EMR

EXPERIMENTAL MEDICINE REVIEWS

Morphophysiological Remarks
in english and italian

Edited by
Aldo Gerbino
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"plumelia"
edizioni

Di.Me.S.

Experimental Medicine Reviews (Eds: A. Gerbino, G. Zummo, G. Crescimanno)
© Plumelia Ricerca (ISBN 978-88-89876-08-4) - Vol. 1 - 2007

CHAPERONOLOGY: A NOVEL RESEARCH FIELD FOR EXPERIMENTAL MEDICINE IN THE XXI CENTURY

[Chaperonologia: un moderno campo di ricerca per la Medicina Sperimentale del XXI secolo]

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Key words: Chaperones, Chaperonins, Hsp60, Hsp10, Chaperonopathies, Chaperonotherapy
Parole chiave: Chaperoni, Chaperonine, Hsp60. Hsp10, Chaperonopatie, Chaperonoterapia

Abstract. We have been studying for some years two mitochondrial heat shock proteins (Hsps), the chaperonins Hsp60 and Hsp10, that are necessary for folding of mitochondrial proteins. In recent times, the interest in these Hsps has been growing since it has been shown that they can also be present in the cytoplasm and secreted outside cells. We still do not know all their functions, but we are aware that they could represent important biomarkers for some tumours and inflammatory diseases.

Riassunto. Da alcuni anni ci occupiamo di studiare due proteine da shock termico mitocondriali, la chaperonina Hsp60 e la sua co-chaperonina Hsp10, necessarie per il folding delle proteine mitocondriali. L'interesse nei loro confronti negli ultimi tempi è aumentato perché si è scoperto che queste molecole possono anche essere presenti nel citoplasma ed essere secrete all'esterno della cellula. Non sono ancora note tutte le loro funzioni, che possono anche essere degli importanti biomarcatori in alcuni tumori e in alcune patologie infiammatorie.

Introduction

For some years, our group has been studying morphology and function of mitochondria both in normal cells and pathological models, in order to achieve a better understanding of the mitochondrial involvement in the pathogenesis of some disease, like cancer. More recently, we have focused our attention on two mitochondrial proteins, namely Hsp60 and Hsp10, that are important for the survival of such organelles and thus of the whole cell.

The Hsps constitute a heterogeneous group of molecules highly preserved during evolution as they are involved in many crucial cellular functions [1-3]. One of the most relevant is the *chaperoning* role, which is responsible not only for the acquisition of functional conformation of other proteins, but also of their preservation after stress caused by a variety of stressors affecting diverse tissues (Table 1). Chaperones are also involved in the

degradation of damaged proteins [4-6]. So, chaperones are proteins whose function is to promote correct folding of nascent polypeptides, refolding of partially denatured proteins, and degradation of irreversibly damaged molecules. Not all Hsps are chaperones, since other molecules, different from Hsps, have such a function, too.

Table 1: Chaperone inducers (Cell stressors)[a]

Type	Description
Physical	Heat, irradiation, UV light, etc
Chemical	Oxygen derived free radicals, hypoxia-anoxia-reperfusion
Biological	Infection, Inflammation
Psychological	Emotions, hormonal imbalance
Mechanical	Compression, shearing stretching
Others: ethanol, methanol, tetracycline, teratogens, mutagens, carcinogens	

[a] Reproduced with permission from reference 9.

Chaperonin and co-chaperonins in normal and pathologic tissues

Hsp60 and Hsp10 are mitochondrial chaperones, commonly named *chaperonin* (Hsp60) and *co-chaperonin* (Hsp10). It is known from studies in prokaryotes that these two chaperonins participate in the folding of nascent polypeptides (Fig.1) [7-8]. Since the mitochondrial Hsp60 and Hsp10 are phylogenetically close to those from bacteria it is generally assumed that the mitochondrial molecules have similar functions than those of the bacterial counterparts and act through similar mechanisms.

A number of studies carried out by different groups have shown that Hsp60 and Hsp10, as well as other chaperones, are associated with several diseases, now referred to as

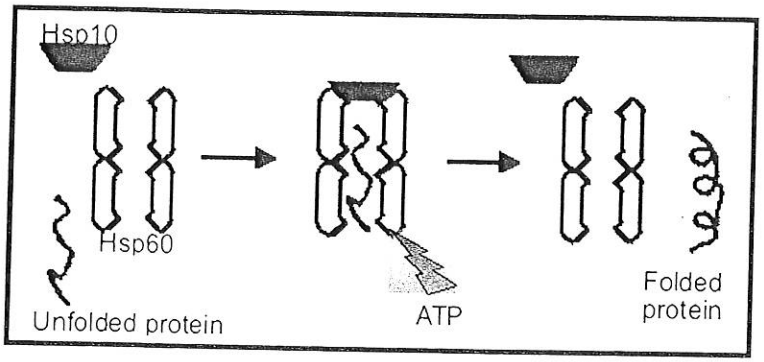

Fig. 1: Schematic representation of Hsp60-Hsp10 performing their protein-folding function as envisioned from data from the prokaryotic system.

chaperonopathies [9-11]. A first classification included two main classes of chaperonopathies, genetic and acquired. Some of the genetic chaperonopathies are, Charcot–Marie–Tooth disease, distal hereditary motor neuropathy, childhood cataracts, distal motor neuropathy, hereditary spastic paraplegia, X-linked retinitis pigmentosa, and Leber congenital amaurosis. Examples of acquired chaperonopathies are Alzheimer disease, amyotrophic lateral sclerosis, Huntington's disease, and other pathologic conditions affecting the vascular, respiratory tract, intestinal tract, and hematopoietic tissues.

More recently, a third class has been proposed, the chaperonopathies "by collaborationism" (or "by mistake") [11], consisting of a number of tumors in which Hsps have been found overexpressed or downregulated and in which Hsps seem to play a role in tumor growth.

Our recent studies have contributed to the understanding of the involvement of both Hsp60 and Hsp10 in the pathogenesis of some solid tumors. In particular, we found an overexpression of such molecules during carcinogenesis of large bowel (Fig. 2), uterine exocervix, and prostate [12-18]. By contrast, we found downregulation during bronchial carcinogenesis and vesical cancer progression [19-20]. Our data have been confirmed by other studies [21-24].

Moreover, our work has highlighted that Hsp60 and Hsp10 may also have a cytoplasmic localisation, even if not always together. We have postulated that these proteins, when present in the cytoplasm, may perform functions that are different from their canonical role in protein folding, For example, cytosolic Hsp60 and Hsp10 could participate in the mechanisms of cell proliferation and differentiation. In addition, we have shown that these molecules occur also in the peritumoral stroma [12-15]; their presence there due, perhaps, to a secretory mechanism.

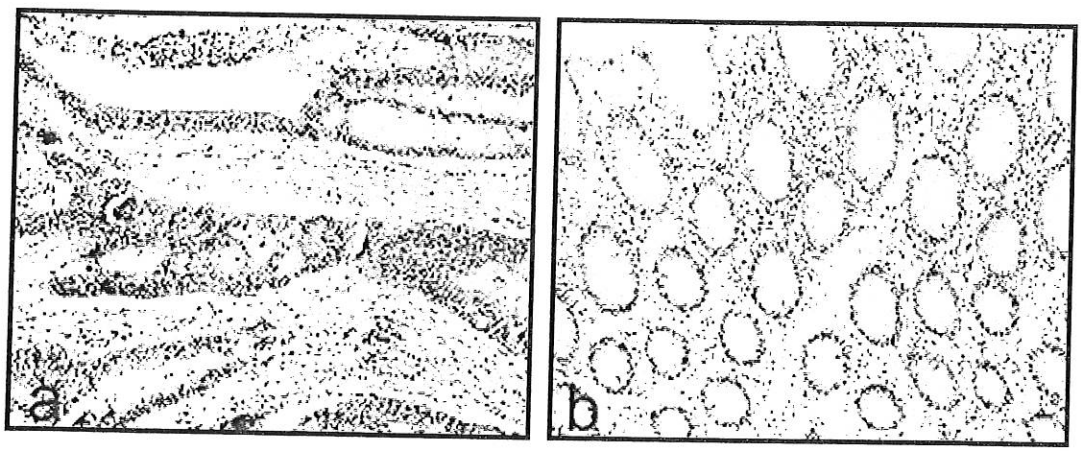

Fig. 2: Hsp60 is overexpressed in colon adenocarcinoma (a), compared to normal colonic mucosa (b).

Perspectives

Our attention is now directed to elucidating the molecular mechanisms by which increased or reduced expression of Hsp60 and Hsp10 determine the development of certain neoplasms [25-27].

Preliminary data suggest that Hsp60 plays both pro- and anti- apoptotic roles, depending on the expression of other molecules, like Hsp70, and p53; therefore, Hsp60 is probably connected to other pro- and anti-proliferative molecules in a complex network with a fine regulation.

Our studies are also focused on the mechanisms underlying the presence of Hsp60 and Hsp10 outside cells, since we assume that such molecules, when released in the interstitium, constitute a powerful pro-inflammatory stimulus [28]. In parallel, we are determining the levels of Hsp60 and Hsp10 in sera of patients with certain types of cancer in the hope that they could become biomarkers useful in clinical oncology.

Conclusions

In summary, our research pertains to the field of *Chaperonology*, encompassing *Chaperonomics* and *Chaperonotherapy. Chaperonology* is the study of intracellular and extracellular chaperones in all their aspects (structure, function, genetics, evolution, pathology) aiming to expand our knowledge on disease pathogenesis, and to use chaperones as diagnostic markers and prognostic indicators. *Chaperonomics* refers to the analysis of chaperone genes in genomes and at their products, including identification and classification of genes and pseudogenes, transcripts, polymorphisms, mutations, and inheritance. These studies should provide a solid basis for further bioinformatics analyses and experimental studies on the role of chaperone genes-proteins in ageing and associated diseases. *Chaperonotherapy* defines the use of chaperones in prevention and treatment of pathological conditions in which malfunctioning or absence of chaperones play a pathogenetic role and that may, therefore, benefit from the use of chaperones as therapeutic agents. *Chaperonotherapy* also includes the development and use of anti-chaperone agents to control conditions in which chaperones play a pro-disease role, like those types of malignant tumors mentioned above that need chaperones to grow and metastasize. We hope that research and clinico-pathological activities in these fields will be useful and will contribute to improve human health in the XXI century.

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ISBN 978-88-89876-08-4

UNIPA BICENTENARIO DELLA FONDAZIONE DELL'UNIVERSITÀ DI PALERMO 1806/2006