lee GS, Yom HC. The proteomics approach to find biomarkers in gastric cancer. J Korean Med Sci 2003; 18:505-9.
79. Fellenberg J, Dechant MJ, Ewerbeck V, Mau H. Identification of drug-regulated genes in osteosarcoma cells. Int J Cancer 2003; 105:636-43.
80. Sato T, Sakamoto T, Takita K, Saito H, Okui K, Nakamura Y. The human prohibitin (*PHB*) gene family and its somatic mutations in human tumors. Genomics 1993; 17:762-4.
81. Cliby W, Sarkar G, Ritland SR, Hartmann L, Podratz KC, Jenkins RB. Absence of prohibitin gene mutations in human epithelial ovarian tumors. Gynecol Oncol 1993; 50:34-7.
82. Jupe ER, Badgett AA, Neas BR, Craft MA, Mitchell DS, Resta R, Mulvihill JJ, Aston CE, Thompson LF. Single nucleotide polymorphism in prohibitin 39 untranslated region and breast-cancer susceptibility. Lancet 2001; 357:1588-9.
83. Wang S, Fusaro G, Padmanabhan J, Chellappan SP. Prohibitin colocalizes with Rb in the nucleus and recruits *N-CoR* and *HDAC1* for transcriptional repression. Oncogene 2002; 21:8388-96.
84. Darmon AJ, Jat PS. BAP37 and Prohibitin are specifically recognized by an SV40 T antigen antibody. Mol Cell Biol Res Commun 2000; 4:219-23.
85. Manjeshwar S, Branam DE, Lerner MR, Brackett DJ, Jupe ER. Tumor suppression by the prohibitin gene 3'untranslated region *RNA* in human breast cancer. Cancer Res 2003; 63:5251-6.
86. Spurdle AB, Hopper JL, Chen X, McCredie MR, Giles GG, Newman B, Chenevix-Trench G. Prohibitin 3' untranslated region polymorphism and breast cancer risk in Australian women. Lancet 2002; 360:925-6.
87. Reddel RR. The role of senescence and immortalization in carcinogenesis. Carcinogenesis 2000; 21:477-484.
88. Jäättelä M. Escaping cell death: Survival proteins in cancer. Exp Cell Res 1999; 248:30-43.
89. Sreedhar AS, Csermely P. Heat shock proteins in the regulation of apoptosis: New strategies in tumor therapy A comprehensive review. Pharmacol Ther 2004; 101:227-257.