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Molecular Chaperones in the Etiology and Therapy of Cancer*

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Molecular chaperones are ubiquitous, well-conserved proteins that account for 2–5 % of all cellular proteins in most cells. The present review summarizes our current knowledge about their involvement in the etiology and therapy of cancer with special emphasis on the expression of chaperones in malignant cells, their role in folding of (proto)oncogene products, cell cycle regulation, cell differentiation and apoptosis, development of metastasis, and their participation in the recognition of malignant cells. We also overview the importance of chaperones in hyperthermia, drug resistance, and recent approaches in chaperone-immunotherapy. (Pathology Oncology Research Vol 4, No 4, 316–321, 1998)

Key words: chaperone, heat shock protein, stress protein, metastasis, immunotherapy, drug resistance

Introduction

Molecular chaperones have been defined as "proteins that bind to and stabilize an otherwise unstable conformer of another protein – and, by controlled binding and release, facilitate its correct fate *in vivo:* be it folding, oligomeric assembly, transport to a particular subcellular compartment, or disposal by degradation".[31] Chaperones are ubiquitous, highly conserved proteins which probably played a major role in the evolution of modern enzymes.[16] Chaperones are vital for our cells during their whole lifetime. However, they are needed even more after environmental stress, which induces protein damage. Stress (heat shock, major changes in the cellular environment after the activation of various pathogens or during the development of disease, etc.) induces the synthesis of many chaperones which therefore are called heat-shock, or stress proteins. Chaperones play an essential role in the etiology of numerous diseases, with a rapidly increasing role in clinical practice.[50,88,92] Lacking a settled view about their exact and specific cellular functions, chaperones are still best classified by their molecular weights. The major chaperone families are listed in *Table 1*.

Induction of molecular chaperones in malignant cells

Chaperones help damaged proteins to re-fold to their native conformation, therefore it is not surprising that they are expressed after the cell experiences various types of environmental stress. Cancer cells have an especially "stressful" life: lack of nutrients, oxygen, space limitations, and hostile environment are all important factors which acting by alone would induce a large number of chaperone proteins. We have summarized some examples of chaperone induction in *Table 2*. Generally, induced levels of chaperones help tumor cell survival. Overexpression of Hsp90-α is usually associated with poor prognosis in breast cancer.[93] However, in some cases such as after the overexpression of Hsp25, proliferation of malignant cells slows down.[47] Thus due to the pleiotropic effects of molecular chaperones. It is often difficult to make simplified statements on their association with poor or better prognosis in various types of cancer.[15]

Molecular chaperones are induced by almost all treatment protocols used to eliminate tumors. Hyperthermia

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Abbreviations: Grp, glucose regulated protein; Hsp, heat shock protein.

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Table 1. **The major molecular chaperone families**

Some common names of eukaryotic chaperone family members	*References for recent reviews*
Hsp25, Hsp27, crystallins, Small heat-shock proteins	10,30
Hsp60, chaperonins	11,31
Hsp70, Grp78, BiP	11,31
Hsp90, Grp94	10,17,63,64
Hsp110	68,91

Neither the co-chaperones (chaperones which help the function of other chaperones listed, such as Hsp10, dnaJ-homologues, Hip, Hop, Hup, etc.), nor the so-called folding catalysts, the peptidyl-prolyl isomerases (immunophilins) and protein disulfide isomerases were included in this table, albeit almost all of these proteins also possess a "traditional" chaperone activity in their own right.

and various forms of chemotherapy are the classical examples of treatment-induced chaperone expression, as we will discuss in later sections of this review. Chaperone induction has also been observed after radiation therapy,[67] or addition photosensitizers and phototherapy.[55]

Association of chaperones with (proto)oncogene products: their role in regulation of the cell cycle, cell differentiation and apoptosis

Chaperones participate in the folding of numerous protooncogene and oncogene products. These target-proteins often form stable complexes with chaperones which keep them in an "activation-competent" state. E.g.: Hsp90 is necessary for the folding of several protooncogene/oncogene protein kinases, such as members of the src and raf families.[17] Chaperones also participate in the maintenance of correct conformation of other proteins involved in signal transduction, such as receptors, G-proteins and transcription factors.[63] Chaperones are necessary for the folding of several proteins of oncogenic viruses, such as those of the hepatitis B virus.[34]

The expression pattern of several chaperones is cell cycle dependent. E.g.: Hsp90-α mRNA is induced at the G_1/S transition of chicken hepatoma cells.[43] In eukaryotic organisms folding of *de novo* synthesized proteins is usually mediated by the ribosome-complex itself. However, folding of several key members of cell cycle regulation requires the help of specific molecular chaperones. E.g.: Hsp90 is necessary for the folding of the cyclin-dependent kinase, CDK4[79] and the cyclin dependent kinase regulator, Wee.[1] Hsp70 participates in the activation of the tumor-suppressor p53 protein.[37] The novel Hsp90 homologue, Hsp75/TRAP-1, as well as the constitutively expressed isoform of Hsp70 associate with the tumor suppressor retinoblastoma protein, most probably stabilizing the conformation of its dephosphorylated, thus tumor suppressive form.[13,38]

Changes in chaperone expression are usually accompanying the differentiation of various cell types. Differentiation of embryonal carcinoma cells leads to elevated levels of small heat shock proteins which may play an important role in various signaling events leading to the differentiated state.[72,78] On the contrary, expression of 70 and 90 kDa chaperones is lowered when the cells leave vigorous proliferation,[3,17,33] which may reflect a decreased need for help in conformational rearrangements.

Induction, or overexpression of various chaperones generally protects host cells from apoptosis.[65] Transgenic mice expressing the inducible form of the 70 kDa molecular chaperone, develop T cell lymphoma due to the severely impaired apoptosis in T cell selection.[70] Similarly, expression of Hsp27 or Hsp70 protects tumor cells against the apoptotic effects of tumor necrosis factor-α.[40,90] On the contrary, overexpression of Hsp90 enhances tumor necrosis factor-α induced apoptosis of U937 cells.[27] This may be related to the possible involvement of an Hsp90-homologue in type-1 tumor necrosis factor receptor signaling as reported by Song et al.[71] Thus the involvement of chaperones in the diversion of the normal cell cycle towards

Table 2. **Induction of molecular chaperones in malignant human cells**

Chaperone family member	*Malignant cell type*	*References*
small heat shock proteins	breast cancer	14,59
	hepatoma	18
	neuroectodermal tumor	39
Hsp60	breast cancer	7
	lymphoma	21
Hsp70	endometrial carcinoma	58
	lung carcinoma	22
	melanoma	22,60
	pancreatic carcinoma	29
	renal carcinoma	66
Grp78	breast cancer	7
Hsp90	breast cancer	22,24,41,93
	endometrial carcinoma	58
	gastrointestinal cancer	20
	hepatoma	22
	leukemia	12,94
	lung carcinoma	22
	melanoma	22,60
	microcytoma	22
	ovarian cancer	54
	pancreatic carcinoma	29
Grp94	breast cancer	7,23,24,93
	colon adenocarcinoma	53
Hsp 110[a]		

[a]Induction of Hsp110 in tumors has not been reported (yet).

apoptosis most probably depends on the type of apoptotic signal. As is obvious from the above, our present knowledge about the involvement of chaperones in the cell cycle and apoptosis is rather fragmentary. However, these areas may well provide significant major advances in the understanding of chaperone function in the near future.

Involvement of chaperones in metastasis development

Induction of various molecular chaperones, such as Hsp27,[80] Hsp70,[45,60] Hsp90,[41] and the collagen-specific chaperone, Hsp47[56] was observed in several metastasis models. The putative heparanase and protease (aminopeptidase) activities of the Hsp90-homologue, Grp94, together with its frequent expression on the surface of tumor cells,[19,28,73,76] may enable Grp94 to act as a mediator of metastasis generation. However, the testing of the putative role of Grp94 and other chaperones in promotion of metastasis formation is a task for future research.

Surface expression of chaperones, their role in antigen-presentation, and in immunorecognition of malignant cells

In the end of the eighties Hsp70, Hsp90 and Grp94 (termed gp96) were identified as tumor-specific antigens expressed on the surface of various tumor cells.[44,76,87] Expression of molecular chaperones on the surface of malignant cells and their secretion to the extracellular fluid has also been reported by numerous other laboratories.[4,17,22,57] Interestingly, extracellular Hsp90-α had a stimulatory effect on the growth of some lymphoid cell lines[48] and Grp78 was identified as a potential intercellular signal-transducing protein between pancreatic cancer cells.[25] Presently neither the molecular details of the surface attachment of molecular chaperones, nor the exact mechanism of their secretion are known.

Though differences in protein structure of various tumor-derived, surface-expressed chaperones were minor, if anything, their immunogenicity showed major differences. This apparent discrepancy led Pramod Srivastava to suggest that the chaperone-related immunogenicity resides in a great variety of peptides, which are non-covalently associated to, and "presented" by the chaperone.[74,75] Endogenously synthesized antigenic determinants are generally presented on major histocompatibility complex (MHC) class I molecules, whereas exogenous antigens are presented by MHC class II molecules. Heat shock and glucose-regulated proteins (Hsp70, Hsp90 and Grp94) may present their bound peptides to MHC class I molecules. Under normal (non-stressed) conditions this may be a helper mechanism for loading of the MHC class I molecules in the endoplasmic reticulum. However, stress proteins may carry their immunogen peptides to MHC class I molecules other than those of their original cells by lysis of the original cell and subsequent phagocytosis by macrophages or by direct macrophage-engulfment of the whole original cell. Since heat shock proteins are highly conserved, transfer of their peptide-load to MHC class I molecules may also occur after the lysis or phagocytosis of foreign cells with different haplotypes. Hence foreign chaperones may "disguise" their bound foreign peptides as self. Thus insertion of the nondiscriminating stress proteins to the peptide/antigen-presenting "relay" may explain the phenomenon of cross-priming, *i.e.* that not all the processing of the antigens occurs *via* the haplotype-restricted MHC class I molecules of the immunized mouse, but at least some of peptide/antigens are salvaged by the macrophages of the immunized mouse *directly* from the chaperones of the immunizing cells (having a different haplotype). During the last years the above hypothesis of Srivastava et al.[77] has been supported by several pieces of experimental evidence.[2,83,85,86]

The involvement of chaperones in antigen-presentation also means that, in an organism developing malignant cells, the MHC non-restricted presentation of tumor antigens becomes more dominant. This mechanism increases the efficiency of immune surveillance. Tumor-derived peptide-loaded chaperones *(via* the peptide-presenting macrophage-MHC class I molecules) may prime cytotoxic lymphocytes even after the death and lysis of the original malignant cells, which extends the cytotoxic response and also makes it more efficient.[77]

Hyperthermia and cancer treatment

Hyperthermia has been utilized in clinical practice as a primary treatment or as an adjuvant to radio-/chemotherapy of cancer for a long time.[8] Hyperthermal treatment protocols are based on proper focusing of thermal damage to cause the selective injury of tumor cells using computer-assisted 3D targeting.[82] The current review warrants for an even sharper (theoretically: all-or-non) discrimination between target and neighboring cells since improper (suboptimal) heating of tumor cells may induce an expression of their molecular chaperones and thus may lead to an increase in their survival and metastatic potential. Induction of Hsp70 may be efficiently used to judge the extent of hyperthermia in the malignant tissue.[51] On the other hand, massive induction of Hsp70 (and other heat shock proteins, such as Hsp110) may indicate the development of tumor-thermotolerance and stress-(drug)-resistance.

Chaperones and drug resistance

Heat treatment leads to increased drug resistance in many tumor cells. In agreement with these early results, in several malignant cell types the simultaneous induction of

various heat shock proteins and multidrug resistance has been observed.[14,36,59] Administration of chemotherapeutic agents often leads to a further increase in the expression of molecular chaperones, such as Hsp25[6] or Hsp70.[35] As a further evidence suggesting the chaperone-induced protection against chemotherapy high level of Hsp60 predicts poor survival of patients treated with cisplatin-containing chemotherapy protocols.[46] However, in some cases the chaperone-induction correlates with the induction of P-glycoprotein, e.g. in case of elevated Hsp90-β levels, where the chaperone has also been shown to associate directly with the multi-drug transporter.[5] In other cases elevated chaperone levels themselves seem to induce a "cross-resistance" against various chemotherapeutic agents irrespectively from the level of multidrug transporter present.[14,36,59] To make the situation even more complex, overexpression of P-glycoprotein has also been observed in heat-resistant hepatoma cells, which are defective in the induction of Hsp70.[61,62]

Molecular chaperones (the cytoplasmic "foldosome", containing Hsp70, Hsp90 and numerous co-chaperones) are actively involved in the folding and activation of steroid receptors.[17,63,64] Thus it is not surprising that they play a pivotal role in the development of steroid resistance in various forms cancer, such as breast, endometrial and prostate cancer. Since length limitatons of the present review do not allow us to review this extensive field, the interested reader is referred to other recent reviews providing an excellent summary of the subject.[26,49,89]

Molecular chaperones in cancer immunotherapy

A large part of tumor immunogenicity resides in the great variety of chaperone-associated tumor-specific peptides. Tumor-specific, chaperone-presented peptides are taken up by macrophages and presented by the macrophage MHC class I molecules. These macrophages are able to prime cytotoxic T lymphocytes for an anti-tumor attack.[2,77,83,86] The chaperone-mediated "escape route" of cytotoxic lymphocyte priming from the restrictive self-MHC molecules has profound consequences in cancer immunotherapy. The vaccination procedure does not necessarily have to use autologous or HLA-matched cells, but a preparation of chaperone-peptide complex from the specific tumor may be used as an effective immunogen to vaccinate the same patient. This vaccination protocol may be extended to shared tumor antigens in the future which may alleviate the need for the costly and time-consuming "personalized" vaccines.[9,32,77,84,85] As an approach of this type, if tumor cells are transfected with the mycobacterial heat shock protein, Hsp65 (which is a major common immunogen in almost all organisms), they loose their tumorigenicity,[52] or can be used as cancer vaccines.[69]

As an alternative chaperone-based immune-related therapy, suppression of the synthesis of certain chaperones, such as the Hsp70 homologue, Grp78, eliminates the tumor resistance to cell mediated cytotoxicity.[42,81]

Conclusions

Overexpression of chaperones in tumor cells is a rather general phenomenon caused by the increased demand of accelerated cell proliferation and the harmful environment. Chaperones protect malignant cells from many of the environmental stresses and render them more resistant against apoptosis, anticancer drugs and immune-attacks. Chaperone-induction may also lead to an increase of the metastatic potential. Tumor chaperones seem to be one of the devils of tumor therapy, thus various selective methods to impair their synthesis in tumor cells have high therapeutic potential. On the other hand, chaperone-peptide vaccination may provide a unique, a very powerful tool in cancer treatment.

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