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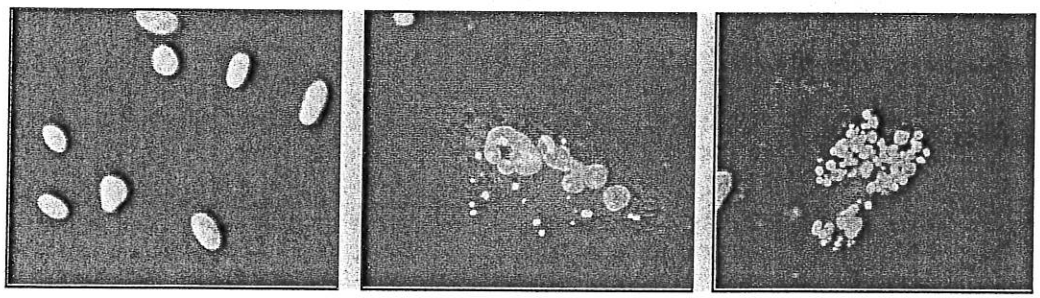

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Chapter III

HEAT SHOCK PROTEINS MAY HAVE A DIRECT EFFECT ON CELLULAR SENESCENCE AND MAY LEAD TO NEOPLASTIC TRANSFORMATION

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ABSTRACT

Heat Shock Proteins (HSPs), known also as chaperones, protect cells against different forms of stress, such as hypoxia, ischemia and hyperoxia. Many cellular roles regarding the four best-known HSPs, HSP27, 60, 70 and 90, different from protein folding have been discovered. In the last few years, we have evaluated the presence and expression of several HSPs in a variety of human carcinogenic models, i.e. the "dysplasia-carcinoma" sequences of uterine exocervix, colorectal mucosa, prostate gland, bladder and bronchi. Moreover we have demonstrated that the HSP60 increases during the initial stages of senescence and that it is localized in cellular compartments, resembling mitochondria. Cellular senescence is considered an important mechanism to irreversibly arrest the growth of cells at risk for tumorigenesis; mutations that disrupt the senescence response in human may lead to increased cancer incidence.

Our results suggest a possible role of HSPs in the replicative senescence and in the regulation of the cell cycle progression through the different stages of cell aging.

Keywords: HSP60, cancerogenesis, replicative senescence, chaperonopathy, stress

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INTRODUCTION

Chaperones are highly conserved proteins protecting cells against different forms of stress, such as hypoxia, ischemia and hyperoxia [1]. They are essential in the maintenance of protein homeostasis inside the cell and have an essential role in the folding of newly synthesized or damaged proteins. This process, ATP-dependent or ATP-independent, drives proteins through degradation or future refolding [2]. Recently, new roles have been discovered and chaperones have been involved into intracellular signalling, protein trafficking and apoptotic events [3]. They can modulate several mutations as well, making them phenotypically silent [4].

In the last few years our research group has evaluated the expression of several HSPs in some human carcinogenic models, revealing a correspondence between the increase in the severity of dysplasia and the increase in HSPs expression levels. These data demonstrate that HSP60 and HSP10 may be used as prognostic markers and suggest a role of these chaperones in the maintenance of the cancerous state. In parallel we studied the expression of HSP60 during the passages of *in vitro* induced replicative senescence in normal human skin fibroblasts. HSP60, known to be involved in the caspase-3 apoptotic pathway, seems to be implicated also in the regulation of the cell cycle progression.

HEAT SHOCK PROTEINS

The cellular response to stress is represented, at the molecular level, by the induced synthesis of the ubiquitous expressed HSPs, as an essential defence mechanism for the protection of the cell from many harmful conditions such as heat shock, oxidative stress or inflammation [5]. The prime experimentation on HSPs was performed on *E.coli*. The simple structure of bacterial chaperones simplified the biochemical characterization of these proteins and permitted the comprehension of the same in eukaryotic cells [6].

HSPs were first identified for their increase during heat shock or oxidative stress, but it was then observed that their expression was also activated during the normal cellular growth. In the cytoplasmic compartment they bind to hydrophobic residues of proteins which have not reached their final conformational state, catalysing their folding [7]. They mediate protein trafficking inside the cell, avoiding irregular aggregations and mismatched proteins interactions. After mRNA translation, unfolded or incorrectly folded polypeptides may enter the degradation (Ubiquitin – Proteasome System) or the folding process, reaching their native state [2,8].

Chaperones have been subdivided into several groups on the basis of their molecular mass. Some HSPs, such as HSP27, HSP70 and HSP90 seem to be involved into the replicative senescence process [9], in cell cycle control, in cancer development and in DNA damage repair processes [10].

HSP60 and HSP10 represent the folding apparatus of mitochondria, HSP10 being a co-chaperone for HSP60 [11]. HSP60 forms a homo-oligomer of fourteen subunits, where seven subunits form a ring. The result is a double ring structure surrounding a central cavity, which can allocate 50 kDa proteins [12]. The preferred substrates of HSP60/HSP10 complex are

unfolded proteins, whose folding is catalysed in an ATP-dependent manner. HSP10 acts as a cap covering the central cavity, opening and closing the structure [11]. This little chaperone coordinates the behaviour of each single HSP60 monomer and regulates the ATP hydrolysis [12]. This hydrolysis has been well studied for the HSP60 and HSP10 bacterial homologues: GROEL and GROES respectively [11]. Shortly after GROEL binding to one ATP molecule, the folding protein interacts with the inner side of the central cavity by means of hydrophobic residues and the ATP hydrolyses induces conformational changes after which GROES and GROEL split and the folded protein is released in the mitochondrial stroma [13]. Exogenous bacterial HSP60 has an effect on epithelial cell vitality [14], activates MAP kinases [14], induces Tumor Necrosis Factor alpha (TNF-α) release in a dose dependent-manner [14] and, during a prolonged inflammatory process, it may cause autoimmunity [15].

In *S. cerevisiae* HSP60 is a gene essential for cellular growth [16]. Each subunit has a C-terminal fragment protruding inside the central cavity of the folding apparatus. This portion is highly conserved among GROEL family members. Deletions inside this fragment lead to cell growth defects. The importance of this C-terminal portion in yeast HSP60 comes from the unique implication in mitochondrial DNA transmission from the mother to the daughter cell. In fact, it seems to be involved in mitochondrial DNA replication for its interactions with ori DNA, probably stabilizing protein binding to the promoter. Other particular roles of yeast HSP60 are in nucleolus replication and mitochondrial DNA segregation [17]. DNA binding capacity has not been proved for bacterial GROEL in E.coli [17].

In eukaryotic cells, HSP60 has been also involved in the apoptotic process. It has been demonstrated by Samali A. et al [18,19] that HSP60 may associate to caspase-3 in an anti-apoptotic manner and Shan Y.X. et al [20] have demonstrated that it may associate to Bak, inhibiting its apoptotic potential and cytochrome c release. Recently, we have suggested the involvement of HSP60 in cell cycle progression and regulation during replicative senescence, probably associating with a 47kDa protein [21], MOK [22,23]. This newly discovered MAP kinase seems to be involved into signal transduction to the nucleus [23].

Considering the alternative roles of HSP60 stated above, this chaperone seems to have an important role inside the cell. A defect in its functions, such as in its anti-apoptotic effect, may favour tumour cell survival and cancer development.

HEAT SHOCK PROTEINS AND CANCER

It has become increasingly clear that disruption of chaperoning mechanisms contributes to aging and disease. Different studies outlines the involvement of defective chaperones in senescence and in several diseases [24]. The chaperonopathies are diseases associated with mutations or post-transcriptional modifications that render a chaperone functionally defective. The manifestations of a chaperonopathy depend on the domain or function that is impaired or abolished. Acquired chaperonopathies are associated with post-translational modifications of the chaperone and usually become clinically evident late in life [24]. Mechanisms of post-translational modifications of proteins are diverse. They include: oxidation of amino acids, deamination and glycation. During senescence, these modifications damage many proteins, including chaperones. As a result, functionally incompetent

chaperones are unable to deal with an excessive demand for the repair of proteins. In the chaperonopathies HSP levels are increased or decreased, or distributed abnormally in tissues and cells. Examples of chaperonopathies that are associated with aging or disease are neurodegenerative diseases, retinophaties, myophaties, cataracts etc. In these disorders, also called protein misfolding diseases, the hallmark is the occurrence of molecules with a tendency to misfold and to precipitate. These abnormal proteins are targeted to refolding, but functionally incompetent chaperones are unable to deal with an excessive demand for the repair of proteins and precipitate together with abnormal polypeptides [24].

A tumour can be considered a chaperonopathy, in fact, during carcinogenesis HSPs show several alterations in their expression levels [25,26]. In some tumors the expression levels of HSPs increase while in other they decrease [27,28]. The contribution of HSPs to tumorigenesis may be attributed to their pleiotropic activities as molecular chaperones that provide the cancer cell with a machinery able to alter protein activities, such as proteins involved into the cell cycle, kinases and other proteins implicated in tumour progression. Some examples of HSPs highly expressed during tumourigenesis are HSP27, HSP60, HSP70 and HSP90 (Figure 1). In a number of cancers such a breast cancer, endometrial cancer and leukaemia, an increased HSP27 level has been detected [29]. Increased levels of HSP70 have also been reported in high grade malignant tumors such as breast cancer and endometrial cancer, osteosarcoma and renal tumors [29-31]. HSP90 and HSP60 are also overexpressed in breast tumours, lung cancer, leukemias and Hodgkin's disease [29,32,33]. The molecular basis for overexpression of HSPs in tumours is not completely understood and may have different aetiologies. For example it may be due to suboptimal cellular environment in the poorly vascularised hypoxic tumour or due to the growth conditions within the solid tumour [29].

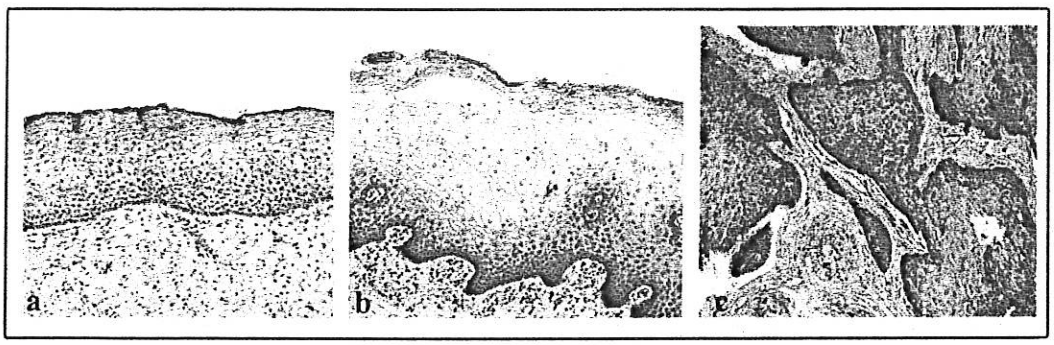

Figure 1 – HSP60 expression in the dysplasia-carcinoma sequence of human uterine exocervix: a) normal epithelium; b) dysplastic epithelium shows positivity in basal, parabasal and intermediate layers; c) carcinoma shows a diffuse positivity in almost all tumoural cells.

HSP60 is one of the chaperones commonly overexpressed in a wide range of malignant cells and tissues; even if its detection could be of limited use in diagnostic immunopathology, its expression levels can help to indicate the presence of abnormal changes during the process of carcinogenesis [34].

In recent studies, we focused our attention on the expression of HSP60 in several carcinogenic models such as the adenoma-carcinoma sequence of colorectum and the

dysplasia-carcinoma sequence of uterine cervix and prostate (Figure 1) [35,36]. Dysplasia is a term that literally means disordered growth. It is encountered principally in epithelia, and it is characterized by a constellation of changes that include a loss of the uniformity of the individual cells as well as a loss in their architectural orientation. Dysplastic cells also exhibit considerable pleomorphism and often contain hyperchromatic nuclei that are abnormally large for the size of the cell. Mitotic figures are more abundant than usual, although almost invariably they conform to normal patterns. Frequently the mitoses appear in abnormal locations within the epithelium and the architecture of the tissue may be disordered. For example, the usual progressive maturation of tall cells in the basal layer to flattened squames on the surface may be lost and replaced by a scrambling of dark basal-appearing cells throughout the epithelium. When dysplastic changes are marked and involve the entire thickness of the epithelium, but the lesions remains confined to the normal tissue, it is considered a preinvasive neoplasm and is referred to as *carcinoma in situ*. Once the tumor cells move beyond the normal confines, the tumor is said to be *invasive* [37]. Using Immunohistochemical and Western blotting analyses, we found that HSP60 expression gradually increases from normal through dysplastic to neoplatic tissues, arguing that its overexpression during carcinogenesis may be functionally correlated to tumour growth [38-45]. Nevertheless, Lebret et al. [10] demonstrated that HSP60 expression disappears suddenly during carcinogenesis, especially in bladder tumours. In accordance with these data, we also demonstrated that HSP60 may also be down regulated during bronchial carcinogenesis [46]. In a series of bronchial biopsies of smoking subjects, we showed that normal and hyperplatic mucosae present HSP60 immunopositivity in most part of the epithelial cells. In patients with squamous metaplasia only a few epithelial elements showed the expression of HSP60, this positivity totally disappeared in dysplastic and tumoural specimen [46].

Whether it is presently unrealistic to affirm that HSP60 overexpression is correlated to cancer development and progression, its levels may be correlated to the expression of other proteins or involved in the activation of cellular signals. The over or down-expression of HSP60 can be used as a molecular marker of the clinical stage and of the patient prognosis in the pre-lesions of a variety of tumours. For example HSP60 expression has also been suggested as a good prognostic marker in human oesophageal squamous cell carcinoma (ESCC) [47]. The literature reported thus far let us considers HSP60 as a carcinogenesis marker, with a potential clinical significance. Although the exact molecular roles of HSP60 have not been defined yet, it is reasonable to think that its expression may serve as a prognostic tool in clinics and histopathologic diagnosis [36]. These studies demonstrate that HSP60 might significantly contribute to tumorigenesis, making it a good candidate target for cancer therapy [48].

It has also been reported the overexpression of HSP10, the HSP60 co-chaperone, in variety of tumours and pre-tumoural lesions, such as large bowel cancer exocervical cancer [21], prostate cancer [21] and mantle-cell lymphoma [21,49]. As for HSP60, this overexpression may serve as a marker in tumour grading and staging.

REPLICATIVE SENESCENCE AND HEAT SHOCK PROTEINS

Many mammalian cells, such as epithelial and β pancreatic cells undergo a limited number of cell divisions *in vitro,* before entering a quiescent state called replicative senescence. In this state cells cannot be induced to divide again [50]. After a limited number of divisions cells stop dividing, suggesting the presence of a biological clock that regulates cell division. During this fine regulation process, some proteins accumulate inside the cell, inducing blocking of the cell division [51]. In normal cultural conditions, after a period of rapid growth, cell division rate decreases progressively until ceases. These cells do not respond to external stimuli, but remain alive for a long period. They change their morphology, loose their original shape and their cytoplasm become flatter. During replicative senescence some modifications of the nucleus structure, gene expression and protein modifications occur [52]. Loss of proliferative potential may be explained partially by the response to growth factors such as Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), Tumour Growth Factor (TGF) and Platelet-Derived Growth Factor (PDGF) [52]. Some studies on normal human fibroblasts have demonstrated that the binding of a ligand to its receptor, the receptor density on the cell surface and the affinity of the ligand for its receptor, do not change during the first passages of senescence, but later on [53]. A characteristic of a senescent cell is an increase in the enzymatic activity of β – galactosidase; this increase persists in aging tissues and this increment goes in parallel with cell doublings (Senescence associated β- galactosidase activity; SA-β-gal) [54].

Some features make it possible to distinguish between senescent cells and normal cells. Senescent cells are resistant to apoptosis in a manner dependent from p53 and undergo necrosis after DNA damage [55-57]. Senescence-induced resistance to apoptosis leads to an increase in the number of senescence cells inside tissues, with consequences on aging, on neoplastic transformation, on the weakness of tissue integrity and on general health [53]. Replicative senescence of T lymphocytes has been suggested to cause rheumatoid arthritis [58], while in vascular cells may be considered responsible for atherosclerotic lesions [59].

Replicative senescence has been extensively studied by many authors but their molecular bases are unknown as why they stop dividing. The progressive telomere-shortening has been proposed as the most important mechanism underlying the senescent state. Telomeres are repeated DNA sequences (TTAGGG in vertebrates) associated with specialized proteins that form a cap over the chromosome ends and are essential for chromosome integrity. During the cell cycle and DNA replication, 50-200 bp of the telomeric DNA are not replicated because the telomerase is not able to replicate the entire chromosome and some sequences must be sacrificed. The presence of telomeres makes it possible to maintain the entire coding sequence, without altering chromosomes homeostasis [60]. Telomeres length in human fibroblasts is an indicator of replicative history and of the cumulative history of oxidative stress [61]. Telomere-shortening also causes T lymphocyte replicative senescence [62]. Recently it has been demonstrated that this phenomenon is not caused only by telomere shortening but also changes in the capping state of chromosomes are needed [63,64].

Also other stimuli, that can have an impact on telomeres length, may induce growth arrest and a senescent phenotype in normal cells. These stimuli can be oxidative stress, chromatin remodelling and activation of oncogenes. For example, mitochondria are the first

targets of oxidative stress, because the aerobic respiratory chain produces Reactive Oxygen Species (ROS). An increase in ROS leads to an increment in the number of mitochondria as a compensatory mechanism, with the consequent increase in the level of mitochondrial proteins [65]. Replicative senescence in Normal Human Oral Keratinocytes (NHOK) is caused by mitochondria alterations [66]. An increase in ROS species as a cause leading to replicative senescence has also been demonstrated for human dermal fibroblasts. Moreover, fibroblasts cultured in the presence of 1-3 % oxygen divide several times before becoming senescent compared to fibroblasts cultured with 20% oxygen [65]. An increase in the level of ROS is also induced by the oncogene *ras*, through the activation of the intracellular signal mediated by MAP kinases [67]. Also Raf and Mek, downstream Ras, can block cell divisions [68].

All these stimuli can even contribute to cancer development. Replicative senescence, in fact, represents a barrier to immortalization and uncontrolled proliferation, and can be used as a tumour suppressive mechanism [69,70]. Recent studies demonstrated that tumour progression can be blocked inducing senescence in cells with oncogene mutations, hence committed to cancer both in human and in mice [71]. This correlation suggests that some onco-suppressor proteins, such as p53 and pRb, may control also replicative senescence establishment [70]. P53 is a transcription factor able to activate or suppress the transcription of genes involved into cell cycle progression or apoptosis as a response to DNA damage. One of the genes regulated positively by p53 during replicative senescence is the Cdk inhibitor p21 [72]. This protein can bind to the Proliferating Cell Nuclear Antigen (PCNA), adjuvant of the DNA polimerase δ. P21 may cause replicative senescence on its own [73]. Moreover, p53 induces cytochrome c release and the activation of mitochondrial apoptosis effectors, one of which is Bax. P300/CBP acetyl-transferase activates p53 by acetylation on the lysine residue of the C-terminus. Acetylation and phosphorylation cascades activates p53 and allows to maintain the cell in the senescent state [73].

Also pRb is implicated in the induction and maintenance of the replicative senescence. This oncosuppressor protein directly regulates transcription binding to the transcription factor E2F during the S phase. E2F in turn activates the transcription of genes whose expression is necessary for DNA replication and cell cycle progression [74]. pRb can be inactivated by phosphorylation by the complex CdK 4/6-CyclinD, inhibited by the p16 CdK-inhibitor. High levels of p16 are expressed during senescence. P16 induction can be explained by chromatin remodelling during senescence [75]. Both pathways p53-p21 and pRb-p16 are important in replicative senescence, in particular p53-p21 pathway establishes the senescence state while pRb-p16 is important in its maintenance [76]. The two pathways are independent but may interact one with each other, since p21 can inhibit CdK and pRb, binding to MDM2, prevents p53 degradation [77]. Other CdK inhibitors accumulate during replicative senescence, p27 and p24. p24 is an inhibitor of the p21 family and accumulates when embryonic fibroblasts undergo replicative senescence in culture [78]. This inhibitor has been considered for long time the real controller of the biological cellular clock [79].

In a recent paper we have demonstrated that in human skin fibroblasts HSP60 increases during the first seven passages of replicative senescence. The suddenly increase in the protein level correlates with cell cycle progression [21]. A correlation HSP60-overexpression/ enescence and a new interaction mtHSP70/HSP60 in human skin fibroblasts have been demonstrated also by Kaul Z et al [80]. They also indicate two different pathways in

replicative senescence for mtHSP70 and HSP60 inside mitochondria [80]. This correlation between HSP60 and senescence confirms our previously published hypothesis that an exogenous HSP60 produced by an external factor, such as a persistent infection by an intracellular bacterium, may block the anti-apoptotic role and the pro-senescence function of the endogenous HSP60, favouring the active proliferation of damaged cells leading to cancer [81]. In particular we hypothesised that Chlamydial HSP60 (CHSP60) produced during exocervix or lung infections may contribute to cancer development and progression [81]. Other authors have demonstrated that also HSP27, HSP70 and HSP90 seems to have an effect on replicative senescence [82-84]. Furthermore some chaperones, like HSP70 may inhibit caspase-dependent and caspase-independent stimuli conferring immortality to cells [85].

CONCLUSION

The replicative senescence is a phenomenon correlated to aging. Old individuals accumulate senescent cells in their body. Two hypothesis can be proposed to explain the importance of replicative senescence in cancer development: damaged cells, with dysfunctional telomeres, enter into a state of cellular senescence as a protective mechanism [52] or cellular senescence promotes aging by exhausting stem cells or progenitor cells late in life, favouring late life cancers [86]. In both cases HSPs may be involved. They can protect cells from cancer development inducing replicative senescence or may favour cancer, if they accumulate inside a senescent cell because of protein mutations. Nonetheless which of the two hypotheses is correct it has to be demonstrated.

REFERENCES

[1] Mayhew, M; da Silva, AC; Martin, J; Erdjument-Bromage, H; Tempst, P; Hartl, FU. Protein folding in the central cavity of the GroEL-GroES chaperonin complex. *Nature*, 1996. 379(6564): p. 420-6.

[2] Wiederkehr, T; Bukau, B; Buchberger, A. Protein turnover: a CHIP programmed for proteolysis. *Curr Biol*, 2002. 12(1): p. R26-8.

[3] Czarnecka, A; Campanella, C; Zummo, G; Cappello, F. Hsp10 and signal trasduction: a "Capsula Eburnea" of carcinogenesis? *Cell Stress Chaperones*, 2006. in press.

[4] Fares, MA; Barrio, E; Sabater-Munoz, B; Moya, A. The evolution of the heat-shock protein GroEL from Buchnera, the primary endosymbiont of aphids, is governed by positive selection. *Mol Biol Evol*, 2002. 19(7): p. 1162-70.

[5] Morimoto, RI. Cells in stress: transcriptional activation of heat shock genes. Science, 1993. 259(5100): p. 1409-10.

[6] Ryan, MT; Naylor, DJ; Hoj, PB; Clark, MS; Hoogenraad, NJ. The role of molecular chaperones in mitochondrial protein import and folding. *Int Rev Cytol*, 1997. 174: p. 127-93.

[7] Lee, S; Tsai, FT. Molecular chaperones in protein quality control. *J Biochem Mol Biol*, 2005. 38(3): p. 259-65.

[8] Imai, J; Yashiroda, H; Maruya, M; Yahara, I; Tanaka, K. Proteasomes and molecular chaperones: cellular machinery responsible for folding and destruction of unfolded proteins. *Cell Cycle*, 2003. 2(6): p. 585-90.

[9] Soti, C; Csermely, P. Molecular chaperones and the aging process. *Biogerontology*, 2000. 1(3): p. 225-33.

[10] Lebret, T; Watson, RW; Fitzpatrick, JM. Heat shock proteins: their role in urological tumors. *J Urol*, 2003. 169(1): p. 338-46.

[11] Richardson, A; Landry, SJ; Georgopoulos, C. The ins and outs of a molecular chaperone machine. *Trends Biochem Sci*, 1998. 23(4): p. 138-43.

[12] Voos, W; Rottgers, K. Molecular chaperones as essential mediators of mitochondrial biogenesis. *Biochim Biophys Acta*, 2002. 1592(1): p. 51-62.

[13] Dubaquie, Y; Looser, R; Rospert, S. Significance of chaperonin 10-mediated inhibition of ATP hydrolysis by chaperonin 60. *Proc Natl Acad Sci U S A*, 1997. 94(17): p. 9011-6.

[14] Zhang, L; Pelech, S; Uitto, VJ. Long-term effect of heat shock protein 60 from Actinobacillus actinomycetemcomitans on epithelial cell viability and mitogen-activated protein kinases. *Infect Immun*, 2004. 72(1): p. 38-45.

[15] Pochon, NA; Mach, B. Genetic complexity of the human hsp 60 gene. *Int Immunol*, 1996. 8(2): p. 221-30.

[16] Hohfeld, J; Hartl, FU. Role of the chaperonin cofactor Hsp10 in protein folding and sorting in yeast mitochondria. *J Cell Biol*, 1994. 126(2): p. 305-15.

[17] Kaufman, BA; Kolesar, JE; Perlman, PS; Butow, RA. A function for the mitochondrial chaperonin Hsp60 in the structure and transmission of mitochondrial DNA nucleoids in Saccharomyces cerevisiae. *J Cell Biol*, 2003. 163(3): p. 457-61.

[18] Samali, A; Orrenius, S. Heat shock proteins: regulators of stress response and apoptosis. *Cell Stress Chaperones*, 1998. 3(4): p. 228-36.

[19] Samali, A; Zhivotovsky, B; Jones, DP; Orrenius, S. Detection of pro-caspase-3 in cytosol and mitochondria of various tissues. *FEBS Lett*, 1998. 431(2): p. 167-9.

[20] 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(9): p. 1135-43.

[21] Di Felice, V; Ardizzone, N; Marciano, V; Bartolotta, T; Cappello, F; Farina, F; Zummo, G. Senescence-associated HSP60 expression in normal human skin fibroblasts. *Anat Rec A Discov Mol Cell Evol Biol*, 2005. 284(1): p. 446-53.

[22] Miyata, Y; Akashi, M; Nishida, E. Molecular cloning and characterization of a novel member of the MAP kinase superfamily. *Genes Cells*, 1999. 4(5): p. 299-309.

[23] Miyata, Y; Ikawa, Y; Shibuya, M; Nishida, E. Specific association of a set of molecular chaperones including HSP90 and Cdc37 with MOK, a member of the mitogen-activated protein kinase superfamily. *J Biol Chem*, 2001. 276(24): p. 21841-8.

[24] Macario, AJ; Conway de Macario, E. Sick chaperones, cellular stress, and disease. *N Engl J Med*, 2005. 353(14): p. 1489-501.

[25] Ciocca, DR; Adams, DJ; Edwards, DP; Bjercke, RJ; McGuire, WL. Distribution of an estrogen-induced protein with a molecular weight of 24,000 in normal and malignant human tissues and cells. *Cancer Res*, 1983. 43(3): p. 1204-10.

[26] Ferrarini, M; Heltai, S; Zocchi, MR; Rugarli, C. Unusual expression and localization of heat-shock proteins in human tumor cells. *Int J Cancer*, 1992. 51(4): p. 613-9.

[27] Morimoto, RI. Heat shock: the role of transient inducible responses in cell damage, transformation, and differentiation. *Cancer Cells*, 1991. 3(8): p. 295-301.

[28] Fuller, KJ; Issels, RD; Slosman, DO; Guillet, JG; Soussi, T; Polla, BS. Cancer and the heat shock response. *Eur J Cancer*, 1994. 30A(12): p. 1884-91.

[29] Garrido, C; Gurbuxani, S; Ravagnan, L; Kroemer, G. Heat shock proteins: endogenous modulators of apoptotic cell death. *Biochem Biophys Res Commun*, 2001. 286(3): p. 433-42.

[30] Nanbu, K; Konishi, I; Mandai, M; Kuroda, H; Hamid, A; Komatsu, T; Mori, T. Prognostic significance of heat shock proteins HSP70 and HSP90 in endometrial carcinomas. *Cancer Detect Prev*, 1998. 22(6): p. 549-555.

[31] Jameel, A; Skilton, RA; Campbell, TA; Chander, SK; Coombes, RC; Luqmani, YA. Clinical and biological significance of HSP89 alpha in human breast cancer. *Int J Cancer*, 1992. 50(3): p. 409-15.

[32] Yufu, Y; Nishimura, J; Nawata, H. High constitutive expression of heat shock protein 90 alpha in human acute leukemia cells. *Leuk Res*, 1992. 16(6-7): p. 597-605.

[33] Hsu, PL; Hsu, SM. Abundance of heat shock proteins (hsp89, hsp60, and hsp27) in malignant cells of Hodgkin's disease. *Cancer Res*, 1998. 58(23): p. 5507-13.

[34] Ciocca, DR; Calderwood, SK. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cell Stress Chaperones*, 2005. 10(2): p. 86-103.

[35] Cappello, F; David, S; Ardizzone, N; Rappa, F; Marasà, L; Bucchieri, F; Zummo, G. Expression of Heat Shock Proteins Hsp10, Hsp27, Hsp60, Hsp70 and Hsp90 in Urothelial Carcinoma of Urinary Bladder. *J Cancer Molec*, 2006. in press.

[36] Cappello, F; Ribbene, A; Campanella, C; Czarnecka, AM; Anzalone, R; Bucchieri, F; Palma, A; Zummo, G. The value of immunohistochemical research on PCNA, p53 and heat shock proteins in prostate cancer management: a review. *Eur J Histochem*, 2006. 50(1): p. 25-34.

[37] Vinay Kumar, M, MD, FRCPath, Abul K. Abbas, MD, MBBS and Nelson Fausto, MD. *Robbins & Cotran Patologia Humana*. Seventh Edition ed. 2005: Elsevier Saunders.

[38] Cappello, F. HSP60 and HSP10 as diagnostic and prognostic tools in the management of exocervical carcinoma. *Gynecol Oncol*, 2003. 91(3): p. 661.

[39] 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(1): p. 35-41.

[40] 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 over-expressed during colorectal carcinogenesis. *Eur J Histochem*, 2003. 47(2): p. 105-10.

[41] Cappello, F; Bellafiore, M; Palma, A; Marciano, V; Martorana, G; Belfiore, P; Martorana, A; Farina, F; Zummo, G; Bucchieri, F. Expression of 60-kD heat shock

protein increases during carcinogenesis in the uterine exocervix. *Pathobiology*, 2002. 70(2): p. 83-8.

[42] 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: p. 139.

[43] 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(2B): p. 1325-31.

[44] Cappello, F; Zummo, G. HSP60 expression during carcinogenesis: a molecular "proteus" of carcinogenesis? *Cell Stress Chaperones*, 2005. 10(4): p. 263-4.

[45] Cappello, F; Zummo, G. HSP60 expression during carcinogenesis: where is the pilot? *Pathol Res Pract*, 2006. 202(5): p. 401-2.

[46] 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(10): p. 816.

[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(18): p. 2804-11.

[48] Czarnecka, A; Campanella, C; Zummo, G; Cappello, F. Mitochondrial chaperones in cancer. *Cancer Biol Ther*, 2006. in press.

[49] Ghobrial, IM; McCormick, DJ; Kaufmann, SH; Leontovich, AA; Loegering, DA; Dai, NT; Krajnik, KL; Stenson, MJ; Melhem, MF; Novak, AJ; Ansell, SM; Witzig, TE. Proteomic analysis of mantle-cell lymphoma by protein microarray. *Blood*, 2005. 105(9): p. 3722-30.

[50] Hayflick, L. The Limited in Vitro Lifetime of Human Diploid Cell Strains. *Exp Cell Res*, 1965. 37: p. 614-36.

[51] Hayflick, L. The illusion of cell immortality. *Br J Cancer*, 2000. 83(7): p. 841-6.

[52] Campisi, J. From cells to organisms: can we learn about aging from cells in culture? *Exp Gerontol*, 2001. 36(4-6): p. 607-18.

[53] Raffetto, JD; Leverkus, M; Park, HY; Menzoian, JO. Synopsis on cellular senescence and apoptosis. *J Vasc Surg*, 2001. 34(1): p. 173-7.

[54] Cristofalo, VJ; Pignolo, RJ. Molecular markers of senescence in fibroblast-like cultures. *Exp Gerontol*, 1996. 31(1-2): p. 111-23.

[55] Seluanov, A; Gorbunova, V; Falcovitz, A; Sigal, A; Milyavsky, M; Zurer, I; Shohat, G; Goldfinger, N; Rotter, V. Change of the death pathway in senescent human fibroblasts in response to DNA damage is caused by an inability to stabilize p53. *Mol Cell Biol*, 2001. 21(5): p. 1552-64.

[56] Sreedhar, AS; Csermely, P. Heat shock proteins in the regulation of apoptosis: new strategies in tumor therapy: a comprehensive review. *Pharmacol Ther*, 2004. 101(3): p. 227-57.

[57] Zhang, J; Patel, JM; Block, ER. Enhanced apoptosis in prolonged cultures of senescent porcine pulmonary artery endothelial cells. *Mech Ageing Dev*, 2002. 123(6): p. 613-25.

[58] Weyand, CM; Goronzy, JJ. Stem cell aging and autoimmunity in rheumatoid arthritis. *Trends Mol Med*, 2004. 10(9): p. 426-33.

[59] Minamino, T; Miyauchi, H; Yoshida, T; Tateno, K; Komuro, I. The role of vascular cell senescence in atherosclerosis: antisenescence as a novel therapeutic strategy for vascular aging. *Curr Vasc Pharmacol*, 2004. 2(2): p. 141-8.

[60] Bailey, SM; Murnane, JP. Telomeres, chromosome instability and cancer. *Nucleic Acids Res*, 2006. 34(8): p. 2408-17.

[61] von Zglinicki, T; Martin-Ruiz, CM. Telomeres as biomarkers for ageing and age-related diseases. *Curr Mol Med*, 2005. 5(2): p. 197-203.

[62] Akbar, AN; Fletcher, JM. Memory T cell homeostasis and senescence during aging. *Curr Opin Immunol*, 2005. 17(5): p. 480-5.

[63] Ben-Porath, I; Weinberg, RA. When cells get stressed: an integrative view of cellular senescence. *J Clin Invest*, 2004. 113(1): p. 8-13.

[64] Bryan, TM; Englezou, A; Gupta, J; Bacchetti, S; Reddel, RR. Telomere elongation in immortal human cells without detectable telomerase activity. *Embo J*, 1995. 14(17): p. 4240-8.

[65] Loeb, LA; Wallace, DC; Martin, GM. The mitochondrial theory of aging and its relationship to reactive oxygen species damage and somatic mtDNA mutations. *Proc Natl Acad Sci U S A*, 2005. 102(52): p. 18769-70.

[66] Kang, MK; Kim, RH; Shin, KH; Zhong, W; Faull, KF; Park, NH. Senescence-associated decline in the intranuclear accumulation of hOGG1-alpha and impaired 8-oxo-dG repair activity in senescing normal human oral keratinocytes in vivo. *Exp Cell Res*, 2005. 310(1): p. 186-95.

[67] Kang, MK; Kameta, A; Shin, KH; Baluda, MA; Kim, HR; Park, NH. Senescence-associated genes in normal human oral keratinocytes. *Exp Cell Res*, 2003. 287(2): p. 272-81.

[68] Lundberg, AS; Hahn, WC; Gupta, P; Weinberg, RA. Genes involved in senescence and immortalization. *Curr Opin Cell Biol*, 2000. 12(6): p. 705-9.

[69] Campisi, J. Cancer, aging and cellular senescence. *In Vivo*, 2000. 14(1): p. 183-8.

[70] Campisi, J. Aging, tumor suppression and cancer: high wire-act! *Mech Ageing Dev*, 2005. 126(1): p. 51-8.

[71] Michaloglou, C; Vredeveld, LC; Soengas, MS; Denoyelle, C; Kuilman, T; van der Horst, CM; Majoor, DM; Shay, JW; Mooi, WJ; Peeper, DS. BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature*, 2005. 436(7051): p. 720-4.

[72] Aliouat-Denis, CM; Dendouga, N; Van den Wyngaert, I; Goehlmann, H; Steller, U; van de Weyer, I; Van Slycken, N; Andries, L; Kass, S; Luyten, W; Janicot, M; Vialard, JE. p53-independent regulation of p21Waf1/Cip1 expression and senescence by Chk2. *Mol Cancer Res*, 2005. 3(11): p. 627-34.

[73] Itahana, K; Dimri, G; Campisi, J. Regulation of cellular senescence by p53. *Eur J Biochem*, 2001. 268(10): p. 2784-91.

[74] Zhang, HS; Postigo, AA; Dean, DC. Active transcriptional repression by the Rb-E2F complex mediates G1 arrest triggered by p16INK4a, TGFbeta, and contact inhibition. *Cell*, 1999. 97(1): p. 53-61.

[75] Itahana, K; Campisi, J; Dimri, GP. Mechanisms of cellular senescence in human and mouse cells. *Biogerontology*, 2004. 5(1): p. 1-10.

[76] Ishikawa, F. Cellular senescence, an unpopular yet trustworthy tumor suppressor mechanism. *Cancer Sci*, 2003. 94(11): p. 944-7.

[77] Bringold, F; Serrano, M. Tumor suppressors and oncogenes in cellular senescence. *Exp Gerontol*, 2000. 35(3): p. 317-29.

[78] Durand, B; Gao, FB; Raff, M. Accumulation of the cyclin-dependent kinase inhibitor p27/Kip1 and the timing of oligodendrocyte differentiation. *Embo J*, 1997. 16(2): p. 306-17.

[79] Mazars, GR; Jat, PS. Expression of p24, a novel p21Waf1/Cip1/Sdi1-related protein, correlates with measurement of the finite proliferative potential of rodent embryo fibroblasts. *Proc Natl Acad Sci U S A*, 1997. 94(1): p. 151-6.

[80] Kaul, Z; Yaguchi, T; Kaul, SC; Wadhwa, R. Quantum dot-based protein imaging and functional significance of two mitochondrial chaperones in cellular senescence and carcinogenesis. *Ann N Y Acad Sci*, 2006. 1067: p. 469-73.

[81] Di Felice, V; David, S; Cappello, F; Farina, F; Zummo, G. Is chlamydial heat shock protein 60 a risk factor for oncogenesis? *Cell Mol Life Sci*, 2005. 62(1): p. 4-9.

[82] Piotrowicz, RS; Weber, LA; Hickey, E; Levin, EG. Accelerated growth and senescence of arterial endothelial cells expressing the small molecular weight heat-shock protein HSP27. *Faseb J*, 1995. 9(11): p. 1079-84.

[83] Holt, SE; Aisner, DL; Baur, J; Tesmer, VM; Dy, M; Ouellette, M; Trager, JB; Morin, GB; Toft, DO; Shay, JW; Wright, WE; White, MA. Functional requirement of p23 and Hsp90 in telomerase complexes. *Genes Dev*, 1999. 13(7): p. 817-26.

[84] Soti, C; Csermely, P. Chaperones come of age. *Cell Stress Chaperones*, 2002. 7(2): p. 186-90.

[85] Nylandsted, J; Rohde, M; Brand, K; Bastholm, L; Elling, F; Jaattela, M. Selective depletion of heat shock protein 70 (Hsp70) activates a tumor-specific death program that is independent of caspases and bypasses Bcl-2. *Proc Natl Acad Sci U S A*, 2000. 97(14): p. 7871-6.

[86] Lim, CS. Cellular senescence, cancer, and organismal aging: a paradigm shift. *Biochem Biophys Res Commun*, 2006. 344(1): p. 1-2.

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