The Unmysterious Roles of HSP90: Ovarian Pathology and Autoantibodies

Eusebio S. Pires

Abstract The heat shock proteins (HSPs) are a group of evolutionarily conserved proteins with important physiological functions, whose synthesis is enhanced by elevated temperature or other stresses. HSPs show high sequence homology between different species, from bacteria to humans. Despite the significant degree of evolutionary conservation, HSPs are highly immunogenic. Of the several HSPs, HSP90 is an abundant, constitutively expressed chaperone constituting around 1–2% of total cellular protein under non-stress conditions. This protein from even the most distantly related eukaryotes has 50% amino acid identity, and all have more than 40% identity with the *Escherichia coli* protein. They are immunodominant antigens for many common microbes, and thus their epitopes are recognized by the immune system. As HSPs are overexpressed at sites of acute and chronic inflammation, individuals are likely to be sensitized during the course of a microbial infection encountered during life. This chapter considers the evidence of a role for HSP90 in autoimmune ovarian failure, where autoantibodies to it have been observed in patients, and has been correlated to infertility.

1 Introduction

As I studied Immunology right from my days of graduation, I kept asking myself one of the most important questions in Immunology—how does the immune system distinguish between "*self*" and "*nonself*"? In a normal, healthy individual, the immune system is able to specifically eliminate unwanted, non-self, and potentially dangerous organisms without attacking its own tissues or cellular components. In some cases, however, this fine-tuning is disturbed leading to autoimmunity: the activation and proliferation of autoreactive lymphocytes or even to an autoimmune disease. In the case of organ-specific autoimmunity, the antibodies produced by the activated B-lymphocytes are directed to self-components expressed only in a

E.S. Pires, Ph.D. (✉)
Department of Obstetrics and Gynecology, School of Medicine, University of Virginia, P.O. Box 800732, Jordan Hall, Charlottesville, VA 22908, USA
e-mail: eusebiopires@gmail.com; ep7d@virginia.edu

D.J. MacPhee (ed.), *The Role of Heat Shock Proteins in Reproductive System Development and Function*, Advances in Anatomy, Embryology and Cell Biology 222, DOI 10.1007/978-3-319-51409-3_2

specific tissue or type of cells. In the case of systemic autoimmunity, the autoantibodies are directed to various autoantigens, which are usually expressed in a wide variety of tissues and, at the cellular level, can be present in the nucleus, cytoplasm, or at the cell surface. Although many autoantigens are ubiquitously expressed, the autoimmune diseases in which they are autoantigenic are often limited.

2 Autoimmunity and Ovarian Autoimmunity

The human body can undergo an autoimmune attack, and autoimmunity of most organs has been reported leading to autoimmune disease which may or may not be life threatening. An increasing number of individuals throughout the world are affected by autoimmune disease, and a large and diverse group of disorders have been categorized by tissue injury or pathology (Lernmark 2001). In general, these diseases are associated with humoral or cell-mediated immune reactions against one or more of the body's own constituents, but it has been customary to divide autoimmune diseases into two categories: systemic and organ-specific. Over the past decades, the list of diseases associated with autoantibodies against tissues, cells, or specific autoantigens has grown enormously (Lernmark 2001). The classification of a disease as autoimmune has traditionally been based on the detection of autoantibodies that could be visualized reacting with an affected tissue or cell. Like all other organs, the reproductive tissues also undergo an autoimmune attack. Many scientists have contributed enormously towards this field of research and to date there have been several differences in opinions. The human ovary is a target of an autoimmune attack in various circumstances, including several organ-specific or systemic autoimmune diseases. Clinically, the ensuing ovarian dysfunction often results in premature ovarian failure (POF), but other pathologies involving the ovaries, such as unexplained infertility, polycystic ovary syndrome (PCOS), and endometriosis, have been associated with anti-ovarian autoimmunity (Luborsky 2002; Ahonen et al. 1987; Anasti 1998; Coulam et al. 1986). POF or premature menopause (or recently rechristened to primary ovarian insufficiency, POI) is a syndrome clinically defined by functional failure of the ovary before the age of 40 years. POF is a heterogeneous disorder with a multicausal pathogenesis, and chromosomal, genetic, enzymatic, iatrogenic, or infectious aberrations may all form the basis for the disappearance of ovarian follicles (Hoek et al. 1997). These aberrations may influence the ovary at any stage of life, including the prepubertal, pubertal, or reproductive stages. There is accumulating evidence that some cases of POF are due to a faulty recognition of self in the ovary by the immune system (Hoek et al. 1997). POF was defined by de Moraes-Ruehsen and Jones (de Moraes-Ruehsen and Jones 1967) as an unphysiological cessation of menses before the age of 40 year and after puberty (hence, in fact, secondary amenorrhea). Women with POF have a hypergonadotropic-hypoestrogenic hormone profile (Hoek et al. 1997). The involvement of autoimmunity has been most extensively studied in

POF. However, the etiological significance of autoimmunity in these pathologies still remains controversial.

3 Antigens Involved in Ovarian Autoimmunity

The diagnosis of an autoimmune mechanism in these pathologies has relied for a long time on the detection of anti-ovarian antibodies (AOA) (Barbarino et al. 2005; Betterle et al. 2002; Damewood et al. 1986). However, little is known about the molecular targets of the autoimmune effectors, and very few autoantigens have been formally identified. The specificity of the available tests has also been questioned (Fenichel et al. 1997; Novosad et al. 2003). Our group developed a novel blocking protocol (Pires et al. 2006) which appreciably reduced the nonspecific reactivity. Using this specific and sensitive test, we have been able to identify a number of specific molecular and cellular targets (Pires et al. 2007) and found that AOA testing has clinical significance (Pires et al. 2011a). From the large number of samples screened, we found that the oocyte is the major cell that is being targeted (Pires et al. 2007). It was also seen that a large number of AOA positive samples reacted with a 90 kDa protein, indicating that this protein (designated EP90) was an immunodominant antigenic target (Pires et al. 2007). This well conserved acidic protein was oocyte specific, serine–threonine phosphorylated, non-glycosylated, and expressed in the day 0 old rat ovary and onwards (Pires and Khole 2009a). LC/MS and MS/MS analysis of this EP90 protein revealed its identity to be human heat shock protein 90 beta (HSP90β) (Pires and Khole 2009a). The identity of EP90 was then reconfirmed using the patient's sera in two sets of experiments. First, most of the EP90 reactive sera of POF patients were seen to react with a recombinant HSP90 protein. Second, a monoclonal antibody to HSP90 showed reactivity with the partially purified EP90 protein. Our data, therefore, suggested that HSP90β could be a major autoantigen of self-reactive antibodies in the study group (Pires and Khole 2009a, b; Pires 2010; Pires et al. 2011a, b; Khole et al. 2012). Antibodies to HSP90 in a female mouse model were generated by active immunization with an immunodominant peptide of HSP90. There was a significant drop in the fertility index due to an increase in pre- and post-implantation loss, associated with an increased incidence of degenerated eggs and embryos. The ovaries showed an increase in the number of empty and degenerated follicles and extensive granulosa cell deaths, which was reflected by the decrease in the levels of Nobox and Gja1 gene expression (Choudhury and Khole 2013). In addition to HSP90 protein as a target, patients are also likely to have several other antigens including P450 side chain cleavage (SCC) enzyme, 17-hydroxylase, α-enolase (Forges et al. 2004; Sundblad et al. 2006), and also different molecular targets (Pires et al. 2007) including reports of alpha actinin, HSP70, and beta actin as targets (Mande et al. 2011).

4 Heat Shock Proteins (HSPs)

HSPs or chaperonins, as they were previously called, are a group of evolutionary conserved proteins that show high sequence homology between different species, from bacteria to humans (Morimoto 1993). They are classified based on molecular size, sequence similarities, and location within the cell and function. Despite the significant degree of evolutionary conservation (Sreedhar et al. 2004), HSPs are highly immunogenic (Calvert et al. 2003). It has been postulated that they could activate antigen-presenting cells, serving as a danger signal to the immune system (Gallucci and Matzinger 2001).

5 Heat Shock Protein-90 (HSP90) in Ovarian Biology, Localization, and Function

Of the several HSPs, HSP90 is an abundant, constitutively expressed chaperone constituting around 1–2% of total cellular protein under non-stress conditions (Falsone et al. 2005; Lindquist and Craig 1988). This protein from even the most distantly related eukaryotes has 50% amino acid identity, and all have more than 40% identity with the *Escherichia coli* protein (Bardwell and Craig 1987). There are two major cytoplasmic isoforms of HSP90: HSP90α and HSP90β, which possibly arose by gene duplication roughly 500 million years ago (Gupta 1995). Sequence similarities between the α and the β forms are 93.4% using the EBI tool: EMBOSS pairwise alignment algorithm. Heat-shock protein-90 is mainly a constitutive dimer (α α or β β); however, monomers (α or β), heterodimers (α β), and higher oligomers of both isoforms also exist (Sreedhar et al. 2004). An important difference is that the α form readily dimerizes, whereas the β form does so with much less efficiency (Sreedhar et al. 2004). Expression of HSP90α is lower compared with HSP90β in most cells, and HSP90α is highly inducible in contrast to HSP90β, whose expression is thought to be constitutive (Hilscher et al. 1974; Gruppi et al. 1991). The isoform specificity is not restricted only to the biochemical level, but extends to the functional role of HSP90 in cell differentiation and development. On the one hand, HSP90α has been shown to play a regulatory role in muscle cell differentiation of zebrafish (Lele et al. 1999), while on the other hand it is shown to inhibit cellular differentiation of embryonal carcinoma cells to trophectoderm (Voss et al. 2000). Studies have shown that HSP90β plays a major role in trophoblast differentiation, and HSP90β-deficient homozygous mice with normal expression of HSP90α fail to differentiate to form placental labyrinths (Voss et al. 2000). Expression of HSP90β is observed throughout the germ cell lineage from very early stages of development to adult oocytes and spermatocytes (Ohsako et al. 1995). Studies have suggested that HSP90β may be required for early

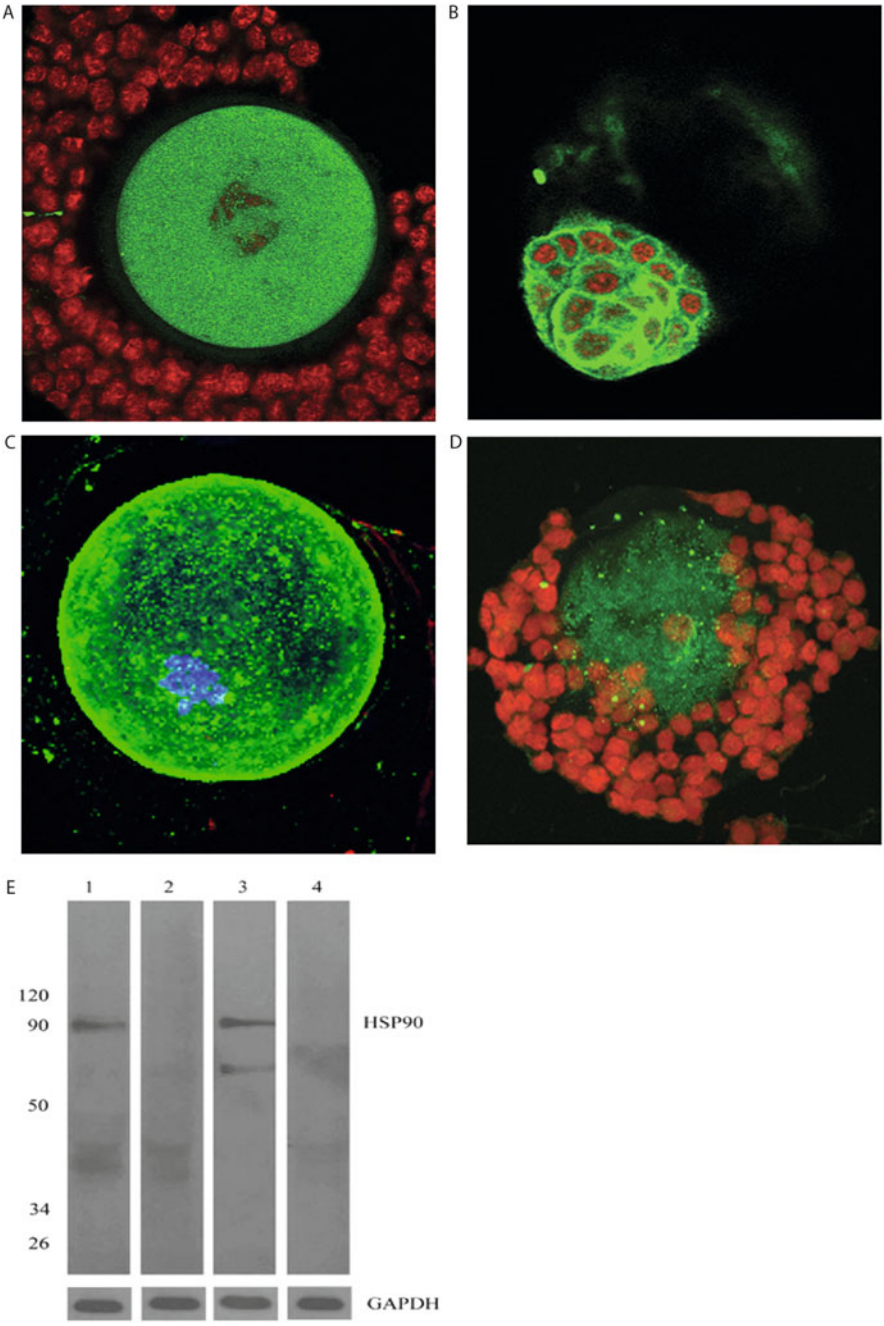

Fig. 1 HSP90 expression in the oocyte. Confocal images of HSP90 expression (*green staining*) in mouse embryogenesis showing immunostaining in the germinal vesicle breakdown oocyte (GVBD; **a**) and in the cells of a blastocyst (**b**). Nuclei were counterstained with propidium iodide as seen in *red*. Indirect immunofluorescence studies using anti-EP6 HSP90 peptide polyclonal antibodies showed surface expression (*green stain*) in an ovulated mouse oocyte (**c**) while the

embryonic development. Experiments from the lab definitively indicated that HSP90β is expressed in the ovary and abundantly in the oocytes and the early embryo (Pires and Khole 2009a). The experiment involved indirect immunofluorescence where a high titer anti-HSP90β positive patient sera was used to stain mouse oocytes and embryos. The sera immunostained the oocyte (green stain) within a germinal vesicle breakdown (GVBD) follicle as well as other stages of embryogenesis till the blastocyst stage, staining the cells of the inner cell mass as well as the trophectoderm (Fig. 1a, b). An anti-EP6 peptide-specific rabbit polyclonal antibody raised in the lab (Pires et al. 2011a) similarly stained the mouse oocytes (Fig. 1c) and embryos while a commercially available HSP90 antibody also stained the oocytes (Fig. 1d). It was interesting to note that the granulosa cells or the cumulus mass were immunonegative. In the same figure, a Western blot analysis was done using ovarian protein extracts and immunoprobed with either commercially available HSP90α or HSP90β polyclonal antibodies. The figure clearly shows that the β form was ovary specific (Fig. 1e, lane 1) while the α form was present in the testes (Fig. 1e, lane 3) and was not in the ovary (Fig. 1e, lane 2).

6 HSP90 Autoantibodies and Ovarian Pathology

HSPs are highly evolutionarily and phylogenetically conserved. They are immunodominant antigens for many common microbes and therefore their epitopes are recognized by the immune system (Van Eden et al. 2002). As HSPs are overexpressed at sites of acute and chronic inflammation (Van Eden 1999), many infertile couples are likely to be sensitized during the course of a microbial infection which they are likely to encounter during life. In view of this, it could be proposed that as a result of prolonged or repeated asymptomatic chronic infections early in the life of these infertile women, they could have developed anti-HSP90 antibodies systemically. These antibodies could thus target the ovarian antigens leading to ovarian failure. This may be relevant to human reproduction, since many couples with fertility problems have had a previously undetected genital tract infection (Witkin et al. 1994). In general, HSPs are among the first proteins produced during embryogenesis (Bensaude and Morange 1983). As a consequence of this, pregnancy outcome may be affected since the constitutive forms of HSP90 (and also

Fig. 1 (continued) nucleus was stained with DAPI (*blue*). Similar immunoreactivity with a commercially available monoclonal antibody to HSP90 was seen in the ooplasm of a mouse oocyte (**d**). No staining in the cumulus granulosa cells was observed. Western blot analysis (**e**) depicted the major isoform in total human ovarian extracts to be the beta isoform of HSP90 as seen at the 90 kDa loci when probed with a commercially available HSP90β antibody (lane 1). No immunoreactivity was seen with a commercially available HSP90α antibody (lane 2). Mouse testicular extracts were used as a positive control for the HSP90α antibody (lane 3). "No primary/secondary alone" antibodies served as negative control (lane 4). Antibody to GAPDH served as loading control to ensure equal amounts of protein per lane

HSP70) are both known to be expressed at high levels during preimplantation mouse embryo development (Loones et al. 1997).

The matured MII egg is transcriptionally an inactive cell and as such is a storehouse of maternal proteins and mRNA required for fertilization and initiation of zygotic development (Calvert et al. 2003). Using the approach of 2D Proteomics and Tandem Mass Spectrometry, this group reported that among the hundreds of proteins that are expressed by the mammalian oocyte, most of them are molecular chaperones and HSPs. Involvement of anti-HSP90 antibodies in pathogenesis of several diseases has been reported by several researchers in various other disease conditions. Faulds et al. (1995) have shown the presence of the antibodies in systemic lupus erythematosus, Hayem et al. (1999) have shown these antibodies in serum of patients with rheumatoid arthritis, Trieb et al. (2000) discussed the presence of this antibody in osteocarcinoma patients, and Vidal et al. (2004) have shown the presence of HSP90 antibodies in women with ovarian cancers. My group were the first to report the presence of anti-HSP90 antibodies in women with infertility (Pires and Khole 2009a). In our study, our data clearly showed that sera from 59 out of the 79 patients who were 90-kDa positive reacted to HSP90 by dot blot as well as ELISA.

The involvement of multiple antigenic targets, the high prevalence of anti-HSP90 antibodies, and the broad gamut of immunological disorders in which anti-HSP90 antibodies are found support the proposal that anti-HSP90 antibodies could be present in patients with a putative defect in immunoregulation. To establish importance of AOA testing in infertile women, a clinical reproductive outcome comparative study was conducted between two groups of women undergoing IVF-ET (Pires et al. 2011b). Group 1 consisted of women who tested positive for AOA, put on corticosteroid therapy, reverted to AOA negative, and then taken up for IVF-ET. Group 2 was seronegative for AOA. Five hundred seventy infertile women enrolled for IVF-ET, AOA testing, corticosteroid therapy, and IVF-ET/ICSI. Around 40% of the patient who were AOA positive made antibodies to HSP90. Comparable clinical outcome and significance of AOA testing were established. AOA positive serum samples were sent periodically to reinvestigate the presence of AOA after corticosteroid therapy and women who turned AOA negative were taken up for IVF-ET. Of the 70/138 women in group 1 who were treated with corticosteroids and turned seronegative for AOA, 22/70 were poor responders and needed donor oocyte-recipient cycles. Results demonstrated that fertilization and clinical pregnancy rates between both groups were comparable. Nevertheless, it was also observed that there was a poor response to the stimulation protocol, smaller number of oocytes retrieved, and more spontaneous abortions in group 1 women. Hence, not all outcomes following the treatment were comparable between the two groups. Based on this data, we proposed that AOA could be used as a diagnostic marker for ovarian failure, and AOA testing could be included in the battery of tests investigating and treating infertility (Pires et al. 2011b).

7 HSP90 Interactome

Proteins rarely act alone and many times they team up and have intricate connections to undertake biological functions at both cellular and systems levels. A critical step towards unraveling the complex molecular relationships in living systems is the mapping of protein-to-protein physical "interactions." The complete map of protein interactions that can occur in a living organism is called the "*Interactome*" (Cusick et al. 2005). Using a complementary proteomics approach directed towards identification of novel proteins that interact with HSP90, an interactome for HSP90

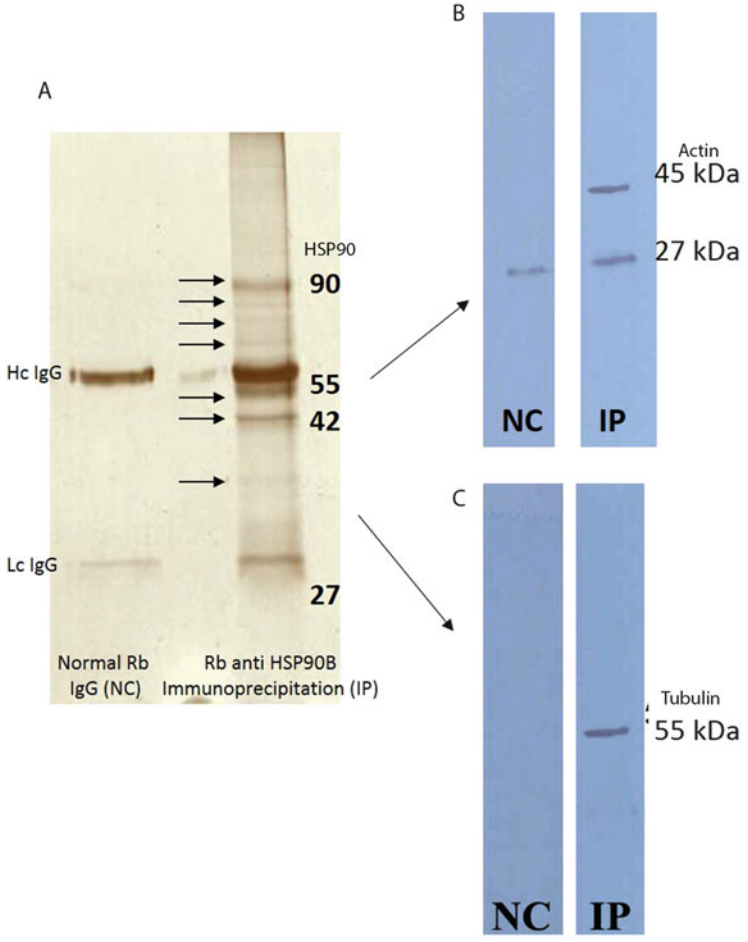

Fig. 2 Cytoskeletal proteins actin and tubulin co-immunoprecipitate with HSP90. Commercially available HSP90B antibody was used to pull down HSP90 protein as well as its binding partners as seen in the silver stained gel (**a**). A Western blot analysis was done on a parallel sample run and probed with actin (**b**) as well as tubulin (**c**) antibodies confirming that they were pulled down in a complex with HSP90

was established. These methods are co-immunoprecipitation, pull-downs with biotinylated geldanamycin, and immobilization of HSP90β on sepharose (Tsaytler et al. 2009). Among the proteins identified by this group, most of these were highly abundant proteins, including major HSP90 co-chaperones, structural proteins, ribosomal subunits, and metabolic and RNA-processing proteins. Also, novel HSP90 substrates at relatively low abundance were identified, such as the signaling proteins cell division protein kinase 3 (Cdk3) and tripartite motif containing 29 (TRIM29). Another study reported on the application of immunoprecipitation (IP) with endogenous HSP90, which yielded 39 interaction partners of HSP90 (Falsone et al. 2005). Of these reported proteins, only nine were previously established as HSP90 partners.

Identification of cytoskeletal proteins, ribosomal subunits, and metabolic and RNA-processing proteins strengthen the hypothesis that, besides the regulation of a specific set of proteins, HSP90 has a central function in several fundamental cellular processes (McClellan et al. 2007; Lotz et al. 2008). Thus, the IP and biotin-GA-mediated purification of structural proteins, including tubulin and kinesin, provides further evidence for the involvement of HSP90 in the assembly of the tubulin-based cytoskeleton network, cytokinesis, and cellular transport (McClellan et al. 2007; Te et al. 2007). Isolation of RNA-binding proteins and ribosomal subunits points to the suggested role of HSP90 in ribosomal subunit nuclear export and RNA processing and maintenance (Schlatter et al. 2002; Zhao et al. 2008). A pilot study done in the lab using a rabbit anti HSP90β as the IP antibody in a total crude ovarian extract (silver stain of the IP reaction shown in Fig. 2a), followed by Western blotting with actin and tubulin antibodies revealed IP of these 2 proteins at their appropriate known masses (Fig. 2b, c, respectively). This indicates that structural proteins such as actin and tubulin do interact with HSP90, and these interactions could be needed to maintain the cytoarchitecture of the cells.

8 Proposed Autoimmune-Mediated Ovarian Infertility via HSP90 as a Game Player

The HSPs play a critical role both in normal function and in the response to stress and are highly conserved in evolution, to an extent even greater than that of evidently essential proteins such as actin or myosin. HSP90 shows 60% amino acid identity with the corresponding yeast protein, 78% identity with the Drosophila protein, and a corresponding protein C62.5 has been identified in *E. coli* (Latchman and Isenberg 1994). Such evolutionary conservation of the HSPs thus results in homologues of the human HSPs being present in bacteria and other organisms such as parasitic protozoa which can infect humans. Such exogenous HSPs constitute the major target of the human immune response to these pathogens, and antibodies and

T cells against the appropriate exogenous HSPs have been detected in individuals infected with organisms as different as the mycobacteria and the protozoan parasites *Plasmodium falciparum* and *Schistosoma mansoni* (Biswas and Sharma 1994; Neumann et al. 1993). Although in these cases the antibodies appear to have a protective effect, in other cases the ability of antibodies and T cells directed against bacterial or protozoan HSPs to also react with the closely related endogenous human HSPs may result in autoimmunity leading to an autoimmune disease. It is more likely that, following the initial priming of the immune system by exposure to exogenous HSPs, some subsequent event involving the endogenous human HSPs is required to trigger the autoimmune response. Such an event could involve either the enhanced expression of the human HSPs or their expression on the cell surface, which can be brought about by a variety of stimuli such as microbial infection. Therefore, initial exposure to exogenous HSPs requires a second event, such as microbial infection, which results in upregulation of the human HSPs and/or their surface localization. This, in turn, induces antibodies and T cells primed against the bacterial or protozoan proteins reacting with the human proteins, leading to autoimmune disease.

In view of this, we propose that a majority of infertile women could have anti-HSP90β antibodies in circulation as a result of prolonged or repeated asymptomatic chronic infections early in life. In the course of a woman's reproductive life, these antibodies could then target the ovarian antigens (e.g., by exposure to the immune system due to accidents, trauma, or immune system memory), leading to early ovarian failure. This may be relevant to human reproduction because many couples with fertility problems have had a previously undetected genital tract infection (Witkin et al. 1994). The constitutive β form of HSP90 is known to be expressed at high levels during preimplantation mouse embryo development. Therefore, the presence of anti-HSP90β antibodies in women during early pregnancy is likely to have detrimental consequences. In parallel, monoclonal antibodies to mammalian heat shock proteins were also shown to impair mouse embryo development in vitro (Neuer et al. 1998).

The precise mechanism of anti-HSP90 antibody-related inhibition of embryo development and ovarian failure has not been reported. Recent reports have suggested that the penetration of autoantibodies into living cells participate in the pathogenesis of diverse autoimmune diseases. For instance, autoantibodies to HSP27 (also known as HSPB1), which are found in patients with glaucoma, have been shown to penetrate into human retinal neuronal cells and induce their active death, most likely by inactivating the ability of HSP27 to stabilize the actin cytoskeleton, suggesting a pathogenic role of these antibodies (Ruiz-Arguelles and Alarcon-Segovia 2001). Also, there is increasing evidence to suggest the presence of HSP90 on the cell surface (Calvert et al. 2003; Eustace and Jay 2004; Sidera et al. 2008) thereby making it accessible to the autoantibodies. Thus, the mere presence of these autoantibodies in the circulation may not only bring detrimental effects by binding to the surface HSP90, but they can also get internalized into the cell and destroy the ovarian cytoarchitecture.

So to reiterate, a persistent microbial infection in some of these women may mount a primary response to microbial HSP90 (Latchman and Isenberg 1994). Any accidental exposure could then account for a secondary immune response (by molecular mimicry). This could reactivate B lymphocytes previously sensitized to microbial HSP90 (by clonal expansion – immune system has memory). The humoral arm of the immune system would then get activated leading to increased production of anti-HSP90 antibodies. This probably could disturb immune regulating mechanisms necessary for oocyte and embryo development and maintenance.

At the cell structural level, it can be speculated that HSP90 associates itself with filamentous actin (Koyasu et al. 1986; Kellermayer and Csermely 1995). It is also well documented that HSP90 also binds to tubulin (Redmond et al. 1989; Czar et al. 1996). In stress conditions, HSP90 acts as a chaperone. During an environmental stress, which is exhibited by the presence of low ATP, this is known to be detrimental to these cytoskeletal frameworks (Loktionova et al. 1996) and if women have antibodies to HSP90 in their circulation, the possible interactions between HSP90 (in this case functioning as a molecular chaperone) and the cytoskeletal proteins may be disturbed and destroyed. Thus, there could likely be a collapse of the ovarian cytoarchitecture as the main role of HSP90 as a chaperone is to maintain this cytoskeletal framework. A proposed model leading to autoimmune infertility via HSP90 as a game player has been schematically depicted in Fig. 3.

9 Summary, Outlook, and Future Directions

HSP90 is a ubiquitous and an essential eukaryotic molecular chaperone that stabilizes a large set of client proteins, many of which are involved in various pathways. This chapter has elucidated that HSP90 plays an important and indispensable role in ovarian biology and pathology (Pires and Khole 2009a, b; Pires 2010; Pires et al. 2011a, b, 2013). The driving theme that is of interest to many is to be able to differentiate in its *housekeeping role*, in comparison to its *pathobiochemical role*. One of the important steps in the quest for HSP90 clients is to ensure its validity and to design experimental system/s to address more specific questions concerning mechanism and physiological importance related to a disease state. Because HSP90 also plays an important role in activation of the immune system, its pharmacological inhibition has increasingly become the focus of research on autoimmune diseases. There is a need to further characterize this chaperone and explore strategies for its utilization as a theranostic (therapeutics and diagnostics) agent.

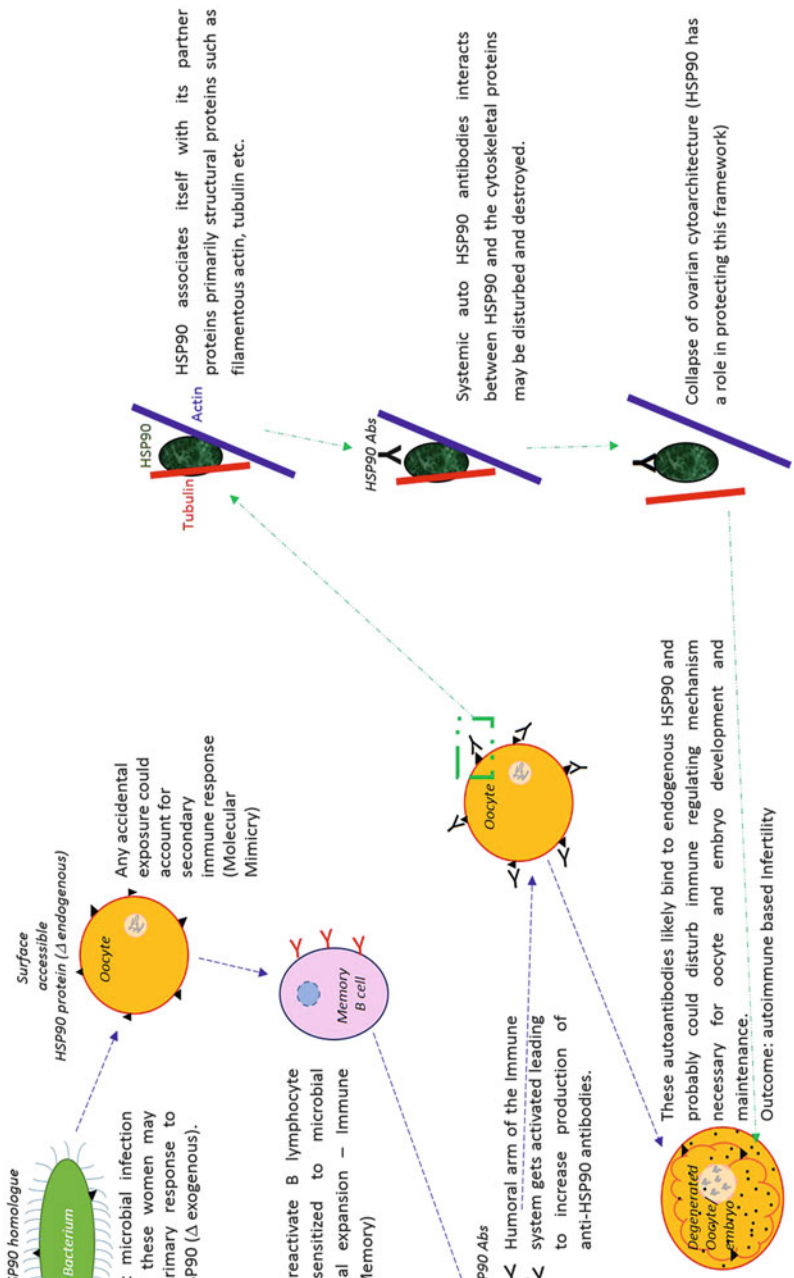

Fig. 3 A proposed model involving HSP90 as a key player in an outcome leading to autoimmune ovarian failure and infertility

Acknowledgements The author would like to place on record the timely help received by the journal Fertility & Sterility for permitting reproduction of 2 figures from his previously published work [Fig. 4. Panel B2 and B5 and Fig. 7. Panel B1 of Pires ES and Khole VV. 2009: A 'block' in the road to fertility: autoantibodies to an immunodominant heat shock protein 90-beta in human ovarian autoimmunity. Fertility & Sterility 92:1395–1409]. He also thanks the journal of Reproductive Biology and Endocrinology for allowing him to reproduce two figures from his earlier work [Fig. 1A and Fig. 5A of Pires ES, Choudhury AK, Idicula-Thomas S, Khole VV. Anti-HSP90 autoantibodies in sera of infertile women identify a dominant, conserved epitope EP6 (380–389) of HSP90 beta protein. Reprod Biol Endocrinol. 2011; 9: 16]. The author would also like to place on record his gratitude to his Ph.D. mentor, the late Dr. Vrinda V Khole from the National Institute for Research in Reproductive Health (ICMR), Mumbai, India for giving him the opportunity to explore and conduct research in this field.

References

Ahonen P, Miettinen A, Perheentupa J (1987) Adrenal and steroidal cell antibodies in patients with autoimmune polyglandular disease type I and risk of adrenocortical and ovarian failure. J Clin Endocrinol Metab 64:494–500

Anasti JN (1998) Premature ovarian failure: an update. Fertil Steril 70:1–15

Barbarino PM, Forges T, Faure GC, Bene MC (2005) Gonadal antibodies interfering with female reproduction. Best Pract Res Clin Endocrinol Metab 19(1):135–148

Bardwell JCA, Craig EA (1987) Eukaryotic Mr 83,000 heat shock protein has a homologue in *Escherichia coli*. Proc Natl Acad Sci USA 84:5177–5181

Bensaude O, Morange M (1983) Spontaneous high Expression of heat-shock proteins in mouse embryonal carcinoma cells and ectoderm from day 8 mouse embryo. EMBO J 2:173–177

Betterle C, Pra D, Mantero F, Zanchetta R (2002) Autoimmune adrenal insufficiency and autoimmune polyendocrinopathy syndromes: auto antibodies, auto antigens and their applicability in diagnosis and disease prediction. Endocr Rev 23:327–364

Biswas S, Sharma YD (1994) Enhanced expression of *Plasmodium falciparum* heat shock protein PFHSP70-1 at higher temperatures and parasite survival. FEMS Microbiol Lett 124:425–430

Calvert ME, Digilio LC, Herr JC, Coonrod SA (2003) Oolemmal proteomics identification of highly abundant heat shock proteins and molecular chaperones in the mature mouse egg and their localization on the plasma membrane. Reprod Biol Endocrinol 1:27

Choudhury A, Khole VV (2013) HSP90 antibodies: a detrimental factor responsible for ovarian dysfunction. Am J Reprod Immunol 70(5):372–385

Coulam CB, Adamson SC, Annegers JF (1986) Incidence of premature ovarian failure. Obstet Gynecol 67:604–606

Cusick ME, Klitgord N, Vidal M, Hill DE (2005) Interactome: gateway into systems biology. Hum Mol Genet 14(Spec No. 2): R171–81

Czar MJ, Welsh MJ, Pratt WB (1996) Immunofluorescence localization of the 90-kDa heat-shock protein to cytoskeleton. Eur J Cell Biol 70(4):322–330

Damewood MD, Zacur HA, Hoffman GJ, Rock JA (1986) Circulating antiovarian antibodies in premature ovarian failure. Obstet Gynecol 68:850–854

de Moraes-Ruehsen H, Jones GS (1967) Premature ovarian failure. Fertil Steril 18:440–461

Eustace BK, Jay DG (2004) Extracellular roles for the molecular chaperone, HSP90. Cell Cycle 3(9):1098–1100

Falsone SF, Gesslbauer B, Tirk F, Piccinini AM, Kungl AJ (2005) A proteomic snapshot of the human heat shock protein 90 interactome. FEBS Lett 579(28):6350–6354

Faulds G, Conroy S, Madaio M, Isenberg D, Latchman D (1995) Increased levels of antibodies to heat shock proteins with increasing age in MRL/MP-LPR mice. Br J Rheumatol 34:610–615

Fenichel P, Sosset C, Barbarino-Monnier P, Bene MC, Hieronimus S, Harter M (1997) Prevalence, specificity and significance of ovarian antibodies during spontaneous premature ovarian failure. Hum Reprod 12(12):2623–2628

Forges T, Monnier-Barbarino P, Faure GC, Bene MC (2004) Autoimmunity and antigenic targets in ovarian pathology. Hum Reprod Update 10(2):163–175

Gallucci S, Matzinger P (2001) Danger signals: SOS to the immune system. Curr Opin Immunol 13:114–119

Gruppi CM, Zakeri ZF, Wolgemuth DJ (1991) Stage and lineage-regulated expression of two HSP90 transcripts during mouse germ cell differentiation and embryogenesis. Mol Reprod Dev 28: 209–217

Gupta RS (1995) Phylogenetic analysis of the 90 kD heat shock family of protein sequences and an examination of the relationship among animals, plants, and fungi species. Mol Biol Evol 12: 1063–1073

Hayem G, De Bandt M, Palazzo E, Roux S, Combe B, Eliaou JF et al (1999) Anti-heat shock protein 70 kDa and 90-kd antibodies in serum of patients with rheumatoid arthritis. Ann Rheum Dis 58:292–296

Hilscher B, Hilscher W, Bulthoff-Ohnolz B, Kramer U, Birke A, Pelzer H, Gauss G (1974) Kinetics of gametogenesis. I. Comparative histological and autoradiographic studies of oocytes and transitional prospermatogonia during oogenesis and prespermatogenesis. Cell Tissue Res 154:443–470

Hoek A, Schoemaker J, Drexhage HA (1997) Premature ovarian failure and ovarian autoimmunity. Endocr Rev 18:107–134

Kellermayer MS, Csermely P (1995) ATP induces dissociation of the 90 kDa heat shock protein (HSP90) from F-actin: interference with the binding of heavy meromyosin. Biochem Biophys Res Commun 211(1):166–174

Khole V, Mande P, Choudhary A, Pires E (2012) Validation of novel candidate biomarkers for diagnosis of ovarian autoimmunity. Advances in developing affordable in vitro molecular diagnostics. Yashraj research foundation and Mahatma Gandhi Mission University, pp 139–151

Koyasu S, Nishida E, Kadowaki T, Matsuzaki F, Iida K, Harada F, Kasuga M, Sakai H, Yahara I (1986) Two mammalian heat shock proteins, HSP90 and HSP100, are actin-binding proteins. Proc Natl Acad Sci USA 83(21):8054–8058

Latchman DS, Isenberg DA (1994) The role of HSP90 in SLE. Autoimmunity 19(3):211–218

Lele Z, Hartson SD, Martin CC, Whitesell L, Matts RL, Krone PH (1999) Disruption of zebrafish somite development by pharmacologic inhibition of Hsp90. Dev Biol 210:56–70

Lernmark A (2001) Series introduction: autoimmune diseases: are markers ready for prediction? J Clin Investig 108(8):1091–1096

Lindquist S, Craig EA (1988) The heat shock proteins. Annu Rev Genet 22:631–677

Loktionova SA, Ilyinskaya OP, Gabai VL, Kabakov AE (1996) Distinct effects of heat shock and ATP depletion on distribution and isoform patterns of human Hsp27 in endothelial cells. FEBS Lett 392(2):100–104

Loones MT, Rallu M, Mezger V, Morange M (1997) HSP gene expression and HSF2 in mouse development. Cell Mol Life Sci 53:179–190

Lotz GP, Brychzy A, Heinz S, Obermann WM (2008) A novel HSP90 chaperone complex regulates intracellular vesicle transport. J Cell Sci 121:717–723

Luborsky JL (2002) Ovarian autoimmune disease and ovarian autoantibodies. J Womens Health Gend Based Med 11:585–599

Mande PV, Parikh FR, Hinduja I, Zaveri K, Vaidya R, Gajbhiye R, Khole VV (2011) Identification and validation of candidate biomarkers involved in human ovarian autoimmunity. Reprod BioMed Online 23(4):471–483

McClellan AJ, Xia Y, Deutschbauer AM, Davis RW, Gerstein M, Frydman J (2007) Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches. Cell 131: 121–135

Morimoto RI (1993) Cells in stress: transcriptional activation of heat shock genes. Science 259(5100):1409–1410

Neuer A, Mele C, Liu HC, Rosenwaks Z, Witkin SS (1998) Monoclonal antibodies to mammalian heat shock proteins impair mouse embryo development in vitro. Hum Reprod 13(4):987–990

Neumann S, Ziv E, Lantner F, Schechter I (1993) Regulation of HSP70 gene expression during the life cycle of the parasitic helminth *Schistosoma mansoni*. FEBS J 212(2):589–596

Novosad JA, Sophia NK, Zhi-Bin T, Nelson LM (2003) Ovarian antibodies as detected by indirect immunofluorescence are unreliable in the diagnosis of autoimmune premature ovarian failure: a controlled evaluation. BMC Womens Health 3:2

Ohsako S, Bunick D, Hayashi Y (1995) Immunocytochemical observation of the 90 KD heat shock protein (HSP90): high expression in primordial and pre-meiotic germ cells of male and female gonads. J Histochem Cytochem 43:67–76

Pires ES (2010) Multiplicity of molecular and cellular targets in human ovarian autoimmunity: an update. J Assist Reprod Genet 27:519–524

Pires ES, Khole VV (2009a) A 'block' in the road to fertility: autoantibodies to an immunodominant heat shock protein 90-beta in human ovarian autoimmunity. Fertil Steril 92: 1395–1409

Pires ES, Khole VV (2009b) Anti-ovarian antibodies: specificity, prevalence, multiple antigenicity and significance in human ovarian autoimmunity. Current Paradigm of Reproductive Immunology Research signpost, Trivandrum, India, pp 159–190. ISBN: 978–81–308-0373-9

Pires ES, Parte PP, Meherji PK, Khan SA, Khole VV (2006) Naturally occurring anti-albumin antibodies are responsible for false positivity in diagnosis of autoimmune premature ovarian failure. J Histochem Cytochem 54(4):397–405

Pires ES, Meherji PK, Vaidya RR, Parikh FR, Ghosalkar MN, Khole VV (2007) Specific and sensitive immunoassay detects multiple anti-ovarian antibodies in women with infertility. J Histochem Cytochem 55(12):1181–1190

Pires ES, Choudhury AK, Idicula-Thomas S, Khole VV (2011a) Anti-HSP90 autoantibodies in sera of infertile women identify a dominant, conserved epitope EP6 (380-389) of HSP90 beta protein. Reprod Biol Endocrinol 9:16

Pires ES, Parikh FR, Mande PV, Uttamchandani SA, Savkar S, Khole VV (2011b) Can anti-ovarian antibody testing be useful in an IVF-ET clinic? J Assist Reprod Genet 28(1):55–64

Pires ES, Hlavin C, Macnamara E, Ishola-Gbenla A, Doerwaldt C, Chamberlain C, Klotz K, Herr A, Khole A, Chertihin O, Curnow E, Feldman SH, Mandal A, Shetty J, Flickinger C, Herr JC (2013) SAS1B protein [ovastacin] shows temporal and spatial restriction to oocytes in several eutherian orders and initiates translation at the primary to secondary follicle transition. Dev Dyn 242:1405–1426

Redmond T, Sanchez ER, Bresnick EH, Schlesinger MJ, Toft DO, Pratt WB, Welsh MJ (1989) Immunofluorescence colocalization of the 90-kDa heat-shock protein and microtubules in interphase and mitotic mammalian cells. Eur J Cell Biol 50(1):66–75

Ruiz-Arguelles A, Alarcon-Segovia D (2001) Penetration of autoantibodies into living cells, 2000. Isr Med Assoc J 3(2):121–126

Schlatter H, Langer T, Rosmus S, Onneken ML, Fasold H (2002) A novel function for the HSP90 kDa heat-shock protein (HSP90): facilitating nuclear export of 60 S ribosomal subunits. Biochem J 362:675–684

Sidera K, Gaitanou M, Stellas D, Matsas R, Patsavoudi E (2008) A critical role for HSP90 in cancer cell invasion involves interaction with the extracellular domain of HER-2. J Biol Chem 283(4):2031–2041

Sreedhar AM, Kalmar E, Csermely P, Shen YF (2004) Hsp90 isoforms: functions, expression and clinical importance. FEBS Lett 562(1–3):11–15

Sundblad V, Bussmann L, Chiauzzi VA, Pancholi V, Charreau EH (2006) α-enolase: a novel autoantigen in patients with premature ovarian failure. Clin Endocrinol 65:745–751

Te J, Jia L, Rogers J, Miller A, Hartson SD (2007) Novel subunits of the mammalian HSP90 signal transduction chaperone. J Proteome Res 6:1963–1973

Trieb K, Gerth R, Holzer G, Grohs JG, Berger P, Kotz R (2000) Antibodies to heat shock protein 90 in osteosarcoma patients correlate with response to neoadjuvant chemotherapy. Br J Cancer 82:85–87

Tsaytler PA, Krijgsveld J, Goerdayal SS, Rüdiger S, Egmond MR (2009) Novel Hsp90 partners discovered using complementary proteomic approaches. Cell Stress Chaperones 14(6):629–638

Van Eden W (1999) Immunity to heat shock proteins and arthritic disorders. Infect Dis Obstet Gynecol 7:49–54

Van Eden W, van der Zee R, van Kooten P, Berlo SE, Cobelens PM, Kavelaars A, Heijnen CJ, Prakken B, Roord S, Albani S (2002) Balancing the immune system: Th1 and Th2. Ann Rheum Dis 61:ii25–ii28

Vidal CI, Mintz PJ, Lu K, Ellis LM, Manenti L, Giavazzi R et al (2004) An HSP90-mimic peptide revealed by fingerprinting the pool of antibodies from ovarian cancer patients. Oncogene 23: 8859–8867

Voss AK, Thomas T, Gruss P (2000) Mice lacking HSP90b fail to develop a placental labyrinth. Development 127:1–11

Witkin SS, Sultan KM, Neal GS, Jeremias J, Grifo JA, Rosenwaks Z (1994) Unsuspected Chlamydia trachomatis infections in the female genital tract and in vitro fertilization outcome. Am J Obstet Gynecol 171:1208–1214

Zhao R, Kakihara Y, Gribun A, Huen J, Yang G, Khanna M, Costanzo M, Brost RL, Boone C, Hughes TR, Yip CM, Houry WA (2008) Molecular chaperone HSP90 stabilizes Pih1/Nop17 to maintain R2TP complex activity that regulates snoRNA accumulation. J Cell Biol 180:563–578