Heat shock protein 10 and signal transduction: a "capsula eburnea" of carcinogenesis?

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Abstract To date, little is known either about the physical interactions of heat shock protein 10 (Hsp10) with other proteins within the cell or its involvement in signal transduction pathways. Hsp10 has been considered mainly as a partner of Hsp60 in the Hsp60/10 protein folding machine. Only recently, Hsp10 was reported to interact with proteins involved in deoxyribonucleic acid checkpoint inactivation, termination of M-phase, messenger ribonucleic acid export, import of nuclear proteins, nucleocytoplasmic transport, and pheromone signaling pathways. At the same time, Hsp10 expression can be up-regulated in cancer cells, because it accumulates as the cell transformation progresses. Recent data suggest that Hsp10 may be not only a component of the folding machine but also an active player of the cell signaling network, influencing cell cycle, nucleocytoplasmic transport, and metabolism, with putative roles in the lack of cell differentiation and in the inhibition of apoptosis. In this review, we revise the involvement of Hsp10 in signal transduction pathways and its possible role in cancer etiology.

INTRODUCTION

To date, heat shock protein 10 (Hsp10) (HSPE1, CPN10, GROES, HSP10) has been considered only as a partner of Hsp60 in the Hsp60/10 protein folding machine. The chaperonin complex structure was elucidated at high resolution, providing data for functional analysis (Xu et al 1997), and detailed descriptions of the chaperonin-mediated folding cycle have also been proposed (Bukau and Horwich 1998; Walter and Buchner 2002). The Hsp60/10 complex is believed to be responsible for accelerating the folding of polypeptides imported into mitochondria, as well as reactivation of denaturated proteins, and diminishing aggregation of nonnative polypeptides and partially unfolded kinetically trapped intermediates. Its potential to smooth the energy landscape necessary for the folding and to prevent intermolecular interactions between nonnative polypeptides has been widely investigated (Ziemienowicz et al 1993; Weissman et al 1995; Bukau and Horwich 1998; Brinker et al 2001; Walter and Buchner 2002).

Hsp10 has been proven to be an essential component

of the protein folding apparatus. Indeed, Hsp10 participates in protein complexes assembly and intra-mitochondrial sorting (Hohfeld and Hartl 1994). Hsp10 binds Hsp60 in the presence of Mg-adenosine triphosphate and suppresses the adenosine triphosphatase (ATPase) activity of the latter. It also participates in the encapsulation of the substrate. The molecular mechanism of Hsp10 chaperone action has been examined (Richardson et al 2001; Gomez-Puertas et al 2004) and functional analyses have proven the necessity of both chaperonins for the biogenesis of mitochondria (Ryan et al 1997).

MANY FACES OF Hsp10

Hsp10 is encoded by a nuclear gene (GeneID, 3336; gene map locus, 2q33.1), translated in the cytoplasm, and transported into mitochondria (Hansen et al 2003). Although in normal cells Hsp10 is mostly localized in the mitochondrial matrix, it has also been found in other subcellular localizations, such as zymogene granules, hormone granules, secretory granules, and mature red blood cells (Sadacharan et al 2001). High levels of Hsp10 in the cytoplasm of cancer cells have been reported in the past (Sadacharan et al 2001; Cappello et al 2003a, 2003b; Han-

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Fig 1. The expression of Hsp10 in normal human colon is lower (a) than in colon cancer (b) and lymph node metastases of colon cancer (c). Immunohistochemistry of tissues delivered by biopsies; red spots represent Hsp10. Magnification, $40 \times$.

sen et al 2003) (Fig 1). The mechanism by which Hsp10 is retained or sequestered in the cytoplasm is not known yet. It could be hypothesized that Hsp10 is accumulated in the cytoplasm directly after the translation or that first it enters into the mitochondria and it is then translocated back into the cytoplasm. Also, Hsp60 has been shown to be overexpressed and localized in the cytoplasm of cancer cells (Cappello et al 2003b, 2005). Nevertheless, Hsp60 may also be down-regulated during bronchial (Cappello et al 2005) and vesical (Cappello et al 2006) carcinogenesis. The finding of a discrepancy between Hsp60 and Hsp10 expression in normal (Cappello et al 2004) and tumoral (Cappello et al 2003c) cells permits the postulation that Hsp10 may play other roles apart from cochaperonin.

The first nonfolding Hsp10 activity to be identified was relative to the function of the early pregnancy factor (EPF) (Summers et al 1998). EPF was identified in 1974 as a factor associated with pregnancy serum, which was active in the rosette inhibition test (Morton et al 1974). Since then, it was characterized to enter the serum and urine of pregnant women during the first and second trimesters (Morton et al 1977) and to be the principal immunosuppressive agent during pregnancy (Morton et al 1992). Recently, EPF has been shown to be an extracellular homolog of the heat shock protein, Cpn10 (Summers et al 1996; Morton 1998). Further experiments have shown that Cpn10 is a potent stimulator of osteoclast recruitment and bone resorption (Summers et al 1996; Meghji et al 1997; Morton 1998). Interestingly additional studies of other heat shock proteins have found that most of the chaperones (groEL, groES, dnaK, and human hsp27, hsp70, and hsp90) are capable of eliciting a bone-resorptive response (Nair et al 1999). In addition to contributing to bone resorption, Hsp10 was also suggested to have a role in bone collagen synthesis (Black et al 2000), because type I collagen synthesis is significantly increased when osteoblasts are treated with Cpn10 (Mansell et al 2002).

EPF has also been shown to suppress the delayed-type hypersensitivity response in mice as well as acute and chronic forms of experimental autoimmune encephalomyelitis in rats and mice. In a human model, it has been shown that EPF binds specifically to CD4⁺, CD8⁺, CD14⁺ (monocytes), and CD56⁺ natural killer cells, and its suppressive effects are driven by inhibition of CD4⁺ T cell activity (Athanasas-Platsis et al 2004). At the same time,



Fig 3. Hsp10 (upper panel) and Hsp60 (lover panel) overexpression in cancer (red denotes high expression): pink, breast cancer; violet, prostate cancer; blue, non–small-cell lung cancer; gray, central nervous system; light green, renal cancer; orange, ovarian cancer; red, leukemia; dark green, colon cancer; brown, melanoma (Ross et al 2000). EPF is known to stimulate the proliferation of many different cells (Cavanagh and Morton 1994). Hsp10 immunosuppressive activity was also detected in sera of ovarian cancer patients. Both sera and ascites of patients with ovarian cancer contained Hsp10, whereas it was not detectable in controls. These sera, containing Hsp10, suppressed T cell CD3- ζ expression, a key component of T cell activation. Moreover, this phenomenon correlated with Hsp10 levels. When Hsp10 was removed from the sera, the serum ability to suppress CD3- ζ was diminished but the immunoprecipitated material was capable of suppressing CD3- ζ . The authors suggested that Hsp10 may be a critical factor in the suppression of T cell activation, allowing the tumor to escape immune surveillance (Akyol et al 2005).

In studies on liver regeneration after partial hepatectomy, Hsp10-EPF was found in large amounts in serum 8 hours after the surgery. If Hsp10 was neutralized with antibodies (passive immunization), a significant decrease in the uptake of [3H]thymidine by the liver remnant measured 4 to 6 hours later was reported. It was proposed that EPF is selectively released from proliferating cells and, in an autocrine or paracrine mode (or both), is involved in deoxyribonucleic acid (DNA) synthesis (Quinn et al 1994). Moreover, Hsp10 has been defined as a potential significant marker in the diagnostics of gestational trophoblastic tumor. A clinical study has demonstrated that diagnosis of malignant trophoblastic tumor can be made with an accuracy of 91.3% by detecting Hsp10-EPF-like activity and that it can also be used as an indicator to distinguish benign from malignant trophoblastic tumor (Fan et al 1999).

EPF has also been characterized as a marker of other types of trophoblastic tumor (invasive mole and choriocarcinoma) (Bojahr et al 1993a), endodermalsinus tumor of the ovary, and rhabdomyosarcoma, as well as adrenal cortex carcinoma (Bojahr et al 1993b), ovarian cancer (Akyol et al 2005), and germ cell tumor of the testis (Rolfe et al 1983).

The production of EPF by a variety of cultured transformed and tumor cell lines, of both germ and non–germ cell origin has been reported (Quinn et al 1990; Akyol et al 2005). Hsp10 production has been found to be associated with cell division, because it was no longer detected after growth arrest or differentiation. Moreover, coculture of tumor cells with increasing doses of anti-EPF monoclonal antibodies resulted in a significant, dose-dependent decrease in the rates of cell growth and viability (Quinn et al 1990). Further neutralization studies with monoclonal antibodies specific for EPF have shown that these antibodies are able to limit the growth of 2 murine tumor lines. Tumors injected with anti-EPF showed significant decrease in the uptake of [³H]thymidine into tumor tissue, and the titration of sera for active EPF showed that a significant reduction in the EPF titer was associated with a significant inhibition of tumor DNA synthesis. It has also been demonstrated that neutralization of EPF retards tumor growth both in vitro and in vivo (Quinn and Morton 1992).

Although many clinical studies have been reported, to date little is known either about the physical interactions of Hsp10 with other proteins within the cell, or its involvement in signal transduction pathways. Most of the literature describes a well-known interaction between Hsp10 and Hsp60 in a barrel-like structure chaperone machine involved in protein folding (Ryan et al 1997; Bukau and Horwich 1998; Brinker et al 2001). This interaction has been widely studied in Escherichia coli, Saccharomyces cerevisiae, and human cells (Ranford et al 2000; Walter and Buchner 2002). Hsp10 has been reported to interact with nuclear pore complex proteins such as NUP2 (ylr335w) or PSE1 (ymr308c), but also to interplay with both a guanine triphosphate–binding protein of the Ras superfamily involved in termination of M-phase-TEM1 (yml064c) and a protein phosphatase involved in DNA checkpoint inactivation-PTC3 (ybl056w) (Table 1) (Uetz et al 2000). In a recent analysis by Stelzl et al (2005), new interactions of Hsp10 in human cells have been reported. The first protein shown to interact with Hsp10 is CRMP1 (DPYSL1) hihydropyrimidinase related protein-1. Collapsin response mediator protein 1 is involved in nervous system development and also in nucleobase, nucleoside, nucleotide, and nucleic acid metabolism (Hamajima et al 1996). The other protein is ERG28 (hspc288), an integral membrane protein localized in endoplasmic reticulum, involved in lipid synthesis, steroid biosynthesis, and sterol biosynthesis. ERG28 is a ubiquitous protein, strongly expressed in testis and some cancer cell lines (Veitia et al 1999). The third protein identified by Stelzl is APLP1 (amyloid precursor-like protein 1), which may play a role in postsynaptic function and which possibly interacts with cellular G protein signaling pathways and can regulate neurite outgrowth through binding to components of the extracellular matrix such as heparin and collagen. Its γ ctf peptide, c30, is a potent enhancer of neuronal apoptosis (Kajkowski et al 2001). Another protein reported to interact with Hsp10 is TLE1-transducin-like enhancer protein 1, a transcriptional corepressor that binds to a number of transcription factors, inhibits NF-KB-regulated gene expression, and inhibits the transcriptional activation mediated by WNT signaling (Levanon et al 1998). TLE 1 is located in the nuclei where it is chromatin-associated and interacts with histone H3 (Palaparti et al 1997). This protein was reported to be involved in signal transduction and organ morphogenesis (Liu et al 1996). The last protein associated with Hsp10 is SAT1-diamine acetyltransferase 1 (EC 2.3.1.57), a spermidine/spermine N^1 -acetyltransferase-1, putrescine acetyltransferase, and

 Table 1
 Yeast (upper panel) and human (lower panel) Hsp10 interactors revealed by proteome analysis

Interactor	Function
PTC3	Type 2C protein phosphatase; dephosphorylates Hog1p to limit maximal kinase activity induced by osmotic stress; dephosphorylates T169 phos- phorylated Cdc28p (see also Ptc2p); role in DNA checkpoint inactivation.
TEM1	GTP-binding protein of the RAS superfamily in- volved in termination of M-phase; controls acto- myosin and sentin dynamics during cytokinesis.
PRE8 NUP116	20S proteasome β-type subunit. Subunit of the nuclear pore complex (NPC) that is localized to both sides of the pore; contains a re- petitive GLFG motif that interacts with mRNA ex- port factor Mex67p and with karyopherin Kap95p; homologous to Nun100n
MDG1	Plasma membrane protein involved in G protein- mediated pheromone signaling pathway; overpro- duction suppresses bem1 mutations
SRP1	Karyopherin α homolog, forms a dimer with kary- opherin β Kap95p to mediate import of nuclear proteins, binds the nuclear localization signal of the substrate during import; may also play a role in regulation of protein degradation
NUP2	Protein involved in nucleocytoplasmic transport, binds to either the nucleoplasmic or cytoplasmic faces of the nuclear pore complex depending on Ran-GTP levels; also has a role in chromatin or-
PSE1	Karyopherin/importin that interacts with the nuclear pore complex; acts as the nuclear import recep- tor for specific proteins, including Pdr1p, Yap1p, Ste12p, and Aft1p.
CRMP1	Protein involved in nervous system development, nucleobase, nucleoside, nucleotide and nucleic
ERG28	Endoplasmic reticulum-bound protein involved in lipid synthesis, steroid biosynthesis, sterol bio- synthesis
APLP1	Protein that may interact with cellular G protein sig- naling pathways, regulate neurite outgrowth through binding to components of the extracellu- lar matrix such as heparin and collagen; involved also in apoptosis, cell adhesion, and endocytosis.
TLE1	Protein involved in signal transduction, organ mor- phogenesis, regulation of transcription, Wnt re- ceptor signaling pathway, and interacts with his- tone H3
SAT1	Enzyme that catalyzes the acetylation of poly- amines; also involved in the regulation of poly- amine transport out of cells.

polyamine *N*-acetyltransferase 1, an enzyme that catalyzes the acetylation of polyamines. This highly regulated enzyme allows a fine attenuation of the intracellular concentration of polyamines and is also involved in the regulation of polyamine transport out of the cells (Xiao et al 1992). Its induction was found in human large cell lung carcinoma (Casero et al 1990). The expression of SAT is regulated by a number of factors, including the natural polyamines and their analogs. The phenotype-specific cytotoxicity that occurs in response to a class of polyamine analogues, the diethylpolyamines, is associated with a phenotype-specific superinduction of SAT in human nonsmall-cell lung carcinomas, whereas in nonresponding cell types, including the small-cell lung carcinomas, the superinduction of SSAT does not occur (Xiao and Casero 1996). All interactions reported by Stelzl do not provide a straightforward explanation for the function of Hsp10, because the functional and structural significance of the reported interactions have not been provided. Nevertheless, Hsp10 action on nonmitochondrial proteins may suggest new functions, outside of the organellum (Stelzl et al 2005).

Two recent reports by Lin et al suggest interaction of human Hsp10 with Ras guanine triphosphatase (GTPase) pathway in myocyte protection after ischemia/reoxygenation insult (Lin et al 2004). This is consistent with previously reported interaction of S cerevisiae Hsp10 with TEM1 (yml064c) (Uetz et al 2000; Lin et al 2004). At the same time, the process of apoptosis following heart muscle hypoxia/reoxygenation has been widely studied. A prominent role has been attributed to Bcl-2/Bax proteins, reactive oxygen species, and mitochondria, but also Janus kinase/signal transducer and activator of transcription and mitogen-activated protein kinase cascades have been recognized as key players in this process (Fryer et al 2001; Gill et al 2002; Krijnen et al 2002). Taking together the Hsp10 and hypoxia reports, it is tempting to postulate the interference of Hsp10 with signaling pathways, playing a crucial role in antiapoptotic protection (Fig 2), which is supported by the involvement of Hsp10 in postischemic cell viability.

HSPs have been proven to have the potential of inhibiting apoptosis and arresting it even if the process is already activated, also in myocardial muscle after hypoxia (Kabakov and Gabai 1997; Preville et al 1999). Most of the research in this field has been focused on Hsp70 and small Hsp families. Hsp72 has been proven to inhibit apoptosis by acting downstream of caspase-3, apoptotic protease activating factor 1 binding, and preventing c-Jun N-terminal kinase activation or inhibiting apoptosis-inducing factor (AIF)-mediated apoptosis, and many others (Gabai et al 1998; Jäättelä et al 1998, 1999; Creagh et al 2000). At the same time, Hsp27 has been shown to protect against programmed cell death triggered by Fas/Apo-1/ CD95 death receptor, oxidative stress, or staurosporine, due to its cytoprotective potential and capacity to prevent apoptosome formation, cytochrome *c* sequestration, and interference with caspase-3 action (Jäättelä 1999; Jolly and Morimoto 2000; Parcellier et al 2003). Overexpression of those proteins has been proven to reduce apoptosis. To date, little attention has been given to the Hsp60/Hsp10 complex in this context. It has only been proven that combined and individual overexpression of chaperonins may protect cells from ischemia-reoxygenation induced cell death. It has also been shown that Hsp10/60 accumula-



Fig 2. Schematic representation of Hsp10/60 roles in apoptosis: (up-down; left-right) Hsp10 interaction with RAF signaling cascade (overexpressed Hsp10 inactivates proapoptotic Raf and ERK and promotes cell survival); Hsp60-Bax interaction in cytoplasm (reduces the protein content of the proapoptotic Bax in mitochondria); Hsp10 interaction with nuclear pores (significance not known); Hsp60/10 machine within mitochondria (involved in protein folding as Hsp60 partner) and in apoptosis (with Hsp60 facilitating caspase-3 maturation); the role of p53, AIF apoptosome, caspase-9, and caspase-3 is shown.

tion is caused by increased transcription and translation of the protein, because Hsp10 messenger ribonucleic acid is induced following global brain ischemia (Hickey et al 2000; Suzuki et al 2000; Lin et al 2001; Ray et al 2001). In the myocardium of patients with chronic atrial fibrillation, the expression of the mitochondrial heat shock proteins Hsp60 and Hsp10 is increased (Schafler et al 2002; Kirmanoglou et al 2004); similar to what happens in the brainstem after subarachnoid hemorrhage (Satoh et al 2003). It has been elucidated that myocyte protection by Hsp10 involves the mobile loop and attenuation of the Ras GTPase pathway (Lin et al 2004). This result is consistent with Hsp10-TEM1 (yml064c) interaction in yeast (Ito et al 2001). Hsp10 overexpression has been shown to inactivate Raf, extracellular signal-regulated kinase (ERK), and p90Ribosomal kinase (p90RSK), which is followed by increased capacity of electron transport chain complex I and preservation of complex I and II function (Lin et al 2004). Raf and ERK are signaling proteins involved in the execution of the growth factor stimulation of the cells-the Ras/Raf/MEK (mitogen-activated protein kinase) also called ER kinase or ERK. This is the pathway that lies at the heart of the signaling networks that govern proliferation, differentiation, and cell survival. Raf is a Ser/Thr protein kinase that catalyzes the phosphorylation of hydroxyl groups on specific Ser and Thr residues (Chong et al 2003). Phosphorylated (active) Raf activates in turn mitogen-activated protein kinase (MAPK)/ERK kinase 1 (MEK1) that activates another protein known as MAPK1 or the MAP kinases ERK1. Upon phosphorylation, nuclear translocation of ERK1 and

ERK2 is critical for both gene expression and DNA replication induced by growth factors. In the nucleus, ERK phosphorylates an array of targets, including transcription factors and a family of RSK-related kinases, the mitogen- and stress-activated protein kinases (MSKs) (Deak et al 1998). This pathway is also involved in maintaining cell survival by modulating the activity of apoptotic molecules including Bad and Bcl-2 (Chang et al 2003a). Depending on the stimulus and cell type, this pathway may transmit signals, which result in the induction of apoptosis or cell survival. This is a complex pathway comprised of multiple members of the kinase and transcription factor families that heterodimerize to transmit different signals. Furthermore, additional signal transduction pathways interact with the Raf/MEK/ERK pathway to regulate positively or negatively its activity, and Hsp10 seems to be one of these molecules, inhibiting the proapoptotic activity and shifting the balance towards cell survival (Lin et al 2001; Chang et al 2003b; Lin et al 2004) (Fig 2).

Additionally, it has been demonstrated that Hsp10 and Hsp60 modulate the Bcl-2 family and mitochondria apoptosis signaling in cardiac muscle cells. According to the studies of Shan and colleagues, the overexpression of Hsp10 increases the abundance of antiapoptotic Bcl-2 and Bcl-xl (Bcl-x protein long isoform), reduces the protein content of the proapoptotic Bax (Bcl-2-associated X protein), but does not alter the expression of Bad (Bcl-2 antagonist of cell death); the overexpression of Hsp10 and Hsp60 stabilizes mitochondrial cross-membrane potential, inhibits caspase-3, and suppresses poly(5'-diphosphate-ribose) polymerase (Shan et al 2003a) (Fig 2).

In the past, we and others have reported the overexpression of Hsp10 in a variety of tumors and pretumoral lesions, such as large bowel cancer and exocervical cancer (Cappello et al 2003a), prostate cancer (Cappello et al 2003c), and mantle cell lymphoma (Ghobrial et al 2005) (Figs 1, 3). Also, the localization of the protein differs between normal and cancer cells. In normal cells, Hsp10 is mostly localized in the mitochondrial matrix, whereas tumor cells present a high accumulation of Hsp10 within the cytoplasm. The role of Hsp10 retained in the cytoplasm is not known yet. We postulate that Hsp10 influences the process of programmed cell death. Indeed, only a few papers discuss the possible involvement of Hsp10 in apoptosis. A first insight into this topic came from the research by Samali et al (1999), who demonstrated an interaction of the Hsp60/10 machine with procaspase-3 in the mitochondria of Jurkat cells and the disruption of this complex when active caspase fragments are released from the intermembranous space of mitochondria, which is followed by the execution phase of cell death. This supports the hypothesis that Hsp60/10 interaction could determine acceleration of caspase-3 maturation (Samali et al 1999) (Fig 2).

The possibility that Hsp10 could influence the function of signaling proteins is further supported by the study of Shan et al (2003b). In their work, the authors report that Hsp10 acts through posttranslational modification. Overexpression of Hsp10 or Hsp60 is shown to increase the abundance of insulin-like growth factor 1 receptor (IGF-1R) and IGF-1-stimulated receptor autophosphorylation, therefore increasing the number of functioning receptors and amplifying activation of IGF-1R signaling. IGF-1 stimulation of MEK, Erk, p90Rsk, and Akt were accordingly amplified under those conditions. Their experiments also showed that Hsp10 and Hsp60 suppressed polyubiquitination of IGF-1 receptor (Shan et al 2003b). Finally, neuronal vesicular cell trafficking and synaptic plasticity also up-regulate Hsp10 expression (Khawaja et al 2004). Once again, all these data suggest that Hsp10 acts by 2 distinct mechanisms, not only as a molecular chaperone involved in protein stabilization and folding but also as a modulator of proteins in signaling cascades. At the same time, not only proteome analyses have shed new lights on the Hsp10 expression and function but also transcription initiation studies have shown that Rpm2p, a component of yeast mitochondrial ribonuclease P, acts as a Hsp10-transcriptional activator in the nucleus (Stribinskis et al 2005). All the data reported herein seem to confirm that Hsp10 is not only a passive component of folding machine but also an active player of cell signaling network, influencing cell cycle, nucleocytoplasmic transport, and metabolism (Table 1).

CONCLUSION

Our data as well as experiments conducted by other research groups (Ross et al 2000; Shan et al 2003b; Ghobrial et al 2005) show that Hsp10 expression is up-regulated in cancer cells. Hsp10 accumulates as the cell transformation progresses, as confirmed by immunohistochemistry, proteomic analysis by protein microarray, and RNAchip technologies. The abundance of Hsp10 in nondifferentiated cancer cells may suggest that it abrogates differentiation, or inhibits apoptosis. Despite this mechanism, it also seems that Hsp10 overexpression promotes clonal evolution. Hsp10 positivity is selective for myeloid and megakaryocytic lineages. The positivity is restricted to precursor cells, whereas mature elements are constantly negative (Cappello et al 2004). Moreover, Hsp10 and Hsp60 are not expressed at stechiometric levels in cells (Fig 3), although the most prominent action of Hsp10 on ATPase activity of the Hsp60 protein is seen at a 1:1 molar ratio (Chandrasekhar et al 1986). Collected data (Fig 3) might indicate that Hsp10 is overexpressed in cancer cells at a higher rate than Hsp60 and might play a role apart from its protein folding partner. In the light of this, it becomes possible to postulate that Hsp10 plays another role in cell life and death, not only as a chaperone. The data reported here encourage further investigations in this field. We suggest that the cellular function of Hsp10 is not restricted to mitochondrial protein folding and may conceal an important role in diverse cellular processes. The recognition of Hsp10 involvement in signal transduction pathway may be important in understanding chaperonin involvement in cellular biology as well as cancer etiology.

REFERENCES

- Akyol S, Gercel-Taylor C, Reynolds LC, Taylor DD. 2006. HSP-10 in ovarian cancer: expression and suppression of T-cell signaling. *Gynecol Oncol* 101: 481–486.
- Athanasas-Platsis S, Somodevilla-Torres MJ, Morton H, Cavanagh AC. 2004. Investigation of the immunocompetent cells that bind early pregnancy factor and preliminary studies of the early pregnancy factor target molecule. *Immunol Cell Biol* 82: 361–369.
- Black AJ, Topping J, Durham B, Farquharson RG, Fraser WD. 2000. A detailed assessment of alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy. J Bone Miner Res 15: 557–563.
- Bojahr B, Straube W, Reddemann H. 1993a. Case observations on the significance of early pregnancy factor as a tumor marker [in German]. Zentralbl Gynakol 115: 125–128.
- Bojahr B, Straube W, Romer T. 1993b. Early pregnancy factor (EPF) as tumour marker in two patients with trophoblastic tumour. *Arch Gynecol Obstet* 252: 215–218.
- Brinker A, Pfeifer G, Kerner MJ, Naylor DJ, Hartl FU, Hayer-Hartl M. 2001. Dual function of protein confinement in chaperoninassisted protein folding. *Cell* 107: 223–233.
- Bukau B, Horwich AL. 1998. The Hsp70 and Hsp60 chaperone machines. *Cell* 92: 351–666.

- Cappello F, Bellafiore M, David S, Anzalone R, Zummo G. 2003a. Ten kilodalton heat shock protein (HSP10) is overexpressed during carcinogenesis of large bowel and uterine exocervix. *Cancer Lett* 196: 35–41.
- Cappello F, Bellafiore M, Palma A, et al. 2003b. 60 kDa chaperonin (HSP60) is over-expressed during colorectal carcinogenesis. *Eur J Histochem* 47: 105–109.
- Cappello F, David S, Ardizzone N, et al. 2006. Expression of heat shock proteins hsp10, hsp27, hsp60, hsp70 and hsp90 in uro-thelial carcinoma of urinary bladder. *J Can Mol* 2: 73–77.
- Cappello F, Di Stefano A, D'Anna SE, Donner CF, Zummo G. 2005. Immunopositivity of heat shock protein 60 as a biomarker of bronchial carcinogenesis. *Lancet Oncol* 6: 816.
- Cappello F, Rappa F, David S, Anzalone R, Zummo G. 2003c. Immunohistochemical evaluation of PCNA, p53, HSP60, HSP10 and MUC-2 presence and expression in prostate carcinogenesis. *Anticancer Res* 23: 1325–1331.
- Cappello F, Tripodo C, Farina F, Franco V, Zummo G. 2004. HSP10 selective preference for myeloid and megakaryocytic precursors in normal human bone marrow. *Eur J Histochem* 48: 261–265.
- Casero Jr RA, Celano P, Ervin SJ, Wiest L, Pegg AE. 1990. High specific induction of spermidine/spermine N¹-acetyltransferase in a human large cell lung carcinoma. *Biochem J* 270: 615–620.
- Cavanagh AC, Morton H. 1994. The purification of early-pregnancy factor to homogeneity from human platelets and identification as chaperonin 10. *Eur J Biochem* 222: 551–560.
- Chandrasekhar GN, Tilly K, Woolford C, Hendrix R, Georgopoulos C. 1986. Purification and properties of the groES morphogenetic protein of *Escherichia coli*. *J Biol Chem* 261: 12414–12419.
- Chang F, Steelman LS, Lee JT, et al. 2003a. Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention. *Leukemia* 17: 1263–1293.
- Chang F, Steelman LS, Shelton JG, Lee JT, Navolanic PM, Blalock WL, Franklin R, McCubrey JA. 2003b. Regulation of cell cycle progression and apoptosis by the Ras/Raf/MEK/ERK pathway. *Int J Oncol* 22: 469–480.
- Chong H, Vikis HG, Guan KL. 2003. Mechanisms of regulating the Raf kinase family. *Cell Signal* 15: 463–469.
- Creagh EM, Carmody RJ, Cotter TG. 2000. Heat shock protein 70 inhibits caspase-dependent and -independent apoptosis in Jurkat T cells. *Exp Cell Res* 257: 58–66.
- Deak M, Clifton AD, Lucocq LM, Alessi DR. 1998. Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. *EMBO J* 17: 4426–4441.
- Fan X, Yan L, Jia S, Ma A, Qiao C. 1999. A study of early pregnancy factor activity in the sera of women with trophoblastic tumor. *Am J Reprod Immunol* 41: 204–208.
- Fryer RM, Patel HH, Hsu AK, Gross GJ. 2001. Stress-activated protein kinase phosphorylation during cardioprotection in the ischemic myocardium. *Am J Physiol* 281: H1184–H1192.
- Gabai VL, Meriin AB, Yaglom JA, Volloch VZ, Sherman MY. 1998. Role of Hsp70 in regulation of stress-kinase JNK: implications in apoptosis and aging. *FEBS Lett* 438: 1–4.
- Ghobrial IM, McCormick DJ, Kaufmann SH, et al. 2005. Proteomic analysis of mantle-cell lymphoma by protein microarray. *Blood* 105: 3722–3730.
- Gill C, Mestril R, Samali A. 2002. Losing heart: the role of apoptosis in heart disease—a novel therapeutic target? *FASEB J* 16: 135– 146.
- Gomez-Puertas P, Martin-Benito J, Carrascosa JL, Willison KR, Valpuesta JM. 2004. The substrate recognition mechanisms in chaperonins. *J Mol Recognit* 17: 85–94.

- Hamajima N, Matsuda K, Sakata S, Tamaki N, Sasaki M, Nonaka M. 1996. A novel gene family defined by human dihydropyrimidinase and three related proteins with differential tissue distribution. *Gene* 180: 157–163.
- Hansen JJ, Bross P, Westergaard M, et al. 2003. 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* 112: 71–77.
- Hickey RW, Zhu RL, Alexander HL, Jin KL, Stetler RA, Chen J, Kochanek PM, Graham SH. 2000. 10 kD mitochondrial matrix heat shock protein mRNA is induced following global brain ischemia in the rat. *Brain Res Mol Brain Res* 79: 169–173.
- Hohfeld J, Hartl F. 1994. Role of the chaperonin cofactor Hsp10 in protein folding and sorting in yeast mitochondria. J Cell Biol 126: 305–315.
- Jäättelä M, Wissing D, Kokholm K, Kallunki T, Egeblad M. 1998. Hsp70 exerts its antiapoptotic function downstream of caspase-3-like proteases. *EMBO J* 17: 6124–6134.
- Jäättelä M. 1999. Escaping cell death: survival proteins in cancer. *Exp Cell Res* 248: 30–43.
- Jolly C, Morimoto RI. 2000. Role of heat shock response and molecular chaperones in oncogenesis and cell death. *J Natl Cancer Inst* 92: 1564–1572.
- Kabakov AE, Gabai VL. 1997. Heat shock proteins and cytoprotection: ATP-deprived mammalian cells. In: *Molecular Biology Inteligence Unit.* R. G. Landes Company, Austin, TX, 5–45.
- Kajkowski EM, Lo CF, Ning X, et al. 2001. β-Amyloid peptide-induced apoptosis regulated by a novel protein containing a g protein activation module. *J Biol Chem* 276: 18748–18756.
- Khawaja X, Xu J, Liang JJ, Barrett JE. 2004. Proteomic analysis of protein changes developing in rat hippocampus after chronic antidepressant treatment: implications for depressive disorders and future therapies. J Neurosci Res 75: 451–460.
- Kirmanoglou K, Hannekum A, Schafler AE. 2004. Expression of mortalin in patients with chronic atrial fibrillation. *Basic Res Cardiol* 99: 404–408.
- Krijnen PA, Nijmeijer R, Meijer CJ, Visser CA, Hack CE, Niessen HW. 2002. Apoptosis in myocardial ischaemia and infarction. J Clin Pathol 55: 801–811.
- Levanon D, Goldstein RE, Bernstein Y, Tang H, Goldenberg D, Stifani S, Paroush Z, Groner Y. 1998. Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. *Proc Natl Acad Sci USA* 95: 11590–11595.
- Lin KM, Hollander JM, Kao VY, Lin B, Macpherson L, Dillmann WH. 2004. Myocyte protection by 10 kD heat shock protein (Hsp10) involves the mobile loop and attenuation of the Ras GTP-ase pathway. *FASEB J* 18: 1004–1006.
- Lin KM, Lin B, Lian IY, Mestril R, Scheffler IE, Dillmann WH. 2001. Combined and individual mitochondrial HSP60 and HSP10 expression in cardiac myocytes protects mitochondrial function and prevents apoptotic cell deaths induced by simulated ischemia-reoxygenation. *Circulation* 103: 1787–1792.
- Liu Y, Dehni G, Purcell KJ, Sokolow J, Carcangiu ML, Artavanis-Tsakonas S, Stifani S. 1996. Epithelial expression and chromosomal location of human TLE genes: implications for notch signaling and neoplasia. *Genomics* 31: 58–64.
- Mansell JP, Yarram SJ, Brown NL, Sandy JR. 2002. 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* 38: 518–522.
- Meghji S, White PA, Nair SP, et al. 1997. *Mycobacterium tuberculosis* chaperonin 10 stimulates bone resorption: a potential contributory factor in Pott's disease. *J Exp Med* 186: 1241–1246.

Morton H. 1998. Early pregnancy factor: an extracellular chaperonin 10 homologue. *Immunol Cell Biol* 76: 483–496.

- Morton H, Hegh V, Clunie GJ. 1974. Immunosuppression detected in pregnant mice by rosette inhibition test. *Nature* 249: 459–460.
- Morton H, Rolfe B, Clunie GJ. 1977. An early pregnancy factor detected in human serum by the rosette inhibition test. *Lancet* 1: 394–397.
- Morton H, Cavanagh AC, Athanasas-Platsis S, Quinn KA, Rolfe BE. 1992. Early pregnancy factor has immunosuppressive and growth factor properties. *Reprod Fertil Dev* 4: 411–422.
- Nair SP, Meghji S, Reddi K, Poole S, Miller AD, Henderson B. 1999. Molecular chaperones stimulate bone resorption. *Calcif Tissue Int* 64: 214–218.
- Palaparti A, Baratz A, Stifani S. 1997. The Groucho/transducin-like enhancer of split transcriptional repressors interact with the genetically defined amino-terminal silencing domain of histone H3. J Biol Chem 272: 26604–26610.
- Parcellier A, Gurbuxani S, Schmitt E, Solary E, Garrido C. 2003. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. *Biochem Biophys Res Commun* 304: 505– 512.
- Preville X, Salvemini F, Giraud S, Chaufour S, Paul C, Stepien G, Ursini MV, Arrigo AP. 1999. Mammalian small stress proteins protect against oxidative stress through their ability to increase glucose-6-phosphate dehydrogenase activity and by maintaining otimal cellular detoxifying machinery. *Exp Cell Res* 247: 61– 78.
- Quinn KA, Athanasas-Platsis S, Wong TY, Rolfe BE, Cavanagh AC, Morton H. 1990. Monoclonal antibodies to early pregnancy factor perturb tumour cell growth. *Clin Exp Immunol* 80: 100–108.
- Quinn KA, Cavanagh AC, Hillyard NC, McKay DA, Morton H. 1994. Early pregnancy factor in liver regeneration after partial hepatectomy in rats: relationship with chaperonin 10. *Hepatology* 20: 1294–1302.
- Quinn KA, Morton H. 1992. Effect of monoclonal antibodies to early pregnancy factor (EPF) on the in vivo growth of transplantable murine tumours. *Cancer Immunol Immunother* 34: 265–271.
- Ranford JC, Coates AR, Henderson B. 2000. Chaperonins are cellsignalling proteins: the unfolding biology of molecular chaperones. *Expert Rev Mol Med* 2000: 1–17.
- Ray PS, Martin JL, Swanson EA, Otani H, Dillmann WH, Das DK. 2001. Transgene overexpression of alphaB crystallin confers simultaneous protection against cardiomyocyte apoptosis and necrosis during myocardial ischemia and reperfusion. *FASEB J* 15: 393–402.
- Richardson A, Schwager F, Landry SJ, Georgopoulos C. 2001. The importance of a mobile loop in regulating chaperonin/co-chaperonin interaction. *J Biol Chem* 276: 4981–4987.
- Rolfe BE, Morton H, Cavanagh AC, Gardiner RA. 1983. Detection of an early pregnancy factor-like substance in sera of patients with testicular germ cell tumors. *Am J Reprod Immunol* 3: 97–100.
- Ross DT, Scherf U, Eisen MB, et al. 2000. Systematic variation in gene expression patterns in human cancer cell lines. *Nat Genet* 24: 227–235.
- Ryan MT, Naylor DJ, Hoj PB, Clark MS, Hoogenraad NJ. 1997. The role of molecular chaperones in mitochondrial protein import and folding. *Int Rev Cytol* 174: 127–193.
- Sadacharan SK, Cavanagh AC, Gupta RS. 2001. 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* 116: 507–517.
- Samali A, Cai J, Zhivotovsky B, Jones DP, Orrenius S. 1999. Presence

of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of Jurkat cells. *EMBO J* 18: 2040–2048.

- Satoh M, Tang J, Nanda A, Zhang JH. 2003. Heat shock proteins expression in brain stem after subarachnoid hemorrhage in rats. *Acta Neurochir Suppl* 86: 477–482.
- Schafler AE, Kirmanoglou K, Pecher P, Hannekum A, Schumacher B. 2002. Overexpression of heat shock protein 60/10 in myocardium of patients with chronic atrial fibrillation. *Ann Thorac Surg* 74: 767–770.
- Shan YX, Liu TJ, Su HF, Samsamshariat A, Mestril R, Wang PH. 2003a. Hsp10 and Hsp60 modulate Bcl-2 family and mitochondria apoptosis signaling induced by doxorubicin in cardiac muscle cells. J Mol Cell Cardiol 35: 1135–1143.
- Shan YX, Yang TL, Mestril R, Wang PH. 2003b. Hsp10 and Hsp60 suppress ubiquitination of insulin-like growth factor-1 receptor and augment insulin-like growth factor-1 receptor signaling in cardiac muscle: implications on decreased myocardial protection in diabetic cardiomyopathy. J Biol Chem 278: 45492–45498.
- Stelzl U, Worm U, Lalowski M, et al. 2005. A human protein-protein interaction network: a resource for annotating the proteome. *Cell* 122: 957–968.
- Stribinskis V, Heyman HC, Ellis SR, Steffen MC, Martin NC. 2005. Rpm2p, a component of yeast mitochondrial RNase P, acts as a transcriptional activator in the nucleus. *Mol Cell Biol* 25: 6546– 6558.
- Summers KM, Fletcher BH, Macaranas DD, et al. 1998. Mapping and characterization of the eukaryotic early pregnancy factor/chaperonin 10 gene family. *Somat Cell Mol Genet* 24: 315–326.
- Summers KM, Murphy RM, Webb GC, Peters GB, Morton H, Cassady AI, Cavanagh AC. 1996. The human early pregnancy factor/chaperonin 10 gene family. *Biochem Mol Med* 58: 52–58.
- Suzuki K, Sawa Y, Kagisaki K, Taketani S, Ichikawa H, Kaneda Y, Matsuda H. 2000. Reduction in myocardial apoptosis associated with overexpression of heat shock protein 70. *Basic Res Cardiol* 95: 397–403.
- Uetz P, Giot L, Cagney G, et al. 2000. A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* 403: 623–627.
- Veitia RA, Ottolenghi C, Bissery MC, Fellous A. 1999. A novel human gene, encoding a potential membrane protein conserved from yeast to man, is strongly expressed in testis and cancer cell lines. *Cytogenet Cell Genet* 85: 217–220.
- Walter S, Buchner J. 2002. Molecular chaperones–cellular machines for protein folding. *Angew Chem Int Ed Engl* 41: 1098–1113.
- Weissman JS, Hohl CM, Kovalenko O, et al. 1995. Mechanism of GroEL action: productive release of polypeptide from a sequestered position under GroES. *Cell* 83: 577–587.
- Xiao L, Casero Jr RA. 1996. Differential transcription of the human spermidine/spermine *N*¹-acetyltransferase (SSAT) gene in human lung carcinoma cells. *Biochem J* 313: 691–696.
- Xiao L, Celano P, Mank AR, Griffin C, Jabs EW, Hawkins AL, Casero Jr RA. 1992. Structure of the human spermidine/spermine N¹acetyltransferase gene (exon/intron gene organization and localization to Xp22.1). *Biochem Biophys Res Commun* 187: 1493– 1502.
- Xu Z, Horwich AL, Sigler PB. 1997. The crystal structure of the asymmetric GroEL-GroES-(ADP)7 chaperonin complex. *Nature* 388: 741–750.
- Ziemienowicz A, Skowyra D, Zeilstra-Ryalls J, Fayet O, Georgopoulos C, Zylicz M. 1993. Both the *Escherichia coli* chaperone systems, GroEL/GroES and DnaK/DnaJ/GrpE, can reactivate heat-treated RNA polymerase. Different mechanisms for the same activity. J Biol Chem 268: 25425–25431.