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The Involvement of Heat Shock Proteins and Related Molecules in the Resistance to Therapies in Breast and Gynecologic Cancer

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Abstract: The HSP response is implicated in conferring to breast and gynecologic malignancies different sensitivities to anticancer therapies including chemotherapy, endocrine therapy and immunotherapy (we are in the need of more studies about radiotherapy). The heat shock proteins are mainly implicated in cell death mechanisms, in cell differentiation including epithelial-mesenchymal transition, in tumor dormancy, in angiogenesis, metastasis formation, and in the escape of immunosurveillance. Considering the ample functions where the HSPs are implicated and that the HSP response is quite complex it is not surprising that the HSP response affects the anticancer therapies. Several of the HSPs have different predominant roles according to the molecular partners with which they interact, thus it is difficult to dissect the molecular mechanisms to find the sensitivity to the therapies. In this review we present the implications of some the major HSPs (HSP27, HSP70 and HSP90) with drug resistance and present some of the main partners that are also implicated in drug resistance like p53, PTEN and MDR. We have given priority to the incorporation of clinical data where the HSPs have been studied using standard chemotherapies and new therapeutic strategies. It is clear that in order to have a significant understanding of the degree of drug resistance/sensitivity presented by a particular patient we need to examine the molecular status of several key molecular markers involved in the drug resistance pathways and that in this context the study of the HSP response should be incorporated. One of the other major problems in this field is that an inhibitor of one particular HSP will not be enough to achieve a significant anticancer response. Now that we know the complexity of this field we need to design strategies aiming to inhibit several molecular HSP pathways simultaneously without significantly affecting the normal cells, this is the principal challenge for the near future.

Keywords: Breast cancer, cervical cancer, chemotherapy, drug resistance, endometrial cancer, heat shock proteins, molecular markers, ovarian cancer.

INTRODUCTION

In cancer cells and cancer tissues the HSPs are usually overexpressed as a consequence of deregulation of the HSFs (mainly HSF1) due to the presence of numerous mutated proteins [1,2]. When a tumor is detectable, it has passed through numerous stresses like low oxygenation, low pH, low nutrient supply, changes in cell organization, exposure to high growth factor concentrations, etc. all of them can awake the HSP response [2,3]. The HSPs play fundamental roles in tumorigenesis, cancer progression and in the response of the cancer cells to anticancer therapies [1-5]. In this review we will deal with the implications of some of the most conspicuous HSP family members with the resistance to therapies in gynecological cancers. Attention has been done to incorporate clinical data. The reader will also see that the HSP response is implicated in some important molecular pathways related to drug resistance and for this reason some of these molecules are also briefly presented here. The nomenclature of the main

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HSPs that will be presented in this review can be seen in Table **1**, we will use the old names due to their ample use.

Table 1. Nomenclature of the main HSPs (for the complete list see Kampinga *et al.* [6]).

Old Name	New Name
Small heat shock proteins	HSPB
HSP27; HSP25; CMT2F	HSPB1
Crystalline alpha A; CRYAA	HSPB4
Crystalline alpha B; CRYAB	HSPB5
HSP40	DNAJ
DJ-2; DjA1; HSDJ	DNAJA1
HSP70	HSPA
HSP70-1; HSP72; HSPA1	HSPA1A (inducible HSP70)
BIP;GRP78; MIF2	HSPA5
HSC70; HSC71; HSP71; HSP73	HSPA8
HSP90	HSPC
HSP90; HSP89; HSP90AA1	HSPC1
HSP90-ALPHA; HSP90AA2	HSPC2
HSP90-BETA; HSP90B	HSPC3
GRP94; GP96; TRA1	HSPC4
HSP110	HSPH
HSP105	HSPH1
HSP110; HSPA4; APG-2	HSPH2
Chaperonins and related	HSPD and others
HSP60; GroEL	HSPD1
HSP10; chaperonin 10; GroES	HSPE1

HSP27 [HSPB1]

Breast Cancer. HSP27

HSP27 is an ATP-independent molecular chaperone that belongs to the small HSP family formed by eleven different isoforms (HSPB 1-11) [6]. In humans, the gene is located on chromosome 7q11.23 and encodes a protein of 205 amino acids. HSP27 provides thermotolerance *in vivo*, cytoprotection and cell survival under stress conditions [7]. However, more specialized functions have been reported [8]. It is involved in cytoskeleton remodeling, cell migration, metabolism, growth, differentiation, apoptosis, proinflammatory gene expression, mRNA stabilization, proteasomal activation and signal transduction pathways. An important fact on HSP27 is its ability to protect against apoptosis which plays a very important role in human pathologies [9]. This allows cells to escape the immune system (based on their ability to counteract death). In addition, HSP27 has been implicated in epithelial to mesenchymal transition and metastasis formation [10]. For these reasons HSP27 is considered a multifunctional protein involved in numerous normal and pathological cellular processes (Fig. **1**). Aberrant levels and induced phosphorylation of HSP27 have been associated with breast tumour growth and resistance to chemo- and radio-therapeutic treatments [11-13]. However, the exact role of this protein in MDR is still under investigation. Contrary to what has been previously reported, HSP27 overexpression has been described as a novel mechanism to sensitize MDR cancer MCF-7/ADR cells to chemotherapy [14], the authors showed HSF1 and HSP27 depletion when MDR1 gene and P-gp-based efflux transporters were functional. HSP27 inhibition is a trigger, at least in part, for the accumulation of transcriptionally active mutant p53, which can either directly or NF-κB-dependently induce an MDR1/P-gp phenotype in MCF-7 cells. This pathway could be abrogated when HSP27 was overexpressed and the acquired MDR was significantly abolished. Consistent with these results, a recent proteomic study suggested the participation of HSP27 in P-gp mediated MDR in DOX-treated cells, a decrease of HSP27 expression was observed in P-gp overexpressed MCF-7/ADR cells [15]. Also HSP27 phosphorylation at Ser82 was induced by DOX in these MCF-7/ADR cells. Supporting these findings, Xu *et al.* [16] have shown that phosphorylated HSP27 activates p53 signaling in an ATM-dependent manner and mediates resistance to DOX-induced apoptosis in MCF-7 human breast adenocarcinoma cells.

On the other hand, alternative therapeutic strategies are being investigated in breast cancer, for example resveratrol, a natural agent that can be an effective adjuvant. It inhibits HSP27 expression and sensitizes tumor cells to DOX treatment [17]. c-Abl/Arg inhibitors (imatinib, nilotinib) may decrease DOX toxicity in breast cancers by decreas-

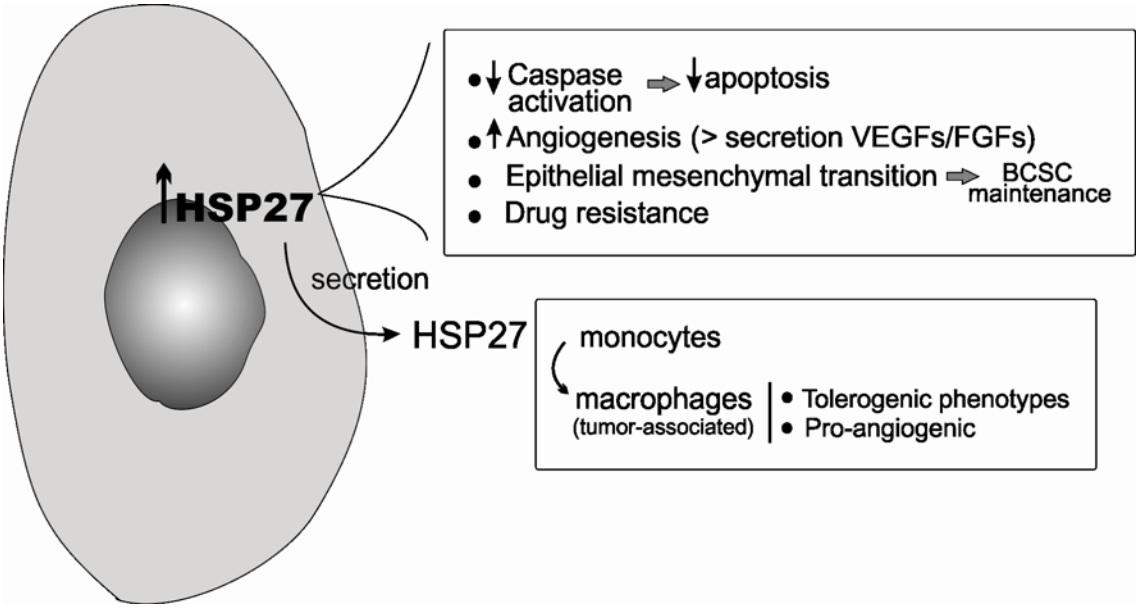

Fig. (1). Schematic representation of the different functions of HSP27 in breast cancer cell biology showing the intracellular and extracellular roles.

ing DOX dose required for effective treatment. Imatinib prevents intrinsic DOX resistance in part by inhibiting STAT3-dependent HSP27/p38/Akt survival pathway and promoting activation of an NF-κB-mediated proapoptotic pathway [18]. Moreover, HSP27 seems to have a role in the cellular response to actinomycin D, the knockdown of HSP27 enhances actinomycin D-induced caspase activation and apoptosis in breast cancer cells [19].

HSP27 also plays a role in the chemoresistant character of BCSCs. Lee *et al.* [20] have demonstrated that HSP27 inhibition potentiates the suppressive effect of HSP90 inhibitors such as geldanamycin in BCSCs. The combination of HSP27 inhibitors with HSP90 inhibitors could serve as a potential solution to prevent the drug resistance and to avoid the toxicity of the high doses of HSP90 inhibitors with clinical applications. Furthermore, HSP27 participates in the maintenance of BCSCs regulating the epithelial-mesenchymal transition; also it is involved in the activation of NF-κB by regulation of IκBα degradation [21].

Finally, it has been demonstrated that HSP27 upregulation in breast cancer cells reduces trastuzumab susceptibility by increasing HER2 protein stability [22].

Relatively few studies have been done on breast cancer patients, our group demonstrated a specific interaction between β-catenin and HSP27 [23]. In human breast cancer biopsy samples, β-catenin was co-expressed in the same tumor areas and in the same tumor cells that expressed HSP27. However, this co-expression was strong when β-catenin was present in the cytoplasm of tumor cells. The interaction between these proteins may explain some of the molecular pathways that influence tumor cell survival and the clinical significance in the prognosis of the breast cancer patients.

A novel function for HSP27 in the balance between tumor dormancy and tumor progression, mediated by tumor-vascular interactions, has been reported in breast cancer [24]. Downregulation of HSP27 in angiogenic tumor cells was followed by long-term tumor dormancy *in vivo* and it was associated with reduced endothelial cell proliferation and decreased secretion of VEGF-A, VEGF-C and basic fibroblast growth factor. The cell lines and mouse model results were validated analyzing human breast cancer tissues, the authors showed that low HSP27 expression levels appeared associated with a less aggressive phenotype and improved survival.

Recently, immune escape and tumor growth-supporting mechanism mediated by extracellular HSP27 has been reported [25]. These authors found highly elevated concentration of HSP27 in the human breast tumor microenvironment of patients, caused by increased intracellular HSP27 expression and high secreted levels of this protein.

They further demonstrated that high levels of extracellular HSP27 released from breast tumor cells could similarly differentiate human circulating monocytes to macrophages, with properties similar to tumor-associated macrophages (tolerogenic phenotypes and extremely pro-angiogenic) which may support human breast tumor progression.

Breast Cancer. PTEN

We have recently reported that downregulation of HSP27 in MCF-7 human breast cancer cells induces upregulation of PTEN [26]. Therefore, we will present the implications of PTEN in breast cancer. The loss of PTEN function is a common genomic event (up to 40 %) due to mutations in its catalytic domain or by reduced PTEN expression through loss of heterozygosity. Epigenetic inactivation of PTEN has been associated to breast cancer progression. PTEN gene is methylated in ductal carcinoma in situ and in early invasive breast cancer [27]. As a consequence of PTEN inactivation, PI3K pathway could be activated inducing oncogenic transformation and also it is associated with the development of drug resistance [28,29]. Targeting PI3K pathway has been an effective strategy to overcome drug resistance in cancer treatment. PTEN loss has been associated with clinical-pathological characteristics such as histological subtype, tumor grade, hormone receptor status and HER2 expression [30, 31]. For example in a study of 547 human breast cancer patient samples, PTEN mutations were determined to be restricted to ER+ tumors [32]. Moreover, PTEN expression has been involved with drug resistance but its clinical validity as a single marker is controversial. Previous studies have reported PTEN as a prognostic indicator and an effector of chemotherapy responses, endocrine therapy and HER2-directed therapies [28, 30, 33, 34]. Decreases in PTEN expression has been implicated in trastuzumab-resistance and hypersensitivity to the mTORC1 inhibitor rapamycin [35, 36]. Cell cultures experiments with HER2 over-expression demonstrated that genetic inactivation of PTEN reverses the effect of HER-2 inhibitors [37]. Also low expression of PTEN was associated with resistance to Herceptin in breast cancer patients. Furthermore, limited clinical trials have shown a decrease in trastuzumab sensitivity in HER2 (+)/PTEN (-) tumors [38, 39]. In a recent study in HER2-positive metastatic breast cancer patients who received taxane plus trastuzumab treatment, HER3 negativity and PTEN loss were identified as independent risk factors for PFS. In particular, PTEN loss was identified as an independent risk factor for OS. The authors suggested that PTEN expression may be a predictive and prognostic biomarker for trastuzumab treatment [40]. In contrast, Perez *et al.* [33] showed benefit of adjuvant trastuzumab for HER2-positive breast cancer patients, regardless of PTEN status. In an editorial comment of this article Rexer *et al.* [35] analyzed the reasons for this discrepancy mentioning for example that "a fraction of HER2 gene–amplified breast cancers that are PTEN positive by IHC may express otherwise nonfunctional PTEN" and that concurrent chemotherapy was added in this study which might reverse drug resistance in HER2 (+)/PTEN (-) tumors. A more recent study found that PTEN loss by itself does not predict resistance to tamoxifen in postmenopausal breast cancer patients and PTEN-negative tumors were associated with negative PR status [41].

The effects of PTEN loss on the efficacy of lapatinib have been evaluated in preclinical and clinical studies. However, the results are controversial. In HER2-positive breast cancer patients with PI3K catalytic subunit alpha gene mutations and PTEN loss were not significantly associated with lapatinib efficacy [42, 43]. In contrast, other studies have associated these aberrations with adverse impact in lapatinib efficacy [44, 45]. Xu *et al.* [46] in a recent report studied a large cohort of patients with HER2-positive metastatic breast cancer receiving first-line treatment with paclitaxel alone or in combination with lapatinib. They found that PIK3CA mutations and the loss of PTEN function were not predictive of lapatinib efficacy regarding OS, PFS, ORR, and CBR. In summary, these molecular aberrations are not conclusively established as conferring resistance to lapatinib in breast cancer patients. Further studies will be required to establish their clinical relevance.

Sequential application of targeted therapies guided by biomarker is a novel approach used to overcome therapy resistance, targeting and reprogramming the signaling networks. It has been reported in PTEN-low/trastuzumab-resistant breast cancers patients that HER2 stabilization and its sustained activation drives trastuzumab resistance. Interestingly, sequential treatment with lapatinib plus PI3K/mTOR dual kinase inhibitor (BEZ235)

combination effectively overcomes trastuzumab + lapatinib resistance with no significant toxicity [47].

Finally, drug resistant breast cancer cells are enriched in populations of cells with characteristics of cancer stem cells referred as cancer initiating cells. *In vitro* and *in vivo* studies have shown that PTEN also is important in their survival. When PTEN expression was knockdown there was an increased in normal and malignant human mammary stem/progenitor cells mediated by Akt activation causing GSK-3β phosphorylation, which in turn led to activation of the Wnt/β-catenin pathway [48]. At this point, we are reporting the association of HSP27 with PTEN in breast cancer cells [26] and we believe that further studies examining the effects of the co-expression of these proteins in cancer patients are needed to know whether they are useful markers of drug resistance and suitable targets for new treatments.

Ovarian Cancer. HSP27

Ovarian cancer is the third most common gynecologic malignancy, a leading cause of death and HSP27 expression is increased and involved with therapy resistance and poor survival. However few studies have been designed to explore the molecular mechanisms involving HSP27 protein with drug resistance in ovarian cancer patients. Recently, Pavan *et al.* [49] suggested that HSP27 has a role as a marker of ovarian cancer progression. They have demonstrated that HSP27 is required for the invasive and metastatic activity of HGF in ovarian cancer cells, this factor induced HSP27 phosphorylation through p38MAPK activation, HSP27 silencing impaired spontaneous metastases. In a xenograft model, HSP27 suppression resulted in the sensitization to low doses of paclitaxel, likely because HSP27 protected microtubules from bundling caused by the drug. Supporting these experiment evidences, epithelial ovarian cancer patients have been studied. HSP27 has been strongly associated with peritoneal metastases. Total serum HSP27 levels were measured in ovarian cancer samples, a significant circulating HSP27 increase was found only in patients with peritoneal metastases and these levels were significantly reduced after combination chemotherapies [50]. In a previous study, these authors analyzed tissues from epithelial ovarian cancer, with or without peritoneal metastasis, HSP27 mRNA, protein levels and HSP27 subcellular localization were determined. The data were consistent with those indicating that higher HSP27 expression correlated with poor clinical outcome, this chaperone was highly expressed in the cytoplasm of epithelial cancer cells with peritoneal metastasis [51]. Opposite to these results Annunziata *et al.* [52] suggested that HSP27 may be a marker of better survival in ovarian cancer patients. They showed that cytoplasmic HSP27 expression decreases in endometrioid ovarian cancer as stage increases. However, the survival could not be established in this study and it did not reach statistical significance for the poor outcome. An earlier investigation in ovarian cancer patients with 60 months of follow-up showed that a decrease in HSP27 immunohistochemical staining was related to decreased survival, suggesting a possible role for HSP27 as independent indicator of prognosis and survival [53].

Increases in HSP27 protein were detected in ovarian tumor biopsies from patients treated with the HSP90 inhibitor geldanamycin analogue 17AAG [54]. HSP27 induction was considered a consequence of HSP90 inhibition and could be of therapeutic significance due to its anti-apoptotic role. Consistent with these results, a previous report suggested that HSP27 contributes to 17-AAG resistance, at least in part, through regulation of glutathione and this modulation may be clinically significant enhancing 17AAG efficacy in patients. They confirmed the increased HSP27 expression by 17AAG in tumor cells and that HSP27 depletion causes sensitization to 17AAG [55].

Ovarian Cancer. PTEN

Research of endocrine responsiveness and resistance in gynecologic cancers have revealed that PI3K/AKT/mTOR pathway becomes activated due to PIK3CA mutation and loss of PTEN and it is used by cancer cells to bypass the hormone therapy effects [56]. In particular, loss of inhibition through inactivating mutations in PTEN has been detected only in 3% to 8% of the endometrioid and lower grade ovarian tumors and may contribute to epithelial ovarian carcinogenesis [57]. However, downregulation of PTEN protein is frequently detected in serous and mucinous epithelial ovarian tumors [58]. Promoter hypermethylation could be involved in the decrease of PTEN expression. Nevertheless, the demethylation agent 5-aza-2′-deoxycytidine failed to restore PTEN protein ex-

pression, suggesting that PTEN is highly regulated at the translational level and that methylation of the PTEN gene plays a subordinate role in ovarian cancer [59]. Several mechanisms of PTEN gene inactivation has been proposed but protein expression deletions may be a significant mechanism [60]. Yang *et al.* [61] have demonstrated that miR-214 induces cell survival and cisplatin resistance through targeting the UTR of the PTEN, which leads to downregulation of PTEN protein and activation of Akt pathway in human ovarian cancer.

The prognostic significance of PTEN is controversial and requires more investigation. In ovarian clear cell adenocarcinomas PTEN inactivation is not an adverse prognostic factor and is not significantly related to PTEN promoter methylation nor LOH at 10q23 locus [62]. Otherwise, decreased PTEN expression is a poor prognostic factor for DFS in patients with epithelial ovarian cancer who were treated with debulking surgery followed by taxane and platinum-based chemotherapy [63].

Recently, multiple inhibition approach to treatment has been investigated using ovarian cancer primary cell cultures, hitting multiple aspects of signaling pathways [64]. The effect of PI3K/AKT/mTOR inhibitors (ZSTK474 and sirolimus) in combination with EGFR inhibitors (erlotinib and gefitinib) was examined. ZSTK474 with EGFR inhibitors showed enhanced activity with some evidence of synergy, whereas sirolimus combinations were less active. However, PTEN study revealed there was no clear pattern in its expression by qRT–PCR or immunohistochemistry and no relationship to sensitivity was noted, probably due to the small number of tumors studied. Glaysher *et al.* [64] suggest that determinants of resistance may not be limited to the target pathways, incorporating both the apoptotic potential of the cell and classical drug resistance mechanisms. An earlier study analyzed the incidence of PTEN deletion in cancer cells isolated from ascites of patients with advanced ovarian cancer. The deletion was in the rate of 27%, similar to the published incidence in samples taken at diagnosis, implying that this is not a major factor regulating secondary resistance to chemotherapy [65]. In addition, it has been evaluated the prognostic effect of concomitant p53 and PTEN status on outcome of 131 patients in FIGO stages I to II with epithelial ovarian cancer. The results showed that presence of PTEN in P53-positive tumors seems to protect from bad prognosis and absence of PTEN seems to worsen prognosis in early stages [66].

Uterine Cancer. HSP27

Endometrial adenocarcinoma is the most common malignant neoplasm of the female genital tract and significant high HSP27 expression in endometrioid tumors has been reported by Geisler *et al.* [67]. HSP27 staining was an independent prognostic indicator in patients with endometrial carcinoma. In spite of these HSP27 evidences and the relative frequency of the disease, the molecular events that contribute to drug resistance as well as the development and progression of the lesion remain poorly understood. In ovarian and uterine cancer cells it has been demonstrated that HSP27 expression was completely depleted by paclitaxel, this result indicated that paclitaxel may possess unique mechanisms able to overcome drug resistance by inhibiting HSP27 expression [68].

Cervical cancer is the second most prevalent malignancy among women and HSP27 is expressed by squamous cell carcinoma of the uterine cervix and its presence is independent of the presence of estrogen and progesterone receptors. HSP27 may be considered a diagnostic marker for CIN and carcinoma [69]. In addition, presence of HSP27 has been associated with more differentiated tumors, whereas its decreased expression has been associated with de-differentiation and transformation to adenocarcinoma. Proteomic based-analysis has shown a downregulation of HSP27, among other proteins, associated with chemotherapy effect in cervical cancer patients studied before and after neoadjuvant chemotherapy with paclitaxel and cisplatin [70]. Another study was focused on protein expression during the sequential steps of SCC and revealed that cytoplasmic expression of HSP27 was more intense in dysplastic lesions than in micro-invasive and invasive SCC [71]. HPV can cause nuclear expression of HSP27 [72] indicating that cervical precursor lesions and invasive cancer were characterized by HSP27 nuclear expression. Weak or strong HSP27 expression was associated with lower PFS and OS than patients with moderate and essentially normal protein expression [71]. Previously El-Ghobashy *et al.* [73] reported early HSP27 upregulation in metaplastic and neoplastic lesions of the cervix. However, no correlation was found between

HSP27 expression with tumor grade, lymph node involvement, and lymphovascular space invasion in invasive adenocarcinoma.

Uterine Cancer. PTEN

Greater than 90% of endometriod cancers are associated with some type of mutation in the PI3K/PTEN/Akt/mTORC1 pathway. PTEN is frequently mutated in sporadic endometrial cancer; up to 80% has been reported for the endometrioid subtype [74]. In 2010, PTEN status was correlated with clinicopathologic variables and survival using data of tumors from women with endometrial cancer enrolled in NCIC Clinical Trials Group. PTEN-negative tumors in women with advanced stage were associated with improved survival [75]. Akiyama-Abe *et al.* [76] demonstrated that loss of PTEN expression by immunohistochemistry is an independent prognostic marker for favorable survival in endometrial carcinomas. Supporting these results, previous study showed both PTEN and p53 staining may be good indicators of clinical stage and probability of tumor recurrence in endometrial carcinomas. Reciprocal abnormality of both proteins occurred at early phase of carcinogenesis; however simultaneous abnormality took places at late phase of the tumorigenic process [77]. Recently, Daniilidou *et al.* [78] found that PTEN and p53 immunoexpression help both in accurate diagnosis and proper therapeutic approach of the various endometrial carcinomas. Both proteins are also prognostic markers for these kinds of tumors. Moreover, PTEN mutations among other mutated genes correlate with high tumor grade, endometrial cancer type and lymph node status [79]. Functional PTEN loss in endometrial carcinoma can be mediated by different mechanisms including PTEN gene promoter methylation, regulation of the PTEN gene or PTEN pseudogene by microRNAs, or alterations of PTEN protein stability and degradation mechanisms [80].

The conservative progestin therapy with MPA has been studied in young patients who desire to preserve fertility with complex atypical hyperplasia or stage Ia, G1 adenocarcinoma (IaG1) of the endometrium. The study suggested that the antitumor action of MPA may be mediated by dephosphorylation of Akt, and that immunohistochemical evaluation of p-Akt and PTEN may be able to predict the outcome of drug therapy [81]. Recently Nagy *et al.* [82] studied promoter hypermethylation of the gene for the DNA repair enzyme MGMT and its association to PTEN mutations, among others, in endometrial tumors from women treated with TAM or unexposed to the drug. Both groups displayed MGMT promoter hypermethylation. However, PTEN mutations were prevalent in TAM-untreated patients affecting the protein functions. The authors have suggested that epigenetic processes may be involved in TAM-inducing mechanisms of endometrial cancer.

The PTEN/AKT pathway and its downstream targets are involved in endometrial carcinogenesis as well as the mTOR pathway [83]. In particular, PTEN has been involved in the maintenance of genomic stability, loss of PTEN function results in defective homologous recombination-mediated repair of DNA double-strand breaks, thus sensitizing cells to inhibition of the PARP. Hence, PTEN-deficient endometrioid endometrial carcinoma cells may also be sensitive to PARP inhibitors like olaparib [84]. Forster *et al.* [85] reported the treatment with olaparib in a patient with PTEN-deficient endometrioid endometrial cancer. During the treatment, a significant reduction in the size of the brain metastases was observed without steroid treatment or radiotherapy, and the patient reported subjective improvement of tumor-related symptoms. In this case, loss of PTEN has been suggested to be a predictive marker for sensitivity to PARP inhibitors. Besides, the mechanisms underlying the anti-proliferative effect of rapamycin have been investigated in human primary cultures from both type I and type II endometrial cancer tumors, but no correlation between PTEN loss and the response to treatment was found [86]. Other molecular markers to predict sensitivity or resistance to mTOR inhibitors have to be assessed.

HSP70 [HSPA]

HSP70 is of considerable importance in maintaining several house-keeping functions such as: a) folding of proteins in the cytosol, endoplasmic reticulum and mitochondria, b) transport of proteins into different subcellular compartments, c) dissolution of protein complexes, d) control of regulatory proteins, and e) refolding of misfolded proteins [1-3]. Inducible HSP70 is known to correlate with poor prognosis in many cancers [12,87]. HSP70 expression is correlated with poor prognosis in breast cancer, endometrial cancer and uterine

cervical cancer. This is consistent with the HSP70 associations with poor differentiation, lymph node metastasis, increased cell proliferation, block of apoptosis and higher clinical stage which are markers of poor clinical outcome. HSP70 confers survival advantage as well as resistance to chemotherapeutic agents, and promotes tumor cell invasion [2]. However there are few reports involving HSP70 with therapy resistance in human breast cancer patients. Thanner *et al.* [88] demonstrated the correlations of HSP70 expression with survival after recurrence in node-negative breast cancer patients. In addition, our group has reported that a high nuclear proportion of HSP70 correlated significantly with drug resistance in biopsies of breast cancer patients treated with induction chemotherapy with FAC [11]. A more recent study described that high HSP70 expression may be a useful prognosis marker in breast cancer patients treated with neoadjuvant doxorubicin mono chemotherapy indicating the resistance to the administered drug and the requirement to change the therapeutic scheme after surgery to CMF [89]. This may be due to the election of a different therapeutic strategy: polychemotherapy *vs.* monochemotherapy before surgery (FAC/FEC *vs.* DOX at a higher dose) and to the addition of a different scheme of chemotherapy after surgery. In fact, the survival of the patients treated first with anthracyclines followed by surgery and CMF was significantly better than those treated with FAC (4 cycles) followed by surgery and then FAC again (to complete 6 cycles in total) [90]. These results need to be replicated in larger studies with other combination of drugs currently in use, for example DOX and C, to confirm the specific role of HSP70 as prognostic molecular markers in breast cancer patients treated with neoadjuvant chemotherapy.

Chemotherapy has been frequently used in neoadjuvant settings but adverse effects and complications are quite common among the patients. Therefore, the idea of using endocrine therapy in these neoadjuvant settings has evolved, at least for ER-positive breast cancer patients. A recent study reported that the downregulation of HSP70 was significantly associated with treatment response of neoadjuvant AI in ER-positive postmenopausal breast cancer patients [91].

Regarding ovarian cancer, the role of HSP70 associated with a poor prognosis and poor response to therapy is still inconsistent and further studies are needed to elucidate its clinical potential in this type of cancer. Annunziata *et al.* [52] showed an association between nuclear expression of HSP70 and aggressiveness in epithelial ovarian carcinomas. Increase cytoplasmic HSP70 expression in effusions (peritoneal and pleural) of ovarian cancer patients did correlate with poor overall survival and could thus be a prognostic marker of poor survival [92]. However, an earlier study showed that HSP70 expression had no prognostic value in epithelial ovarian carcinoma, although it correlated with stages of cancer (such as defined by FIGO) [93]. In this study HSP27 expression was found associated with worse overall survival. The identification of novel targeted therapies and the consequent selection of treatment based on tumor profile may have a major impact on the management of this cancer.

HSP90 [HSPC]

HSP90 is a highly conserved molecular chaperone expressed abundantly in most living organisms, in eukaryotes members of the HSP90 family are found in the cytoplasm, in the nucleus and in organelles. Five human isoforms of HSP90 has been identified which differ in domain structure, cellular location, and substrate specify. In the human there are two cytosolic HSP90 isoforms: an inducible and a constitutive form: HSP90α and HSP90β, respectively; and HSP82 and HSC82 in yeast [2, 6, 94]. There are organelle-specific HSP90 forms, for example: TRAP1 in mitochondria [95], HSP90C in chloroplasts [96], Grp94 in endoplasmic reticulum [97] and isoform HSP90N [98]. HSP90 is also secreted and found on the surface of cells [99, 100]. In order to maintain the normal function of HSP90 chaperone machinery, there exist cochaperone molecules, such as Aha1, Cdc37, Hop, PP5 and p23. Post-translational modifications of HSP90 and its cochaperones are essential for their functions. HSP90 participates in cellular homeostasis and tightly command environmental stress responses by assisting correct folding and maturation processes of its client proteins. Actually more than 200 of these client proteins have been described which include several tumor-related proteins. HSP90 can help tumor cells to maintain the malignant *status* with the support of important oncoproteins required to tumor development, including signal-tansduction proteins (Bcr-Abl, B-Raf, Alk, Akt), tyrosine-

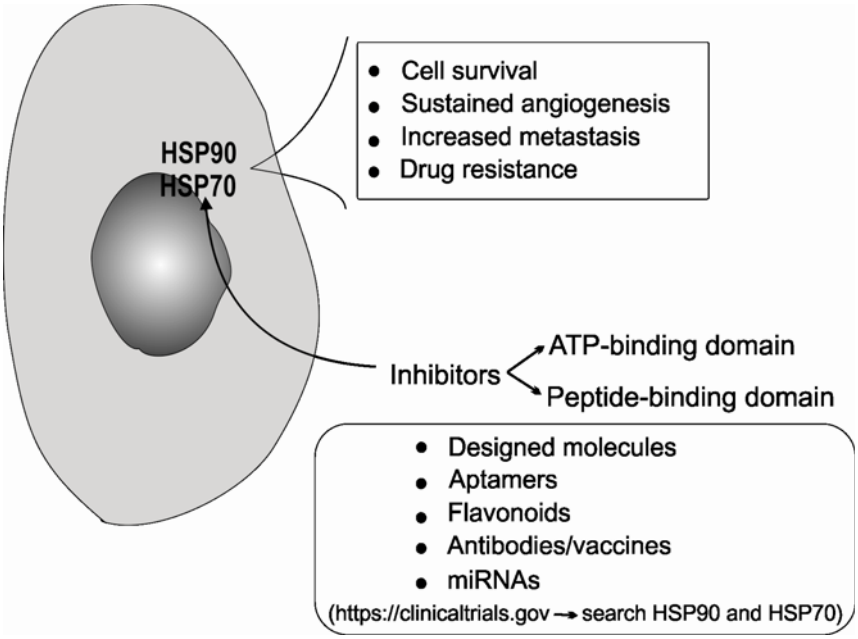

Fig. (2). Schematic representation of the different functions of HSP70 and HSP90 and the inhibitors developed to interfere with their functions. In the web address shown the reader can find the updated information on the clinical trails targeting these proteins.

kinase receptors (mutated EGFR, HER2, c-Kit, IGFR1), transcription factors (p53, HIF-1α, steroids receptors), cell-cycle regulatory proteins (Rb, Cyclin D, CDK4), antiapoptotic proteins (Bcl-2, Apaf1, survivin) and many others, in a similar manner to normal cells (telomerase hTERT, Fak1, MMP2) [101-104]. Hence, HSP90 appeared as a novel molecular target for cancer therapy and HSP90 inhibitors were investigated as cancer chemotherapeutic drugs. Recent studies revealed that HSP90 inhibitors have the potential to target multiple hallmark cancer processes such as insensitivity to anti-growth signals, evading apoptosis, sustained angiogenesis, tissue invasion and metastasis, limitless replicative potential and self-sufficiency in growth signals [105]. A variety of HSP90 inhibitors have been tested in preclinical and clinical trials (Fig. **2**). The first-generation of HSP90 inhibitors are benzoquinone ansamycins including natural products like geldanamycin and radicicol and the synthetic derivatives of geldanamycin: tanespimycin (17-AAG), alvespimycin (17-DMAG), retaspymicin hydrochloride and KF58333. However, the clinical efficacy of these compounds has been very poor owing to several disadvantages including limited solubility, formulation troubles, potential multidrug efflux activities, and hepatotoxicity [106, 107]. Furthermore, as monotherapeutic agents these molecules have displayed modest efficacies in the clinical setting [108, 109] indicating that they may be useful only in combination with other anticancer drugs. In an attempt to reverse these restrictions several second-generation HSP90 inhibitors are under study. Among them various synthetic small molecules inhibitors such as ganetespib and NVP-AUY992, which are engaged in active clinical trials. HSP90 inhibitors have shown early promising results in molecularly defined subgroups of solid tumours (ALK-rearranged non-small-cell lung cancer and HER2-amplified breast cancer) and some haematological malignancies (eg, multiple myeloma) [110]. For a comprehensive review of HSP90 implications in diagnosis, prognosis, predictive and treatment in gynecological cancer before 2005 please refer to Ciocca and Calderwood [12].

Breast Cancer

In the last ten years it has been a rapid advance in the field of development of HSP90 inhibitors with translation to several clinical trials, however, none of them have reached a phase III and therefore the inhibitors have not been approved for use in the clinic yet. The main activity of these compounds was seen in metastatic HER2-positive breast cancer. For a complete and exhaustive review please refer to Zagouri *et al.* [111]. The authors suggest that HSP90 inhibitors may play an important role in the treatment of triple negative

and aromatase-inhibitor breast cancer resistance subtypes.

Studies conducted in breast cancer cell lines have shown relevant interactions of HSP90 with important molecules such as those related to hereditary breast cancer (among others). Most inherited cases of breast cancer are associated with abnormalities in two genes: BRCA1 and BRCA2, women with inherited mutations or abnormal alterations in one of these genes have a higher risk of developing breast and ovarian cancer. Cells with mutations in BRCA1gene have compromised DNA repair mechanisms and are sensitive to PARP inhibitors. Johnson *et al.* [112] showed an HSP90-mediated stabilization of a BRCA1 C-terminal domain. The stabilized BRCA1 mutant protein was essential for conferred PARP inhibitor as well as cisplatin resistance. Treatment of resistant cells with the HSP90 inhibitor 17-dimethylaminoethylamino-17-demethoxygeldanamycin reduced mutant BRCA1 protein levels and restored their sensitivity to PARP inhibitors. Resistant cells also acquired a TP53BP1 mutation that facilitated DNA end resection in the absence of a BRCA1 protein capable of binding CtIP. Ultimately, concomitant increased mutant BRCA1 and decreased 53BP1 protein expression occur in clinical samples of BRCA1-mutated recurrent ovarian carcinomas that have developed resistance to platinum. These results would provide evidence for a two-event mechanism by which BRCA1-mutant tumors acquire anticancer therapy resistance.

On the other hand, Lee *et al.* [113] proved that CLC604, an isosteric analogue of YC-1 [1-benzyl-3-(5-hydroxymethyl-2-furyl)indazole], reduced HER2 expression through a post-transcriptional mechanism and involvement of proteasomal activity. CLC604 disrupted the association of HSP90 with HER2 resulting from the inhibition of HSP90 ATPase activity. Moreover, these authors found that CLC604 significantly enhanced the antitumor efficacy of clinical drugs against HER2-overexpressing tumors and efficiently reduced HER2-induced drug resistance *in vitro* and *in vivo*.

At present, there are several *in vitro, in vivo* and preclinical studies in breast cancer experimental models with the aim of elucidate which molecules are involved in HSP90-mediated resistance to develop new therapies based on specific inhibitors of this protein. We are still waiting new results to move these compounds into the clinical practice.

Ovarian Cancer

Most ovarian cancers are epithelial ovarian carcinomas (cancer that begins in the epithelial superficial cells) or malignant germ cell tumors (cancer that begins in egg cells). Epithelial ovarian cancer is often detected at an advanced stage and is frequently deadly. While at the first moment many patients respond to initial standard treatments: surgery or chemotherapy (consisting of a platinum-based agent and a taxane), most suffer recurrence and eventually treatment-resistant disease. Although there have been several efforts to develop protein-targeted agents, these studies have so far documented little efficacy. Liu *et al.* [114] conducted data-mining meta-analyses integrating results from multiple siRNA screens to identify gene targets that showed significant inhibition of cell growth. These authors established that many genes with such activity were client proteins of HSP90. Based on these findings ganetespib, a second-generation small-molecule HSP90 inhibitor, was evaluated against epithelial ovarian cancer, both as a single agent and in combination with cytotoxic and targeted therapeutic agents. The results obtained by these authors allowed them to conclude that ganetespib, a single-targeted agent with effects on numerous proteins and pathways, is augmenting standard epithelial ovarian cancer therapies.

Hendrickson *et al.* [115] evaluated the efficacy and biological effects of the gemcitabine/tanespimycin combination in patients with advanced ovarian and peritoneal cancer. To assess the effect of tanespimycin on tumor cells, levels of the chaperone proteins HSP90 and HSP70 were examined in PBMC and in paired tumor biopsy lysates. This study was a two-cohort phase II clinical trial. Patients were grouped according to prior gemcitabine therapy. All participants received tanespimycin 154 mg/m^2 on days 1 and 9 of cycle 1 and days 2 and 9 of subsequent cycles. Patients also received gemcitabine 750 mg/m^2 on day 8 of the first treatment cycle and days 1 and 8 of subsequent cycles. The tanespimycin/gemcitabine combination induced a partial response in 1 gemcitabine naïve patient and no partial responses in gemcitabine resistant patients. Stable disease was seen in 6 patients (2 gemcitabine naïve and 4 gemcitabine re-

sistant). Immunoblotting demonstrated limited upregulation of HSP70 but little or no change in the levels of most client proteins in PBMC and paired tumor samples. Although well tolerated, the tanespimycin/gemcitabine combination exhibited limited anticancer activity in patients with advanced epithelial ovarian and primary peritoneal carcinoma, perhaps due to lack of significantly downregulation of the client proteins at clinically achievable exposures.

MDR and HSPs

We will first present MDR and then the links between proteins/genes of the ABC transporters and the HSP response. A major problem of chemotherapeutic cancer treatment is intrinsic or acquired MDR [116]. In 1950 acquired drug resistance was noted for the first time in a laboratory leukaemia model in mouse cells [117]. In 1972 Dano described that the drug resistance was produced by the active outward transport of chemotherapy drugs [118]. Drug resistance, both intrinsic and acquired, remains the primary cause of the failure of cancer therapy. Intrinsic drug resistance is a disturbing problem responsible for the refractory nature of various types of cancer while acquired resistance is the result of the failure to chemotherapy after an initial successful response. This resistance emerges at irregular intervals after a successful outcome and confers tolerance to previously effective treatments and other untested treatments [119]. The MDR phenotype was thus characterized as the resistance to a number of structurally and functionally unrelated chemotherapeutic agents, display of active outward transport, and overexpression of a 170 kDa plasma membrane associated glycoprotein P-gp, so-called "classical" or P-gp-depending MDR [120]. P-gp is coded by the MDR1 (ABCB1) gene and functions as an energy-dependent multidrug membrane transporter that rapidly extrudes a variety of hydrophobic anticancer drugs from target cancer cells. In tumor cells expressing P-gp there is reduced intracellular drug concentrations which decrease the cytotoxic effects of a broad spectrum of anti-tumor drugs. P-gp is a member of the ATP-binding cassette (ABC) transporter family of proteins that includes several members that confer drug resistance. A total of 48 ABC transporters have been identified in humans. These are classified in 7 subfamilies, based on sequencing of the highly conserved ATP-binding domains [119, 120].

Breast Cancer

Anthracycline chemotherapy is nowadays the standard treatment against breast cancer with doxorubicin widely used in combination with 5-fluorouracil and cyclophosphamide. All these drugs are substrates for ABC transporters. The role of these proteins in the breast cancer multidrug-resistant phenotype was investigated by evaluation of gene and protein expression in tumour samples using RT-PCR, Western blot and immunohistochemistry [121]. Different studies have reported that about 50% of breast tumors express P-gp, and this expression was associated with a higher likelihood of treatment failure [122-124]. Despite these findings, it was not possible to associate the expression of P-gp prior to chemotherapy with shorter progression-free survival (PFS), therefore the clinical relevance of this observation remains unclear [122]. In 1981 Tsuruo *et al.* [125] discovered verapamil, an inhibitor of P-gp function, raising the possibility to reverse the MDR phenotype. Verapamil was the first of many inhibitors that emerged later (cyclosporin A, dofequidar fumarate, tariquidar, etc) [126]. Based on this fact a large number of clinical trials were developed, but due to several considerations including potency of the agents and the design of the trials, the results were not satisfactory [127-129]. Saeki *et al.* [130] performed a Phase III randomized double blind control study of six cycles of CAF (100 mg cyclophosphamide with 25 mg/m2 adriamycin and 500 mg/m2 fluorouracil) with or without 900 mg dofequidar in 221 patients with metastatic breast cancer. Although both the overall response rates of 42.6% and 53.1% ($p = 0.077$) and median progression-free survivals of 241 days and 366 days ($p = 0.145$) for CAF alone and CAF plus dofequidar, respectively, suggested a benefit from adding dofequidar, the results were not statistically significant [130]. Both clinical trials Phase II and Phase III showed no significant improvement in the response rate [131]. Even though clinical results failed to show correlation, a meta-analysis demonstrated that the expression of P-gp increases after chemotherapy and that the expression of this protein correlates with a poorer response to treatment [121]. Therefore, the concern that has arisen in the last years is whether the expression of P-gp

is related to decreased drug accumulation or is a marker for another feature of poor outcome, such as invasiveness [130]. As previously described the overexpression of P-gp in tumor tissue has been associated with the clinical outcome, but the mechanism by which this upregulation occurs has still not been revealed. Several studies showed that epigenetic changes (acetylation and methylation) could be responsible for the rise in the expression of P-gp [130-132]. In conclusion, is important to note that although the expression of P-gp or other drug efflux pump in tumors have not achieved successful results in clinical trials, this does not mean that these proteins are not important in the development of new therapeutic strategies, an effort should be directed toward developing real test for ABC transporters expression [129].

Ovarian Cancer

The most common regimen for primary treatment of ovarian cancer is the combination of cytoreductive surgery followed by the doublet of a taxane (paclitaxel 135–175 mg/m2) and carboplatin [136]. Several types of drug resistance mechanisms have already been identified [137, 138], but the overexpression of P-gp is the most frequent reason for therapy failure [139]. P-gp is involved in the transport of paclitaxel [140, 141] and this mechanism is the most frequent reason for therapy failure. P-glycoprotein expression correlates with unfavourable prognosis and is suggested as a marker for chemotherapy resistance in advanced ovarian cancer [142]. The detection of the expression level and function of MDR1/P-gp should provide useful information for the more efficient treatment of malignant diseases by properly adjusting the chemotherapeutic protocol. However, resistance to chemotherapy remains a major challenge to treatment, and although MDR has been extensively studied in *in vitro* models, the translational utility of this research remains an uncertainty [143]. Moreover, until now, many P-gp inhibitors failed to show efficacy during preclinical and clinical studies [144]. Recently, lapatinib, a novel member of the family of kinase inhibitors that inhibits the tyrosine kinases of HER2 and EGFR, has been shown to inhibit the function of ABC transporters by binding to their ATP-binding sites, including P-gp [145].

In past years, many efforts have been made to overcome drug resistance associated with PTX treatment [146]. Moreover, PTX effectiveness is decreased due to their adverse side effects. In a study in 2012, Vergara *et al.* [146] developed nanoformulation of two drugs in one nanocapsule locating PTX in the core and lapatinib on the shell periphery. These formulations have been used to test the efficiency of lapatinib/PTX nanocolloids in comparison with that of PTX given alone. Such combination (PTX and lapatinib) enhanced the cytotoxic efficacy of PTX. Overall, the results of this study show that PTX-nanocolloids increased *in vitro* antitumor efficacy of PTX and that the combination with lapatinib can significantly overcome multidrug resistance in ovarian cancer cell lines. These results are encouraging for the development of multifunctional nanocolloids that could be used in the clinical practice. Preclinical studies in animal tumor models are necessary, including a detailed evaluation of pharmacokinetics and pharmacodynamics [146].

On the other hand, the identification and analysis of genes relevant to MDR1 in patients may contribute to a better understanding of this phenomenon and potentially help circumvent this obstacle [147]. MDR1 (ABCB1), located at 7q21.12, is genetically very variable, and it is likely that SNPs in this gene may have significant effects on the expression and function of P-gp, and hence the absorption, metabolism and clearance of P-gp substrates [147-149].

Johnatty *et al.* [143] analysed the largest study to date of MDR1 SNPs and clinical outcome among women with invasive epithelial ovarian cancer treated with paclitaxel-based chemotherapy. The authors didn't found significant associations between SNPs analyzed and OS in patients with nil residual disease treated with any first-line chemotherapy regimen. Then they proposed that this SNP is modifying the effect of residual disease, a significant predictor of outcome, on overall survival independently of chemotherapy regimen, and warrants further investigation. There was no convincing evidence of an association between PFS or OS and any of the 1562 SNPs analysed in either the 'standard' or 'all chemo' subsets.

The lack of a standard approach to study the cancer transcriptome as well as in analysis of gene expression is a major impediment to formulating hypotheses about the etiology and effective treatment of cancer [148]. Gillet *et al.* [149] recently

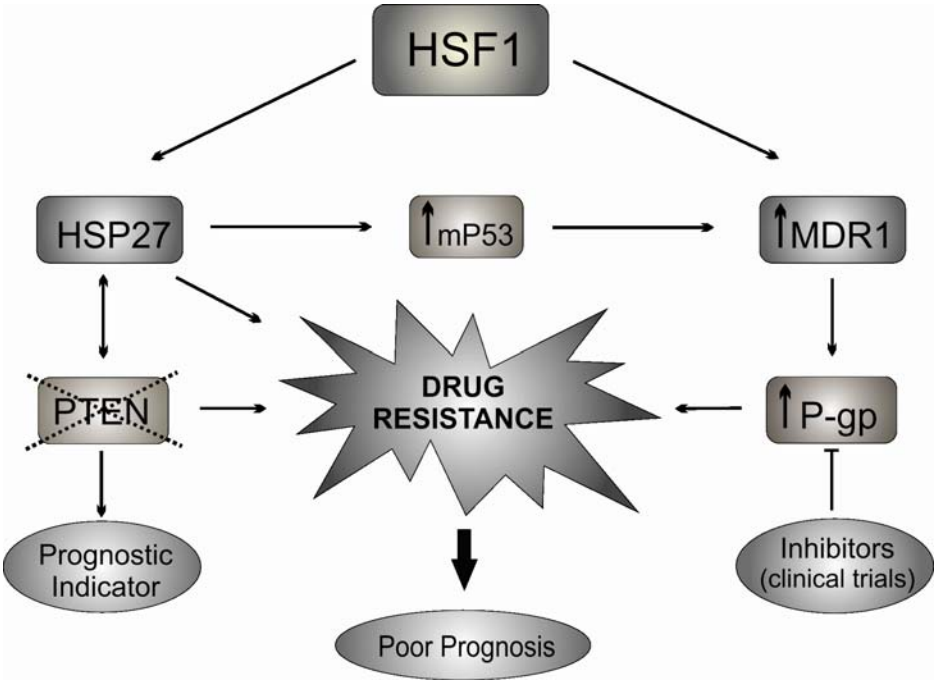

Fig. (3). Participation of HSP27, PTEN, p53 and P-gp in drug resistance. HSF1 can induce HSP27 expression and the constitutive activation of the multidrug resistance gene 1 expression in stress-dependent and stress-independent manners [154]. HSP27 is involved in the accumulation of transcriptionally active mutant p53 (mP53) resulting in the MDR1/P-gp phenotype. P-gp expression correlates with unfavorable prognosis and chemotherapy resistance. Currently, P-gp inhibitors are being tested in preclinical and clinical studies. HSP27 produces downregulation of PTEN and the loss of this protein has been suggested to be an independent prognostic marker although its role in drug resistance remains controversial.

showed the superiority of Taq-Man-based quantitative reverse transcriptase PCR (qRT-PCR) over established technologies in assessing the expression profiles of highly homologous ABC transporter genes. They used the TaqMan low density array (TLDA), a medium throughput TaqMan-based qRT-PCR assay, to evaluate the clinical relevance of 380 MDR-related genes. Most of these genes have been reported to be involved in MDR in studies of cultured cancer cells and may be involved in either intrinsic or acquired MDR, in many cancer types. Also they found that the expression signature of a group of 11 genes statistically improves the prognostic power of 4 clinical covariates (age, stage, residual tumor status after cytoreductive surgery, and CA-125 level) for OS. Data reveal that the expression level of the 11 genes associated with MDR define an intrinsic drug resistance signature that significantly improves prediction of OS in patients with ovarian cancer compared with predictions based only on clinical covariates. The influence of these genes on drug resistance and its relationship with the malignant phenotype and/or growth rate of the tumor remains to be determined.

MDR and HSPs

HSF1 is an inducer of the MDR response in cell culture [2]. HSF1 is the transcription factor responsible for the transcriptional response of vertebrate cells to different stresses including heat shock, ischemia, and aging all of which produce accumulation of misfolded proteins as a central feature [150]. The data suggest that HSF1 modulates the MDR-1 mRNA maturation pathway and could act either on splicing or on the stability of some MDR-1 mRNA precursors. The induced MDR phenotype occurs in the absence of heat shock or cellular stress, independently from the canonical heat shock–activated mechanism involved in HSP induction [151, 152]. In addition, yeast HSF can activate the expression of PDR3, encoding an MDR transcription factor that also directly activates RPN4 gene expression, which in term encodes a transcription factor that activate the expression of a number of genes encoding proteasome subunits [153]. These authors suggest a close linkage between stress responses and pleiotropic drug resistance, at least in yeast the regulatory overlap among HSF, Yap1 and Pdr3 suggest that stress responses and MDR are not independent re-

sponses, that they are tightly coordinated events. Another link between MDR and HSPs has been mentioned earlier in doxorubicin-resistant MCF-7 cells [14].

CONCLUSIONS

It is clear that the HSP polypeptides are implicated in conferring to gynecologic malignancies different sensitivities to anticancer therapies including chemotherapy, endocrine therapy and immunotherapy (we are in the need of more studies about radiotherapy). One of the main problems in this field is that the HSP response is quite complex, there are several HSPs with different predominant roles and with different molecular partners that make it difficult to dissect the molecular mechanisms responsible for the different degree of sensitivity to the therapies. Among the main molecular routes are those where members of the HSP response meet PTEN, p53 and MDR1. Figure **3** presents some of these relationships taken as example HSF1 and HSP27. Cancer cells have redundant mechanisms to cope the challenge imposed by the different anticancer therapies. Therefore, to have a significant understanding of drug resistance presented by a particular patient we need to examine the molecular status of several key markers involved in the drug resistance pathways. The other major problem in this field is that an inhibitor of one particular HSP will not be enough to achieve a significant anticancer response, now that we know the complexity of this field we need to design strategies aiming to inhibit several molecular pathways simultaneously. This is the big challenge for the near future.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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LIST OF ABBREVIATIONS

HSPs	=	Heat shock proteins
HSF	=	Heat shock factor
MDR	=	Multidrug resistance
ADR	=	Adriamycin
P-gp	=	P-glycoprotein
DOX	=	Doxorubicin
ATM	=	Ataxia Telangiectasia Mutated
BCSCs	=	Breast cancer stem cells
PTEN	=	Phosphatase and tensin homolog
PI3K	=	Phosphatidylinositol 3-kinase
ER	=	Estrogen receptor
PFS	=	Progression free survival
OS	=	Overall survival
PR	=	Progesterone receptor
ORR	=	Overall response rate
CBR	=	Clinical benefit rate
HGF	=	Hepatocyte growth factor
17AAG	=	17-allylamino-17-demethoxygeldanamycin
UTR	=	3'-untranslated region
LOH	=	Loss of heterogozity
CIN	=	Cervical intraepithelial neoplasia
SCC	=	Squamous cervical cancer
MPA	=	Medroxyprogesterone acetate
MGMT	=	O6-methylguanine DNA methyltransferase
TAM	=	Tamoxifen
PARP	=	Poly (adenosine diphosphate ribose) polymerase
FAC	=	5-fluorouracil, adryamicin, and cyclophosphamide
CMF	=	Cyclophosphamide, methotrexate, and 5-fluorouracil
AI	=	Aromatase inhibitors
TRAP1	=	Tumor necrosis factor receptor-associated protein 1

Grp94 = 94 kDa glucose- regulated protein

PBMC = Peripheral blood mononuclear cells

SNPs = Single nucleotide polymorphisms

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