Metadata of the chapter that will be visualized online

Chapter Title	Grp94 (HSP90B1)	
Copyright Year	2012	
Copyright Holder	Springer Science+Business Media, LLC	
Corresponding Author	Family Name	**Gorza**
	Particle	
	Given Name	**Luisa**
	Suffix	
	Division/Department	Department of Biomedical Sciences
	Organization/University	University of Padova
	Street	viale G. Colombo 3
	City	Padova
	Postcode	35121
	Country	Italy
	Email	luisa.gorza@unipd.it
Author	Family Name	**Vitadello**
	Particle	
	Given Name	**Maurizio**
	Suffix	
	Division/Department	CNR-Institute of Neuroscience
	Organization/University	Padova section
	City	Padova
	Postcode	35121
	Country	Italy
	Email	mvitadel@bio.unipd.it

G

Grp94 (HSP90B1)

Luisa Gorza[1] and Maurizio Vitadello[2]
[1]Department of Biomedical Sciences, University of Padova, Padova, Italy
[2]CNR-Institute of Neuroscience, Padova section, Padova, Italy

Synonyms

94 kDa glucose-regulated protein; Endoplasmin; Erp99; gp96; Grp94; Heat shock protein 90 kDa beta member 1; TRA1; Tumor rejection antigen gp96

Historical Background

Grp94 is the endoplasmic reticulum (ER) paralog of heat shock protein (▶ Hsp) 90. This chaperone/stress protein was identified together with the other members of the family of glucose-regulated proteins because its levels significantly increased after treatment of mammalian cells with culture medium lacking glucose. Subsequent evidence showed that other ER stressors, such as calcium depletion, inhibition of glycosylation, and reducing agents, similarly affected Grp94 protein levels, making it one of the hallmarks of ER stress response (Lee 1987). Grp94 protein levels increase also after exposure to low-doses of endotoxin, hypoxia, and mild ischemia and in most tumors (Srivastava 2006; Glembotski 2008). The last feature, which is accomplished by Grp94 binding of tumor-specific peptides, explains the other widely used synonyms gp96 and TRA1 (Srivastava 2006).

The apparent Mr of Grp94 is around 94–100 kDa in most Vertebrate species and is the product of a single gene. Whereas Grp94 orthologs are detected in Metazoans, plants enclosed, no corresponding gene or protein has been so far identified in yeast and other monocellular eukaryotes, except for *Leishmania* (Eletto et al. 2010). The protein shows multiple Mr isoforms depending on the variable degree of glycosylation and on the presence of Ser/Thr- or Tyr-phosphorylation (Frasson et al. 2009 and references within). Whereas the functional significance of glycosylation remains still obscure, Ser/Thr-phosphorylation by Golgi▶ casein kinase and Tyr-phosphorylation by ▶ Fyn Src-kinase are involved in Grp94 subcellular localization. Detailed analyses of Grp94 distribution in adult mammalian tissues and primary cells indicate relatively low expression levels of mRNA and protein in most tissues, except for lung bronchial epithelium, pancreatic islets, kidney tubular cells, and dendritic cells (Mao et al. 2010).

Structurally, Grp94 contains four domains (Eletto et al. 2010 and Fig. 1a). The N-terminal (N) domain contains a nucleotide binding site, a peptide binding site, unmapped sites responsible for interaction with dendritic cells during antigen presentation (Jockheck-Clark et al. 2010), the binding site for the co-chaperone Murine canopy 3 (CNPY3, Liu et al. 2010), and a putative transmembrane domain (Frasson et al. 2009 and references within). The second domain, as in Hsp90, is a charged linker between the N and middle (M) domains, but it is longer in Grp94 and functionally important for binding of both nucleotide and calcium. The M-domain contains residues involved in ATP hydrolysis after interaction with the N-terminal domain, and the C-terminal domain mediates the dimerization of

S. Choi (ed.), *Encyclopedia of Signalling Molecules*, DOI 10.1007/978-1-4419-0461-4,

Grp94 and ER localization via the C-terminal KDEL peptide. Grp94 displays a "twisted V" conformation as crystal structure (Fig. 1b), whereas in solution it has an extended, "chair-like" conformation, which shifts toward less extended, more closed conformations after nucleotide binding (Eletto et al. 2010).

Like other ER chaperones, such as Grp78 and calreticulin, Grp94 is a putative low affinity-high capacity Ca^{2+}-binding protein. Grp94 binds about 16–28 moles Ca^{2+} per mole protein with a few high affinity (Kd 1–5 μM) binding sites and the rest, lower affinity sites (Kd ∼600 μM). Thus, Grp94 may bind about 4 moles of calcium/mole of protein in the presence of 1 mmol/L calcium, namely the range of free calcium concentration considered to be present within the ER (Eletto et al. 2010).

ER localization of Grp94 is regulated, like other ER chaperones, through the C-terminal KDEL sequence. The KDEL sequence is recognized by a specific receptor, which is mainly localized in the cis-Golgi. Dimers-oligomers of Grp94 interacting with the aminoacyl-tRNA synthetase-interacting multifunctional protein 1 (AIMP1; previously known as p43) bind to the receptor and lead to its oligomerization and rapid transport out of cis-Golgi (Kim et al. 2010). The KDEL receptor-Grp94 complex returns to the ER, where it dissociates, thus freeing the receptor for further cycling of transport. In addition to the ER, Grp94 is detected at the cell surface of several tumors and of immature cells (Srivastava 2006; Gorza and Vitadello 2000). Two signaling pathways responsible for Grp94 exit outside the ER have been so far identified (Fig. 2): (1) in differentiating myoblasts, the Tyr phosphorylation of Grp94 by Fyn Src-kinase is required for the chaperone translocation to Golgi and cell surface (Frasson et al. 2009) and (2) in LPS-stimulated monocytes and splenocytes, the Ser-phosphorylation of AIMP1 by c-Jun N-terminal kinase (▶ JNK) releases the binding of the protein from Grp94 and the interaction with the KDEL receptor (Kim et al. 2010).

Another distinctive feature of Grp94 is the transmembrane configuration, which apparently involves a minor proportion of molecules and coexists in the ER compartment with the luminal configuration (Frasson et al. 2009 and references within). The precise mechanism of anchorage of Grp94 molecules to the cell surface is presently unknown; however, data suggest that it occurs through noncovalent, nonionic binding with other proteins (Srivastava 2006).

Physiological Functions

Protein Chaperone

Grp94 exists as a homodimer or higher-order oligomer. Although it has been shown to bind to some nascent ER proteins, Grp94 participates to the second major ER chaperone system, which operates, in addition and/or in alternative to the lectin system, on unfolded regions of proteins containing hydrophobic residues and is involved in folding and assembly of multimeric proteins, whose first and most studied example is the immunoglobulin heavy chain (Ma and Hendershot 2004). A large ER-localized multi-protein complex is initiated by the ER chaperone Grp78 that recognizes protein hydrophobic residues and recruits Grp94 and other molecular chaperones, among which are protein-disulfide isomerase, cyclophilin B, Grp170. As revealed by studies performed using chemical cross-linking, the complex forms also in the absence of protein synthesis (Ma and Hendershot 2004). The mechanism through which Grp94 exerts its chaperone function is still obscure, due to controversial evidence concerning ATPase activity, to the lack of co-chaperones and the paucity of client proteins. Conservation of amino acids involved in ATP binding and hydrolysis between Hsp90 and Grp94 would suggest a similar mechanism of nucleotide utilization. Grp94 binds adenosine nucleotides in vitro and mutations of either the ATP binding site, or the putative catalytic one, hamper chaperone activity in vivo (Randow and Seed 2001). However, structure analysis methods, including x-ray crystallography, mass spectrometry, and optical techniques showed that the ATP and ADP-bound forms of Grp94 are equivalent, that is, they display identical twisted "V"-shape conformation with closed C domains and open N domains in opposing orientation (Eletto et al. 2010; Fig. 1b). Therefore, ATP binding is not sufficient to drive Grp94 into a hydrolytically productive conformation: the open N domain and the relaxed N–M orientation leave the catalytic residues too distant from the gamma-phosphate of the ATP, at variance with Hsp90 and other proteins of the GHKL ATPase/kinase superfamily, whose ATPase activity results from the contact of residues localized in both the N and M domains with the bound nucleotide. As argued by the structural analysis, co-chaperone(s) would be required by Grp94 to promote ATP hydrolysis by rotating the catalytic loop, and to regulate binding and release of client proteins.

The ER resident protein CNPY3 has been recently identified as the first Grp94 co-chaperone, specifically involved in TLR folding (Liu et al. 2010). CNPY3 binds Grp94 close to the ATP pocket and in competition with nucleotides, suggesting that it regulates the folding of conformational intermediates without nucleotides, as shown for some Hsp90 co-chaperones (Eletto et al. 2010; Liu et al. 2010). Indeed, the same single point mutation at the highly conserved amino acid 103 (E103A) of Grp94, which was found to abolish ATPase activity and insulin-like growth factor (► IGF) folding (Eletto et al. 2010), hampered the interaction with CNPY3 (Liu et al. 2010) and folding of Toll-like receptors (► TLR) (Randow and Seed 2001), but it did not affect the folding of other client proteins, such as ► integrins (Randow and Seed 2001). At present, no recognition moiety has been identified for Grp94. Its small population of client proteins does not display common features other than the presence of disulfide bonds, and, in the case of integrins and TLR, shows selectivity even among family members (Table 1 and McLaughlin and Vandenbroeck 2011).

An unexpected role for Grp94 has been described in the ER quality control, that is, in targeting misfolded proteins to ER-associated degradation (ERAD). Misfolded and demannosylated protein substrates are bound by OS-9, a ER lumenal lectin, which associates with Grp94 in order to present substrates to the ubiquitination and dislocation apparatus. The role of Grp94 is presumably to facilitate or mediate substrate recognition, since degradation of mutated glycosylated proteins is impaired in Grp94-depleted cells (Eletto et al. 2010).

Immune Regulator

Grp94 is involved in both innate and adaptive immune responses.

In addition to play a key role in the folding of several TLR, Grp94 was proposed to activate professional antigen-presenting cells (pAPC) by interacting with TLR2 and ► TLR4. Contaminating endotoxin, either derived from recombinant Grp94 preparations or released even at very low levels from commensal flora, appears to have confounded the interpretation of these studies. However, after stringently controlling the level of endotoxin contamination and evaluating the inhibition of the Grp94 interaction by means of specific antibodies, a recent study demonstrated that this chaperone activates human macrophages primarily through TLR2 and induced both IL-6 and TNF-α release (Huang et al. 2009) (Fig. 3a). Some activation occurs through TLR4, concerning only IL-6 production. Besides, Grp94 appears to regulate the pathway activated by the TLR2 receptor downstream the mitogen extracellular kinase (► MEK)► -ERK1/2. In kidney tubular cells, this chaperone binds and maintains in active conformation the serine-threonine protein phosphatase 5 (PP5), a negative modulator of ► Raf-1 (Mkaddem et al. 2009).

In addition to initiate the innate immune responses by interacting with TLR2 and TLR4, Grp94 contributes to the adaptive one. Several in vitro studies demonstrated that the N-terminal and charged linker domains of Grp94 comprise a peptide-binding domain, which is inhibited when small molecules such as radicicol, geldanamycin and NECA occupy the nucleotide pocket at the opposite site (Eletto et al. 2010). Although it is presently debated whether Grp94-peptide binding is physiologically relevant in vivo, it is fully recognized that Grp94 can direct the associated peptides into the Major Histocompatibility Complex (► MHC) class I cross-presentation pathway of pAPCs (Srivastava 2006; Jockheck-Clark et al. 2010). Peptides bound to Grp94 are internalized by pAPCs by endocytosis and trafficked to a post-ER endosomal compartment, where they are processed and loaded onto mature MHC class I molecules (Fig. 3b). The mechanism of endocytic uptake of the Grp94-peptide complex remains controversial. A large body of evidence indicates the requirement of receptor-mediated internalization, via the low-density lipoprotein receptor-related protein 1 (► CD91) and/or the scavenger receptors, whereas recent results obtained in pAPC, engineered to knockdown or knockout CD91 expression, support a prominent role of the pathway of nonspecific fluid-phase uptake (Jockheck-Clark et al. 2010). In any case, the interaction of Grp94-peptide complex with pAPC elicits proinflammatory cytokine secretion and MHC class I/II up-regulation, leading to subsequent priming and activation of peptide-specific CD8+ T lymphocytes.

Calcium Binding Protein

Although each Grp94 molecule can bind between 16 and 28 Ca^{2+}, only one high affinity calcium-binding site has been mapped with certainty in the N-terminal portion of Grp94, in the charged linker domain (Eletto et al. 2010). In vitro studies showed that Ca^{2+} binding

at this site stimulates Grp94 peptide binding. Due to its relative abundancy in the ER, Grp94 is expected to provide about 30 μM of Ca^{2+} storage capacity. This property, which is shared with other ER chaperones, like Grp78 and calreticulin, might imply the participation of Grp94 to the maintenance of calcium homeostasis. In addition to protein folding, ER is deputed to the storage and utilization of calcium. Release and uptake of calcium by the ER is involved in many signaling pathways and physiological responses, like protein secretion and muscle contraction. Depletion of calcium from the stores induces an ER stress response. Besides, alterations in ER free calcium concentration strongly influence cell survival. If the ER has less free calcium to release upon stress, then cytosolic calcium levels will not rise to the critical levels needed for triggering deleterious downstream effectors. Therefore, the increased expression of ER chaperones with calcium-binding properties should protect cells by decreasing the amount of releasable calcium. Such a hypothesis finds support from several studies showing that Grp94 overexpression counteracts the increase in intracellular Ca^{2+} evoked by exposure either to calcium ionophore or to oxidants, whereas cells engineered to decrease Grp94 expression appear unable to cope with it (Pizzo et al. 2010 and references within). Passive calcium release from the stores, induced in myogenic cells by means of sarcoendoplasmic calcium ATPase (SERCA) inhibition, showed that cellular levels of Grp94 inversely correlate to the rise in intracellular Ca^{2+} (Pizzo et al. 2010). This body of evidence points toward a role for Grp94 in preserving cell viability, when challenged by perturbations of Ca^{2+} homeostasis, suggesting that Grp94 may modify intracellular Ca^{2+} content, probably by interaction with calcium cycling proteins.

Physiological Function in Embryonic Development

Consistently with the observation that Grp94 is absent from most unicellular organisms, knockdown of Grp94 in different cell culture systems shows that low basal expression levels of the protein are sufficient to support cell growth and proliferation (Eletto et al. 2010). Similarly, two Grp94 gene mutations causing knocking out of the protein (the *Shepherd* mutation in *A. thaliana* and the frameshifts terminating Grp94 prematurely in the pre-B lymphocyte 70Z/3 cell line) are not cell-lethal, but rather affect selected processes. Although these data suggest that Grp94 does not appear to be essential for growth of individual mammalian cells in culture, survival of grp94$^{-/-}$ embryonic stem cells (ES) is strictly dependent from the presence of serum and IGFs. In addition, a parallel independent study showed that grp94$^{-/-}$ ES cells display an increased, compensatory, expression of ER chaperones, whereas in the presence of a 50% reduction of Grp94 levels, as it occurs in hemizygosis, there is no compensatory response (Mao et al. 2010). Furthermore, grp94$^{-/-}$ ES cells show a decreased ability to activate the XBP-1 pathway of the ER stress response. This observation reveals the involvement of Grp94 in the ER stress signaling, a function up to now mainly attributed to Grp78 (Glembotski 2008). In grp94$^{-/-}$ ES cells, the decreased expression of the ER stress-induced transcription factor XBP-1 might be due to the misfolding of the Grp94 client proteins TLR4 and TLR2, which in macrophages are known to activate the ER-stress sensor, inositol-requiring enzyme 1a (▶ IRE 1), and its downstream target XBP-1 (Mao et al. 2010).

Conversely, Grp94 absence is responsible for the occurrence of lethal phenotypes in plants, nematodes, fruit flies, and mice (Eletto et al. 2010). In mammals, Grp94 transcripts are found in oocytes and, in 5–8.5-d embryos, protein expression is highest in the embryonic and extraembryonic ectoderm and lower in the visceral endoderm. Grp94 is also detected in mesoderm cells emerging from the primitive streak. At later stages during organogenesis (E9.5–13.5) Grp94 is expressed within the developing heart, neuroepithelium, and branchial arches (Fig. 4). Despite of the widespread and early expression of Grp94 during embryonic development, its requirement during differentiation and organogenesis differs depending on the pattern of expression and the essential function of the client proteins.

One relevant example is given by skeletal muscle progenitors, where Grp94 expression is required for differentiation and maturation (Eletto et al. 2010; Gorza and Vitadello 2000). The phenotype of grp94$^{-/-}$ mice is lethal at E7.5 and embryo bodies generated by grp94$^{-/-}$ ES cells show any type of tissue differentiation, except the formation of striated and smooth muscles. The loss of ability to give origin to muscle is due to the absolute requirement of Grp94 in folding of IGFs. When supplemented with IGF,

grp94$^{-/-}$ ES cells are able to differentiate and express myogenin and contractile proteins; they can also fuse into myotube-like syncitia, although with far less efficiency of wild type ES cells. Since Grp94 depletion does not affect myoD levels, the chaperone is not required for the commitment to myogenesis. Conversely, Grp94 appears to be involved in myogenesis initiation. Few hours after switching the myogenic cell line C2C12 to differentiation medium, the protein translocates from the ER to the Golgi compartment and the cell surface, after interacting with and being Tyr-phosphorylated by the Src-kinase Fyn (Frasson et al. 2009 and Fig. 2a). It is presently unknown whether this re-localization is related to delivery of specific protein cargo; an identified relevant client is IGF-II, whose secretion and autocrine interaction with IGF-I and II receptors (► IGF-R1) is required to proceed along the differentiation program. Eventually, Grp94 appears to be involved in myotube formation. Knocking down Grp94 protein levels to 40% of wild type C2C12 cells is permissive for myogenic differentiation, but not for myotube formation (Gorza and Vitadello 2000). Since IGF supplementation does not fully rescue the myotube phenotype, it is likely that other Grp94 client proteins play a relevant role at this later stage.

Another example of the tissue specificity of Grp94 involvement in tissue differentiation and maturation has been recently provided by the study of murine hematopoietic and lymphopoietic precursors after conditional knock out of Grp94 (Staron et al. 2010). Despite the essential role of the chaperone in the folding of most TLRs and integrins, only lymphopoiesis, that is, maturation of pro-B lymphocytes and development of thymocytes, is severely affected. Furthermore, this defect cannot be rescued by wild type precursors, indicating that the chaperone regulates B- and T-lymphopoiesis in a cell-intrinsic fashion.

Pathophysiological Functions

Cancer

Most neoplastic cells express high levels of chaperone/stress proteins. ER chaperones are upregulated probably because hypoxia and hypoglycemia are two characteristics of the tumor environment and inducers of the ER stress response. A direct consequence of this upregulation is the enhancement of neoplastic cell survival; an indirect one is the increase and the release of tumor-specific peptides bound to chaperones. Srivastava and coworkers first identified a series of structurally related, but distinct, Grp94 molecules associated with specific immunogenicity of chemically induced mouse sarcoma. Specific antitumor immunity, raised after purification of the Grp94-peptide complex from a highly metastatic variant of murine lung carcinoma, is mediated by CD4$^+$ and CD8$^+$ T lymphocytes and natural killer cells and suppresses tumor metastasis. Furthermore, vaccination with Grp94-peptide complexes isolated from different tumors elicits antitumor response against all the tumors (Srivastava 2006). These results, which have been confirmed experimentally by other laboratories, represent the starting point for the development of anticancer immunotherapy, which is presently tested in several clinical trials (Woods and Mulders 2009).

Grp94 is also involved in anticancer therapies aimed to disrupt Hsp90 chaperone function on several oncogenic proteins by means of molecules like geldanamycin and analogues, which inhibit ATPase activity (Fig. 1a). The effects of these molecules on Grp94 function in tumor cells are rarely investigated, although they might play a role in reducing active cell surface levels of transmembrane receptors implicated in cancer, such as the receptor for the epidermal growth factor (► EGF receptor) and, most importantly, the release of IGFs (McLaughlin and Vandenbroeck 2011).

Autoimmune Diseases

Only immature cells and some neoplastic ones express Grp94 molecules at the cell surface. Spontaneous systemic lupus erythematosus-like autoimmune phenotype was experimentally obtained by inducing persistent expression of cell surface Grp94, via transgenic overexpression of an obligatory transmembrane form of Grp94 (Liu et al. 2006) or knock out of AIMP1, which allows the interaction of Grp94 with the KDEL receptor (Kim et al. 2010). A subsequent study excluded the direct role of the enforced cell surface expression of Grp94 demonstrating that disease phenotype occurs secondary to the concomitant increased expression of TLR4 and the enhanced sensitivity of the receptors to low levels of endotoxin released by the commensal flora (Liu et al. 2006). On the other hand, the inhibition of Grp94 localization to the surface of AIMP$^{-/-}$ cells, achieved by restoring KDEL receptor

uptake, appeared efficient in ameliorating the disease phenotype (Kim et al. 2010). Despite these controversial findings, the participation of this chaperone in the pathogenesis of autoimmune diseases has been recently demonstrated for rheumatoid arthritis. Released Grp94 is increased in synovial fluid from the joints of human rheumatoid arthritis patients, where it interacts with the extracellular domains of both TLR2 and TLR4, promoting the self-perpetuating activation of synovial macrophages (Huang et al. 2009).

A major nonimmune role in the pathogenesis of autoimmune myositis is played by the ER stress response accompanying transgenic MHC class I upregulation in mouse skeletal muscle fibers and leading to ▶ NF-kB activation and increased expression of ER chaperones. Extensive analysis of patients' biopsies revealed that Grp94 overexpression was restricted to regenerating myofibers, which recapitulating muscle differentiation overexpressed also MHC class I molecules and other autoantigens (Vitadello et al. 2010). In this context, the immunomodulatory and pro-inflammatory effects of Grp94 upregulation appear to play a secondary role, bound to muscle regenerative events, which, however, may stimulate further the immune response.

Nevertheless, Grp94 involvement in the inflammatory and the adaptive immune response represents an interesting therapeutical target for autoimmune disorders. Grp94 chaperone activity on client proteins, such as TLRs and the p40 subunit of the interleukin 12 family of cytokines (▶ IL-12), might be inhibited by radicicol and geldanamycin derivatives, which have been shown to reduce inflammatory markers and capillary leakage and modulate IL-12 family secretion levels (McLaughlin and Vandenbroeck 2011).

Ischemia-Reperfusion

Ischemia-reperfusion represents a strong inducer of the ER stress response. Several studies have been performed in the brain, where the activation of the ER stress response upregulates ER chaperone expression and is protective. Such an issue is far more relevant for mammalian cardiomyocytes, where control of intracellular calcium concentration $[Ca^{2+}]_i$ becomes critical during ischemia. Although evidence obtained in vitro and in vivo confirmed the activation of the ER stress response in ischemic-reperfused cardiomyocytes, it is less clear what function the ER stress response serves in the cardiac context. Several studies show that ER stress response contributes to ischemic damage, by inducing both apoptosis and autophagy-mediated cell death; conversely, others support the protective role of the ER stress response and of ER chaperones, among which is Grp94, in decreasing size of infarct or maintaining cardiomyocyte viability (Glembotski 2008). Such a discrepancy might depend upon the context of the study, in that protective aspects of the ER stress response predominate early after the ischemic injury, whereas pro-apoptotic features occur later. Although the mechanism through which Grp94 exerts cardioprotection remains to be determined, a body of evidence point to its role in the maintenance of calcium homeostasis. Grp94 overexpression improves myocyte survival after exposure to oxidative stress, a usual consequence of ischemia-reperfusion, because it reduces the degree of protein oxidation (Pizzo et al. 2010). Calcium dyshomeostasis is generated by and generate further oxidative stress. Grp94 overexpression, which can also be pharmacologically upregulated, appears to disrupt this pathway, by reducing the amount of releasable calcium from the stores.

Ischemia/reperfusion injury also induces an innate immune response, leading to an inflammatory reaction and tissue damage that have been attributed to engagement of TLR2 and 4. In the ischemic kidney, Grp94 contributes to the activation of ERK1/2 downstream pathway by dissociating from PP5, and thus inactivating this negative modulator. Although multiple pathway activated by ischemia-reperfusion act upon the ERK1/2 cascade of renal tubular cells, the dissociation of Grp94 from PP5 is dependent only from TLR2 activation (Mkaddem et al. 2009).

Inhibitors and Expression Regulators

The small number of client proteins and the involvement in specific cellular events make this chaperone an interesting target to be inhibited or increased, depending of the desired outcome.

Known inhibitors of both ATP hydrolysis and peptide binding, such as radicicol, geldanamycin, and analogues, are already tested in clinical trials mostly as inhibitors of Hsp90 chaperone activity (McLaughlin and Vandenbroeck 2011). The nucleotide analog 5′-*N*-ethylcarboxamidoadenosine (NECA), a broad-spectrum adenosine A_2 receptor antagonist, which binds Grp94

and not Hsp90, is presently used only in vitro. Recently, the molecule S-methyl 2-(4,6-dimethoxypyrimidine-2-yloxy)-3-methylbutanoate (GPM1) has been shown to directly interact with hydrophobic residues of Grp94 within the dimerization domain (Fig. 1a), facilitating its oligomerization and retrograde transport to endoplasmic reticulum via the KDEL receptor. In vivo administration of this compound reduced maturation of pAPCs and activation of B and T cells, alleviating the symptoms associated to an experimental model of systemic lupus erythematosus in mice (AIMP1$^{-/-}$) (Han et al. 2010).

Although upregulation of Grp94 is achieved pharmacologically together with that one of the other ER chaperones – one example is the ER stress response induced by celecoxib, a nonsteroidal anti-inflammatory drug, and its derivatives (McLaughlin and Vandenbroeck 2011) – a more selective Grp94 involvement apparently follows the administration of curcumin (Pizzo et al. 2010). Similarly to celecoxib, also curcumin is a SERCA inhibitor and able to induce an ER stress response by depleting calcium stores. A single pulse of curcumin at low dosage selectively upregulates Grp94 expression and contains the effects of oxidative stress on protein carbonylation and cell survival, whereas the benefits of such a short curcumin treatment cannot be observed, when Grp94 is knocked down by antisense cDNA (Pizzo et al. 2010).

Summary

Grp94 is a chaperone/stress protein of the ER and is expressed only in multicellular organisms. At variance of the paralog Hsp90, Grp94 is involved in the folding of a limited population of client proteins and only one co-chaperone has been identified so far. Grp94 absolute requirement for embryonic development derives from the specific role of the client proteins played in critical contexts, like IGFs for differentiation of muscle tissues, and TLR and integrins for lymphopoiesis.

In addition to chaperone activity, Grp94 serves different functions. It binds calcium and participates, through still unknown mechanisms, to the control of intracellular calcium homeostasis. When increased, consequent to the ER stress-response, it exerts cytoprotection against different stressors, such as calcium overload and reactive oxygen species. Grp94 may change its subcellular localization, migrating to Golgi and the cell surface, depending on the cell type and the activation of signal transduction pathways involving Src-kinase or JNK. Cell surface and released Grp94 molecules act as immune regulator of both the innate and adaptive responses by allowing maturation of pAPCs. The protein interacts with TLR2 and initiates inflammation promoting IL-6 and TNF-α secretion from pAPCs. It participates to the adaptive immune response by binding peptides and being internalized in post-endoplasmic reticulum endosomal compartment of pAPCs, where the carried peptide is loaded onto mature MHC class I molecules and can recruit specific $CD4^+$ and $CD8^+$ T lymphocytes.

Although this last property suggested the use of peptide-bound Grp94 as anticancer vaccine, the other features identify this ER chaperone as an interesting target for pharmacological therapy. Hampering the chaperone activity of Grp94 or increasing the protein retention into the ER reduces the release of pro-inflammatory and immune modulatory signals. Future attempts to change selectively Grp94 protein levels would permit to vary cell resistance against lethal stresses.

References

Eletto D, Darsh D, Argon Y. GRP94 in ER quality control and stress responses. Sem Cell Dev Biol. 2010;21:479–85.

Frasson M, Vitadello M, Brunati AM, La Rocca N, Tibaldi E, Pinna LA, Gorza L, Donella-Deana A. Grp94 is Tyr-phosphorylated by Fyn in the lumen of the endoplasmic reticulum and translocates to Golgi in differentiating myoblasts. Biochim Biophys Acta-Mol Cell Res. 2009;1793:239–52.

Glembotski CC. The role of the unfolded protein response in the heart. J Mol Cell Cardiol. 2008;44:453–9.

Gorza L, Vitadello M. Reduced amount of the glucose-regulated protein GRP94 in skeletal myoblasts results in loss of fusion competence. FASEB J. 2000;14:461–75.

Han JM, Kwon NH, Lee JY, Jeong SJ, Jung HJ, Kim HR, Li Z, Kim S. Identification of gp96 as a novel target for treatment of autoimmune disease in mice. PLoS One. 2010;5(3):e9792. doi:10.1371/journal.pone.0009792.

Huang QQ, Sobkoviak R, Jockheck-Clark AR, Shi B, Mandelin AM, Tak PP, Haines GK, Nicchitta CV, Pope RM. Heat shock protein 96 is elevated in rheumatoid arthritis and activates macrophages primarily via TLR2 signaling. J Immunol. 2009;182:4965–73.

Jockheck-Clark AR, Bowers EV, Totonchy MB, Neubauer J, Pizzo SV, Nicchitta CV. Re-examination of CD91 function in GRP94 (glycoprotein 96) surface binding, uptake, and peptide cross-presentation. J Immunol. 2010;185:6819–30.

Kim G, Han JM, Kim S. Toll-like receptor 4-mediated c-Jun N-terminal kinase activation induces gp96 cell surface expression via AIMP1 phosphorylation. Biochem Biophys Res Commun. 2010;397:100–5.

Koo BH, Apte SS. Cell-surface processing of the metalloprotease pro-ADAMTS9 is influenced by the chaperone GRP94/GP96. J Biol Chem. 2010;285:197–205.

Lee AS. Coordinated regulation of a set of genes by glucose and calcium ionophores in mammalian cells. Trends Biochem Sci. 1987;12:20–3.

Liu B, Li Z. Heat shock protein HSP90b1 (grp94, gp96) optimizes B cell function via chaperoning integrins and Toll-like receptors but not immunoglobulins. Blood. 2008;112(4):1223–30.

Liu B, Yang Y, Dai J, Medzhitov R, Freudenberg MA, Zhang PL, Li Z. TLR4 up-regulation at protein or gene level is pathogenic for lupus-like autoimmune disease. J Immunol. 2006;177:6880–8.

Liu B, Yang Y, Qiu Z, Staron M, Hong F, Li Y, Wu S, Li Y, Hao B, Bona R, Han D, Li Z. Folding of Toll-like receptors by the HSP90 paralogue gp96 requires a substrate-specific cochaperone. Nat Commun. 2010;1:79. doi:10.1038/ncomms1070.

Ma Y, Hendershot LM. ER chaperone functions during normal and stress conditions. J Chem Neuroanat. 2004;28:51–65.

Mao C, Wang M, Luo B, Wey S, Dong D, Wesselschmidt R, Rawlings S, Lee AS. Targeted mutation of the mouse grp94 gene disrupts development and perturbs endoplasmic reticulum stress signaling. PLoS One. 2010;5:e10852. doi:10.1371/journal.pone.0010852.

McLaughlin M, Vandenbroeck K. The endoplasmic reticulum protein folding factory and its chaperones: new targets for drug discovery? Br J Pharmacol. 2011;162:328–45.

Mkaddem SB, Werts C, Goujon JM, Bens M, Pedruzzi E, Ogier-Denis E, Vandewalle A. Heat shock protein gp96 interacts with protein phosphatase 5 and controls toll-like receptor 2 (TLR2)-mediated activation of extracellular signal-regulated kinase (ERK) 1/2 in post-hypoxic kidney cells. J Biol Chem. 2009;284:12541–9.

Nganga A, Bruneau N, Sbarra V, Lombardo D, Le Petit-Thevenin J. Control of pancreatic bile-salt-dependent-lipase secretion by the glucose-regulated protein of 94 kDa (Grp94). Biochem J. 2000;352:865–74.

Ostrovsky O, Makarevich CA, Snapp EL, Argon Y. An essential role for ATP binding and hydrolysis in the chaperone activity of GRP94 in cells. Proc Nat Acad Sci USA. 2009;106:11600–5.

Ostrovsky O, Eletto D, Makarewich C, Barton ER, Argon Y. Glucose regulated protein 94 is required for muscle differentiation through its control of the autocrine production of insulin-like growth factors. Biochim Biophys Acta Mol Cell Res. 2010;1803:333–41.

Pizzo P, Scapin C, Vitadello M, Florean C, Gorza L. Grp94 acts as a mediator of curcumin-induced antioxidant defence in myogenic cells. J Cell Mol Med. 2010;14:970–81.

Randow F, Seed B. Endoplasmic reticulum chaperone gp96 is required for innate immunity but not cell viability. Nat Cell Biol. 2001;3:891–6.

Srivastava PK. Therapeutic cancer vaccines. Current Opin Immunol. 2006;18:201–5.

Staron M, Yang Y, Liu B, Li J, Shen Y, Zúñiga-Pflücker JC, Aguila HL, Goldschneider I, Li Z. gp96, an endoplasmic reticulum master chaperone for integrins and Toll-like receptors, selectively regulates early T and B lymphopoiesis. Blood. 2010;115:2380–90.

Vitadello M, Doria A, Tarricone E, Ghirardello A, Gorza L. Myofiber stress-response in myositis: parallel investigations on patients and experimental animal models of muscle regeneration and systemic inflammation. Arthritis Res Ther. 2010;12:R52. doi:10.1186/ar2963.

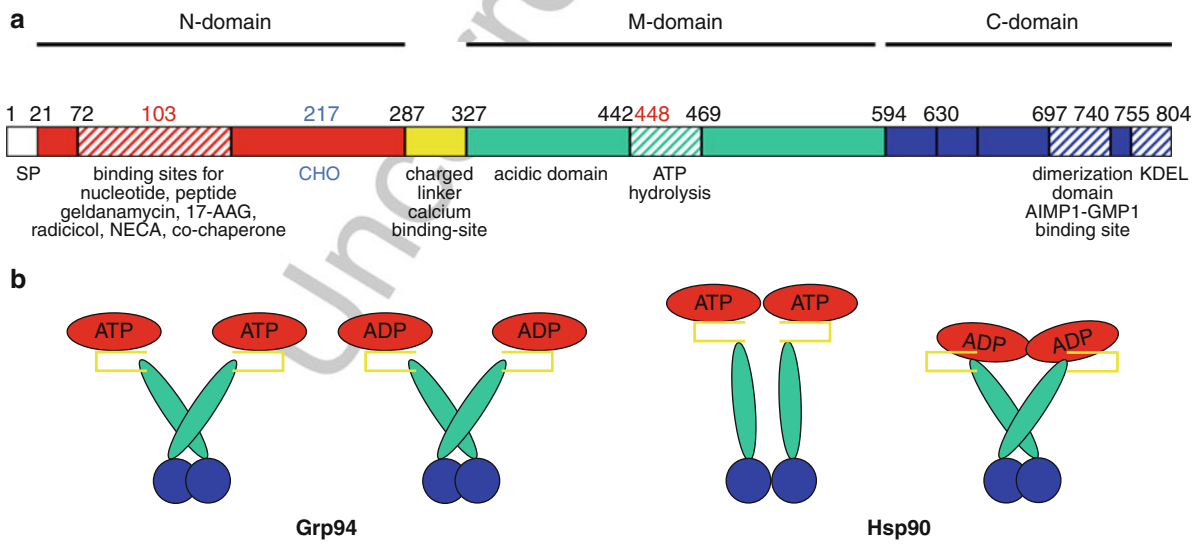
Wanderling S, Eletto D, Makarewich C, Barton ER, Argon Y. Grp94 is essential for mesoderm induction and muscle development because it regulates insulin-like growth factor secretion. Mol Biol Cell. 2007;18:3764–75.

Woods CG, Mulders P. Vitespen: a preclinical and clinical review. Future Oncol. 2009;5:763–74.

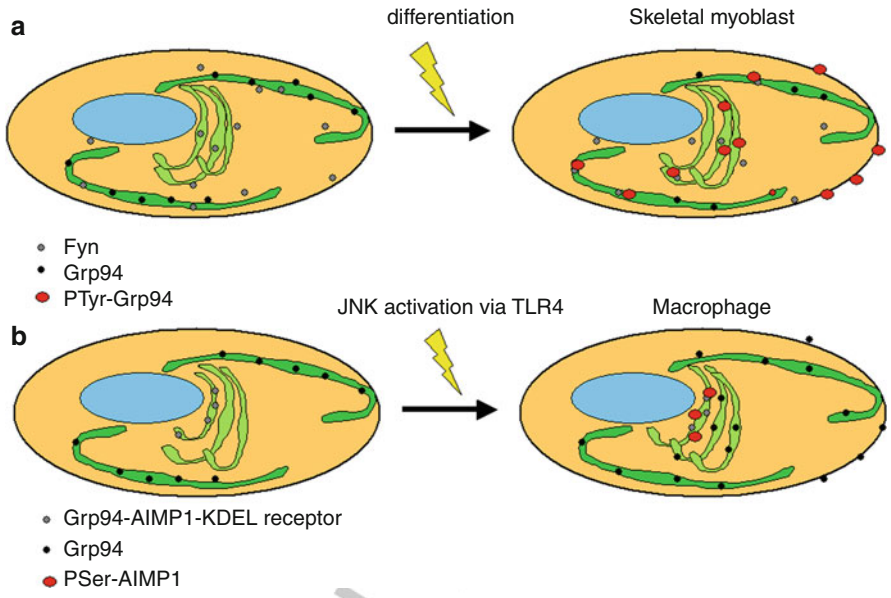
Yang Y, Liu B, Dai J, Srivastava PK, Zammit DJ, Lefrancois L, Li Z. Heat shock protein gp96 is a master chaperone for toll-like receptors and is important in the innate function of macrophages. Immunity. 2007;26:215–26.

Grp94 (HSP90B1), Table 1 List of proteins requiring the Grp94 chaperone for folding, as shown by Grp94 knocking out or down experiments

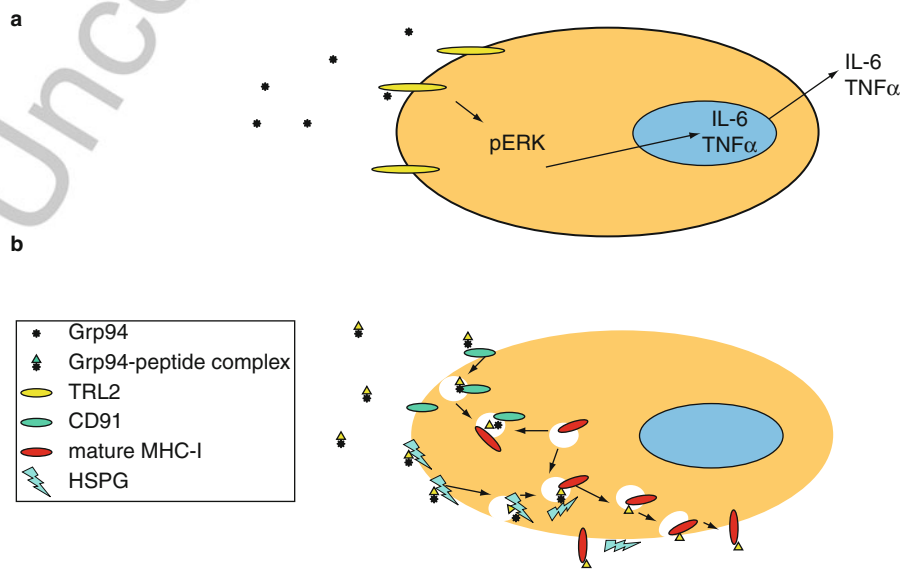
Proteins	References
Membrane receptors	
TLR (TLR1, TLR2, TLR4, TLR5, TRL6, TLR7, TLR9, TLR11, but not TLR3)	Randow and Seed (2001), Yang et al. (2007), Liu and Li (2008), Liu et al. (2010)
Integrins (α1, α2, α4, αD, αE, αL, αM, αV, αX, β2, β5, β6, β7, β8)	Randow and Seed (2001), Liu and Li (2008), Staron et al. (2010)
Secreted enzymes and molecules	
ADAMTS9 metalloprotease	Koo and Apte (2010)
Bile-salt-dependent lipase	Nganga et al. (2000)
IGFs (IGF-I, IGF-II)	Wanderling et al. (2007), Ostrovsky et al. (2009), Ostrovsky et al. (2010)



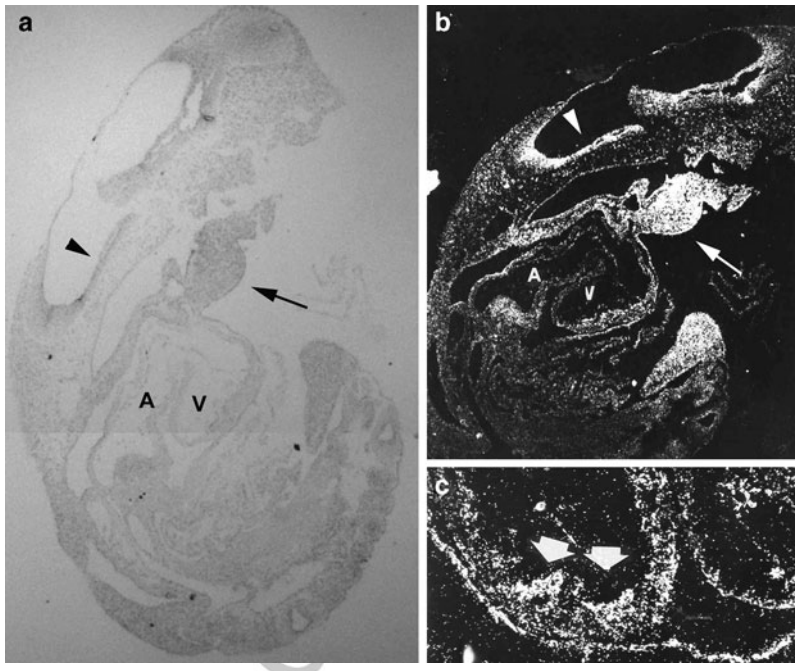
Grp94 (HSP90B1), Fig. 1 Diagrams illustrate Grp94 structure and configuration. (**a**) Primary structure of Grp94 and domain boundaries identification. Numbers identify amino acids. Numbers 103 and 448 identify amino acid residues involved in ATP hydrolysis; number 217 indicates an identified site of glycosylation. *Crosshatched* regions identify functions specified below and described in the text. SP: signal peptide; CHO: oligosaccharide. (**b**) Schematic representations of domain interactions of Grp94 and Hsp90 proteins observed after binding with ATP or ADP. At variance with Hsp90, whose conformation is changed to a catalytically active one by nucleotide binding, Grp94 ground-state conformation is apparently not affected



Grp94 (HSP90B1), Fig. 2 Signals responsible for Grp94 cell-surface localization. (**a**) Differentiation of skeletal myoblasts induced by low serum culture medium activates an ER-resident Fyn, which interacts with and Tyr-phosphorylates Grp94, promoting its migration to the Golgi compartment and the cell surface. (**b**) Exposure of circulating or spleen macrophages to bacterial lipopolysaccharide activates TLR4 and JNK-mediated AIMP1 phosphorylation, which dissociates Grp94-KDEL receptor complex and favor Grp94 export to the cell surface



Grp94 (HSP90B1), Fig. 3 Schematic representations of the pathways activated by exposure of pAPCs to Grp94 in innate and adaptive immune responses. (**a**) Interaction of soluble Grp94 with TLR2 induces IL-6 and TNF-α secretion in human macrophages through ERK1/2 pathway activation. (**b**) Peptides complexed to Grp94 are internalized by pAPCs after either receptor-mediated or fluid-phase endocytosis, and loaded onto mature MHC class I molecules in a post-ER compartment. HSPG: heparan sulfate proteoglycan



Grp94 (HSP90B1), Fig. 4 In situ hybridization analysis of Grp94 mRNA distribution in the E10 rabbit embryo. (**a**) and (**b**) show bright and dark field micrographs, respectively, of a sagittal section of the whole embryo. A and V indicate primordium of the atrial and ventricular myocardium, respectively. Strong hybridization signals are detectable at the level of branchial arches (*thin arrow*) and neuroepithelium (*arrowhead*). (**c**) The micrograph shows the myocardium of the ventricular primordium at higher magnification. *Large arrows* point to stronger hybridization signals at the level of ventricular trabeculae