Review

Capsula Eburnea

THE ROLE OF HUMAN HEAT SHOCK PROTEINS IN CARCINOGENESIS

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SUMMARY

Recently, the interest towards the role of heat shock proteins (HSPs) in carcinogenesis has been steadily increasing. Indeed, it seems that these proteins contribute to the development of some cancers, including colorectal cancer, that can now be considered "chaperonopathies" by mistake". The specific roles of the different HSPs, in particular Hsp60 and Hsp10, during carcinogenesis are still unclear. However, a number of studies suggest that they could be useful tools for the diagnosis, staging, prognosis and treatment of many tumors.

Introduction

The physiological functions of a protein are attributable to the folding process that is essential to establish the functional conformation of any protein.

Most proteins are aided in their folding by other proteins called "chaperones" [1]; these are special multimolecular complexes able to catch defectively folded or unfolded proteins, in order to ensure their correct folding [2,3]. Chaperones are constitutively present in all cells and are believed to be among the oldest proteins synthesized by cells billions of years ago [4]; however, their expression can also be induced by several types of cellular stress (alcohol, UV radiation, infectious agents, anoxia, etc). These chaperones induced by stressors [5] belong to a group of proteins called "Heat shock proteins" (HSPs), after the name of the first inductor agent studied: thermal shock [6]. The human HSPs are classified into groups according to their molecular mass in kilodalton: Hsp60, Hsp70, Hsp90, Hsp100 and small HSPs (Tab. 1). The main protagonists of the cellular reactions involved in proteic folding are the components of the Hsp60 and the Hsp70 families [7]. Chaperones act as a part of a multimolecule team, including chaperones and co-chaperones. Examples of such multimolecular complexes are the complex consisting of Hsp70 and Hsp40, or the Hsp60 and Hsp10 complex. The former can be generally found in the cytosol, while the latter works inside the mitochondria to ensure the correct execution of the protein folding process [8]. Inside the mitochondria, Hsp60 forms a heptameric ring-like structure; two rings join together to form a barrel with a hole in the center, in which the folding process takes place. Hsp10 instead forms a heptamer, a kind of cap that closes the barrel-like structure formed by Hsp60, thus starting the folding process. This action requires seven ATP molecules [9, 10], and therefore the chaperone changes its shape and hides hydrophobic residues in the central cavity (Fig. 1). This causes the central cavity to become more hydrophilic, and the polypeptidic chain, no longer bound to the hydrophobic groups of the chaperone, starts to fold. A missed or defective folding of cellular proteins can determine a metabolic dysfunction of the cell, and its death by apoptosis or necrosis [11].

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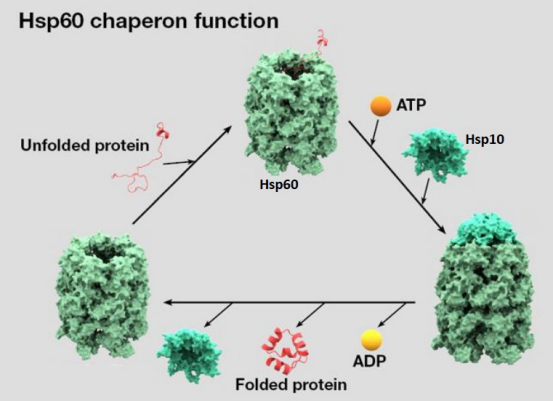
Figure 1: Figure 1: Scheme on Hsp60 and Hsp10 structure and function. Reproduced with modification from: www.pdbj.org.



The multi-step process of carcinogenesis

Carcinogenesis can be considered as a multi-step process in which normal cells are transformed into cancerous cells through the involvement of various molecular and morphological changes. This process is caused by an alteration of the normal balance between proliferation and cellular death, due to mutations in cellular DNA. Such alteration results in uncontrolled cell division, and the consecutive formation of a malignant mass. An altered cell proliferation can lead to benign tumors; some types of these may successively turn into malignant tumors (cancer). The majority of tumors originate from an initial alteration, evolving gradually to a more severe state, characterised by uncontrolled growth, modification of the local environment, invasion of surrounding tissues and metastatic spreading through the circulatory system.

In many tumors, high levels of heat shock proteins (HSPs) can be found and this seems to be associated with most of the carcinogenetic events. Moreover, these high levels of HSPs are closely linked to a poor prognosis and resistance to therapy [12].

Role of molecular chaperones during carcinogenesis

Many research groups are interested in the process of folding and in the chaperone machine in general, since several diseases can be caused by defective protein folding [2]. Furthermore, over the last few years many authors have been trying to identify the distinct ("non-canonical") roles of molecular chaperones; for example, it is now

Table 1: Main chaperones and their functions

Chaperone	Subcellular localization	Known function
Hsp10	Mitochondria, cytosol, zymogen granules	Protein folding; modulation of immune system
Hsp27	Cytosol	Anti-apoptotic function; cytoprotection
Hsp40	Cytosol	Folding and refolding of denatured proteins, together with Hsp70/ Hsc70
Hsp47	ER	Synthesis/assembly of various collagens
Hsp60	Mitochondria, cytosol, cell membrane, vesicles, cell surface	Cytoprotection; protein folding; macrophage activator possibly through Toll-like receptors
Hsc70	Cytosol, nucleus	Protein folding; clathrin uncoating; peptide binding
Hsp72	Cytosol, nucleus	Cytoprotection and anti-apoptotic function. Implicated in spermatogenesis
Hsc74	Mitochondria	Antigen presentation; radioresistance
Hsp90α	Cytosol	Protein folding; cytoprotection; intracellular signalling (e.g., steroid receptor); cell-cycle control
Hsp90β	Cytosol	Protein folding; Cytoprotection; Intracellular signalling (e.g., steroid receptor); Cell-cycle control
Hsp110	Cytosol/ nucleus	Binds to Hsc70 to form a high-molecular-weight complex; involved in protein folding; thermotolerance; involved in embryogenesis

known that they are involved in various processes, such as the regulation of the innate immune system, gene expression, cell differentiation, DNA replication and signal transduction, as well as participating in programmed cell death, cellular senescence and carcinogenesis [13, 14]. The increased transcription of HSPs in tumor cells is a critical step in carcinogenesis, caused by a loss of p53 function as well as an increase in the expression of the proto-oncogenes HER2 and c-Myc. HSPs are involved in tumor growth both by stimulating autonomous cell proliferation and inhibiting apoptotic pathways [12]. Therefore, the assessment and quantification of these proteins in tissues and biological fluids can be useful for diagnostic and prognostic aims [2], and even during disease follow-up, as well as for the development of novel antineoplastic strategies.

Is cancer a chaperonopathy?

The number of diseases potentially caused by dysfunctional chaperones is steadily growing. These diseases are called "chaperonopathies" and can be divided in "congenital" or "acquired" according to their aetiology, or in "by excess", "by defect" or " by mistake" [15, 16] depending on their pathogenesis. Several types of cancers are classified as "chaperonopathies by mistake"; in these, the chaperones, regardless of whether they are increased or reduced [17-22], act by favoring the tumoral mass instead of the organism where the tumor is growing in. Recently, many research groups have turned their attention to studying the extracellular chaperones. In fact, several recent studies have shown that cells are able to release these proteins into the extracellular space, by "unconventional" secretion pathways (meaning that the release takes place without the participation of the endoplasmic reticulum and Golgi apparatus), including "lipid rafts" and "exosomes" [24]. After their release outside the cells, chaperones can act in an autocrine, paracrine or endocrine manner, due to the fact that these mechanisms are recognised by various receptors located in the cellular membrane. These receptors are also expressed in immune cells, and this is very interesting, especially considering the role of extracellular chaperones in the modulation of the immune response [25]. The presence of HSPs in the extracellular fluid seems to imply that they play an immunoregulatory role [26]. The involvement of the chaperones in anti-stress mechanisms can be considered as a predecessor of the immune system and there are now clear indications that both systems interact at various levels [25]. In view of the considerations above, HSPs could be responsible for carcinogenesis.

An outlook on the role of HSPs in colon cancer pathogenesis

Colon cancer is still one of the most frequent cancers in the world, and a common cause of death and therefore, it is paramount to develop new diagnostic and therapeutic methods.

Many authors have studied the role of various HSPs in the pathogenesis of different tumors. In particular, some researchers have focused on Hsp60 and its co-chaperonin Hsp10, whose expression is increased also in colon cancer. As regards to Hsp60, it was previously thought that in eukaryotic cells, this protein was only located inside the mitochondria [27], but its extramitochondrial localization (e.g. in cytosolic vesicles or cellular membrane) has since been discovered thanks to the employment of immunohistochemistry and immunoelectron microscopy (IEM) [28]. Cytosolic Hsp60, whose genesis has not yet been clarified, can be involved in a complex of molecules that regulate the activation of the apoptotic pathway [29-32], since in the cytosol Hsp60 binds to pro-caspase 3 and blocks its activation after pro-apoptotic stimuli, thus producing an anti-apoptotic effect [33]. On the contrary, the presence of Hsp60 in the cellular membrane implies that the protein is being released into the extracellular space via the exosomal pathway. In fact, from the membrane, Hsp60 is internalized in cellular vesicles by lipid rafts, and successively secreted through the exosomes; in some cases, Hsp60 can even reach the bloodstream in this manner [34, 25]. However, the reason for this migration is still not clear. Hsp60 can also be found in the blood circulation, integrated in exosomes or in soluble form, and this suggests the presence of other mechanisms of secretion, not yet well understood.

Hsp10, that is usually localized inside the mitochondria, has also been found in extramitochondrial localizations such as zymogen granules, secretory granules of different cell types (e.g. pancreatic cells) [35] and red blood cells [36], in precursors of normal human bone marrow, specifically myeloid and megakaryocytic precursors [37] as well

as in various pre-cancerous lesions (e.g. colon, exocervical and prostate cancer) [19, 20]. It has also been shown that in normal cells, Hsp10 is generally localized in the mitochondrial matrix, while in tumoral cells, it is found especially in the cytosolic space; however, the role of this cytosolic rising is not yet clear [38].

Conclusions

The understanding of the specific role of HSPs in carcinogenesis is still incomplete. It would be important to clarify the mechanisms by which the chaperones reach the non-canonical locations discussed above, and to elucidate their function(s) in each of these locations. This knowledge will contribute to a better understanding of the molecular mechanisms of carcinogenesis, leading to the development of new, valuable diagnostic and therapeutic tools for clinical oncology and surgical pathology [39].

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