Research Article

Heat Shock Protein HSP27 Secretion by Ovarian Cancer Cells Is Linked to Intracellular Expression Levels, Occurs Independently of the Endoplasmic Reticulum Pathway and HSP27’s Phosphorylation Status, and Is Mediated by Exosome Liberation

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The heat shock protein HSP27 has been correlated in ovarian cancer (OC) patients with aggressiveness and chemoresistance and, therefore, represents a promising potential biomarker for OC diagnosis, prognosis, and treatment response. Notably, secretion of soluble HSP27 has been described by a few cell types and may take place as well in OC cells. Therefore, we studied HSP27 secretion mechanisms under diverse cellular conditions in an OC cell model system. Secretion of HSP27 was characterized after overexpression of HSP27 by transfected plasmids and after heat shock. Intra- and extracellular HSP27 amounts were assessed by Western blotting and ELISA. Protein secretion was blocked by brefeldin A and the impact of the HSP27 phosphorylation status was analyzed overexpressing HSP27 phosphomutants. The present study demonstrated that HSP27 secretion by OVCAR-3 and SK-OV-3 cells depends on intracellular HSP27 concentrations. Moreover, HSP27 secretion is independent of the endoplasmic reticulum secretory pathway and HSP27 phosphorylation. Notably, analysis of OC cell-born exosomes not only confirmed the concentration-dependent correlation of HSP27 expression and secretion but also demonstrated a concentration-dependent incorporation of HSP27 protein into exosomes. Thus, secreted HSP27 may become more important as an extracellular factor which controls the tumor microenvironment and might be a noninvasive biomarker.

1. Background

Ovarian cancer (OC) therapy is restricted to a great extent by a limited understanding of the driving force of OC breakout and progression. OC represents the eighth most common female malignancy and first most common cause of death amongst gynaecological malignancies in the Western hemisphere [1]. However, an unsatisfactory characterization of the underlying molecular and cellular dynamics primarily prevents the development of new diagnosis and treatment approaches.

Heat shock proteins (HSP) are cellular factors whose expression levels are frequently upregulated in carcinoma tissue with respect to the nonmalignant tissue counterpart. Recently, accumulating evidence has emphasized the pivotal role of HSP in initiation and progression of cancer. Cytoprotective and thus frequently prooncogenic activities of HSP are particularly in demand when cellular proteins are disordered. Moreover, HSP are mostly stress-inducible cell survival factors that are upregulated by chemical and physical stresses (e.g., oxidative stress, hypoxia, and heat shock) as well as radio- and chemotherapy. HSP functions are closely
linked to bind specifically to client proteins and subsequently to control turnover and activity of these proteins [2, 3]. While the two most abundant HSP group members, HSP70 and HSP90, have been investigated most extensively, only little is known about the small HSP family member, HSP27, and its effect in OC progression.

Elevated levels of HSP27 are associated with treatment resistance and poor prognosis in numerous types of malignancies including breast, prostate, liver, and gastric carcinoma [4–9]. HSP27 function in OC progression, however, has not been studied yet in detail. In 1995, Langdon and coworkers have determined HSP27 levels in primary OC tissue samples and correlated expression levels of the protein with the aggressiveness and chemoresistance of OC [10]. Recently, histological studies have shown that elevated HSP27 levels are important predictors of peritoneal metastasis [11, 12]. Furthermore, an experimental approach utilizing an established OC cell line revealed that HSP27 activity governs resistance to paclitaxel treatment [15]. It is known that HSP27's prooncogenic capacity is due to its properties as a cell survival factor; consequently, HSP27 represents a promising biomarker for OC diagnosis and treatment benefit.

Notably, a few studies have demonstrated that HSP27 is liberated by a so far unknown cellular machinery [14, 15] and that secreted HSP27 also may exhibit paracrine properties in tissues microenvironment. Secreted HSP27 was found to induce angiogenesis and to take effect in the regulation of NF-kB signaling [16–18]. Moreover, secreted HSP27 suppresses the release of proinflammatory factors while activating the production of anti-inflammatory factors [19]. In blood samples from OC patients, increased levels of HSP27-specific antibodies were described suggesting the presence of the corresponding antigen and secreted HSP27 protein itself [20]. Zhao and coworkers have shown an increase of circulating HSP27 levels in OC patients exclusively in peritoneal metastasis-positive patients, meanwhile the authors failed to demonstrate differences between the groups of healthy women and metastasis-negative OC patients [12].

The objective of the present study was to examine HSP27 secretion by OC cells against the background of future applications in gynecological diagnosis and prognosis. Thus, analysis of intra- and extracellular HSP27 levels was performed in an OC cell culture model.

2. Methods

2.1. Cell Culture. The human OC cell lines OVCAR-3 and SK-OV-3 were both received from Cell Lines Service (Eppelheim, Germany) and propagated in RPMI 1640 medium (Biochrom, Berlin, Germany) containing 10% foetal bovine serum (Biochrom), 0.125% gentamicin (Ratiopharm, Ulm, Germany), and 0.1% insulin (Novo Nordisk, Mainz, Germany) and DMEM/F12 (Life Technologies, Darmstadt, Germany) and propagated in RPMI 1640 medium without foetal bovine serum and gentamicin. 3.0 × 10^5 cells/well for lysates; GAPDH1 was the loading control in exosome preparations. HSP27 expression levels were normalized to controls (control = 1.0).

2.5. Overexpression of HSP27 and HSP27 Phosphomutants. 24 h before transfection OC cells were harvested by trypsinization and replated at a density of 3.0 × 10^5 cells/well into a 6-well cell culture plate containing the appropriate medium without foetal bovine serum and gentamicin. 3.0 μg of plasmid DNA/well (pHSP27 wt, pHSP27-3A, pHSP27-3D [21]; empty control vector pcDNA3.1, Life Technologies) was transfected into OVCAR-3 cells using Lipofectamine 2000
Figure 1: Basal intracellular expression and secretion of HSP27 in OVCAR-3 and SK-OV-3 cells. (a) OVCAR-3 and SK-OV-3 cells were propagated and relative intracellular HSP27 expression was determined by Western blotting. HSP27 signal was normalized to β-actin. (b) Secreted HSP27 concentrations were determined in OVCAR-3 and SK-OV-3 cell culture supernatants by ELISA. Graphs of samples were calculated as the mean ± SD. P values were determined by Student’s t-test with P ≤ 0.05 (∗) given as significant.

(Life Technologies) according to the manufacturer’s instructions. Transiently transfected cells were harvested 24 h, 48 h, and 72 h after transfection and analyzed by Western blotting and ELISA.

2.6. Heat Shock Experiments. For heat shock experiments SK-OV-3 cells were plated at a density of 1.5 × 10^5 cells/well into a 6-well cell culture plate and after an incubation of 24 h two protocols of heat shock were performed. Cells were exposed to a temperature program (heat shock cycle: 37°C for 24 h, 39°C for 24 h, and 37°C of 24 h) or cells were uniquely exposed to 39°C for 4 h. Control cells were treated at 37°C for 72 h and 4 h, respectively.

2.7. Inhibition of the Endoplasmic Reticulum Secretory Pathway. Cells were plated at a density of 1.5 × 10^5 cells/well into a 6-well cell culture plate containing the appropriate medium. After 24 h brefeldin A was supplemented at a final concentration of 80 nM using ethanol vehicle as control. At indicated time points, cells as well as cell culture supernatant were harvested and intracellular and extracellular HSP27 concentrations were determined by Western blotting and by ELISA analysis.

2.8. Exosome Preparation. Preparation of exosomes was followed as previously described [22]. Briefly, cells were incubated in T75 cell culture flasks with 20 ml cell culture medium and exposed to the heat shock cycle. Subsequently, cell culture supernatant was harvested and cleared by centrifugation (100 xg; 5 min; 4°C), and exosomes were pelleted by ultracentrifugation (100,000 xg; 1 h; 4°C; Sorvall RC M120 MX Micro-Ultracentrifuge with Ultra-Clear Centrifuge Tubes Beckman, 11 × 34 mm). Exosome sediments were resuspended in Laemmli buffer and analyzed by Western blotting.

2.9. Statistics. The statistical analysis was performed using SPSS 13.0 software (SPSS, Chicago, IL, USA). Data were evaluated using the graphics and statistics software program Graph Pad Prism 5.01 (GraphPad Software, La Jolla, CA, USA). Statistical comparisons were performed using Student’s t-test with P ≤ 0.05 (∗), P ≤ 0.01 (∗∗), and P ≤ 0.001 (∗∗∗) given as significant.

3. Results

3.1. HSP27 Expression in OVCAR-3 and SK-OV-3 Cell Lines Is Correlated with Differentially Secreted HSP27 Protein Levels. Secretion of the low molecular weight protein HSP27 from OC tissue has been described previously [12, 20]. However, further studies are required to unravel the cellular circumstances that promote HSP27 secretion with all of the attendant effects on tumor microenvironment control. In context of this, we aimed at analyzing basal expression of HSP27 in OVCAR-3 and SK-OV-3 cells by Western blotting (Figures 1(a) and 1(b)). Intracellular HSP27 amount in SK-OV-3 cells (HSP27/β-actin: 2.71 ± 0.80) was only slightly enhanced compared to OVCAR-3 cells (HSP27/β-actin: 1.82 ± 0.28, P = 0.1425). Notably, the subtle distinction between intracellular HSP27 expressions in both cell lines was linked with significantly elevated levels of secreted HSP27 protein in OVCAR-3 cells (Figure 1(c)). In OVCAR-3 cell culture supernatant, a significant increase in secreted HSP27 protein (1,004.9 ± 137.4 pg/ml) compared to SK-OV-3 cells (322.5 ± 9.9 pg/ml; P = 0.0197) has been exhibited.

3.2. Upregulation of Intracellular HSP27 Expression Leads to Enhanced Secretion of the Protein. Genetically engineered overexpression and heat shock induction of intracellular HSP27 protein have been used in order to explore a potential...
connection of intracellular HSP27 expression and secreted HSP27 levels. As shown in Figures 2(a) and 2(b), transfection of OVCAR-3 cells with the DNA plasmid pHSP27 wt led to a clear overexpression of HSP27 over a period of 72 h (24 h: 3.12 \pm 1.38, P = 0.0131; 48 h: 3.50 \pm 2.90, P = 0.0203; 72 h: 1.68 \pm 0.48, P = 0.0041) compared to pcDNA3.1 transfected controls. Parallel determination of HSP27 protein in the cell culture supernatant by ELISA demonstrated...
elevated amounts of secreted HSP27 compared to control transfections (24 h: 1.25 ± 0.58, P = 0.3625; 48 h: 1.68 ± 0.95, P = 0.1506; 72 h: 2.38 ± 1.28, P = 0.0418) (Figure 2(c)).

To verify these data, a comparable approach was used in which the endogenous HSP27 expression in SK-OV-3 cells was activated by heat shock induction. A 37°C/39°C/37°C heat shock cycle for 24 h each temperature (cycle) and a unique 39°C heat shock for 4 h (4 h) were performed and analyzed by Western blotting (Figures 3(a) and 3(b)). In both experimental setups, HSP27 levels rose significantly (cycle: 2.10 ± 0.72, P = 0.0037; 4 h: 1.71 ± 0.61, P = 0.0315) compared to 37°C control incubations. As shown in Figure 3(c), elevated intracellular HSP27 levels during heat shock cycle were linked to the significant increase in HSP27 secretion (cycle: 1.80 ± 0.42, P = 0.0001). After 4 h heat shock, however, no changes in secreted HSP27 concentrations were detectable (4 h: 0.99 ± 0.12, P = 0.8258).

3.3. HSP27 Secretion Is Insensitive to Brefeldin A Treatment. The molecular route through endoplasmic reticulum and Golgi apparatus to the extracellular space is the main secretory pathway for secreted proteins in eukaryotic cells and sensitive to inhibition by brefeldin A [23, 24]. To prove whether endoplasmic reticulum secretory pathway functionality may be critical for basal HSP27 secretion, we performed brefeldin A incubation experiments using interleukin 6 (IL-6) as positive control (Figures 4(a) and 4(d)). As expected, IL-6 secretion was suppressed in OVCAR-3 cells propagated in vehicle treated cells over a period of 48 h (5h: 0.53 ± 0.29, P = 0.0497; 24 h: 0.73 ± 0.16, P = 0.0056; 48 h: 0.76 ± 0.18, P = 0.0086) (Figure 4(a)). In SK-OV-3 cells, however, IL-6 secretion was blocked after 5 h (0.48 ± 0.19, P < 0.0001) followed by significantly increased levels of secreted IL-6 after 24 h and 48 h of incubation (24 h: 1.28 ± 0.19, P = 0.0278; 48 h: 1.65 ± 0.32, P = 0.0065) (Figure 4(d)).

Except the 5 h incubation of OVCAR-3 cells, brefeldin A treatment did not affect intracellular levels of HSP27 neither in OVCAR-3 cells (5 h: 1.30 ± 0.20, P = 0.0048; 24 h: 0.97 ± 0.21, P = 0.7389; 48 h: 1.00 ± 0.19, P = 0.9567) (Figure 4(b)) nor in SK-OV-3 cells (5 h: 1.02 ± 0.08, P = 0.6342; 24 h: 1.09 ± 0.77, P = 0.7599; 48 h: 0.98 ± 0.26, P = 0.8327) (Figure 4(e)).

ELISA analysis of cell supernatant of OC cells propagated in the presence of brefeldin A revealed no alterations of secreted HSP27 in OVCAR-3 (5 h: 0.92 ± 0.05, P = 0.7686; 24 h: 0.97 ± 0.39, P = 0.9141; 48 h: 0.98 ± 0.34, P = 0.9013) (Figure 4(c)) and SK-OV-3 cells (5 h: 0.95 ± 0.57, P = 0.8097; 24 h: 0.98 ± 0.68, P = 0.9534; 48 h: 1.45 ± 0.47, P = 1.040) (Figure 4(f)) compared to controls.

3.4. HSP27’s Phosphorylation Status Does Not Control Protein’s Secretion. Protein phosphorylation governs the oligomerization state of HSP27 and thus may be an initial step in following HSP27 transport into the extracellular space. Phosphorylated species of HSP27 exist in the form of monomers and low molecular weight oligomers and, therefore, are predominantly available for cellular protein secretion [25]. Transfection experiments using plasmids encoding for wild-type HSP27, the nonphosphorylatable triple substitution mutant HSP27-3A (substitution of serine-15, serine-78, and serine-82 with alanine-15, alanine-78, and alanine-82), and the phosphomimicking triple mutant HSP27-3D (substitution of serine-15, serine-78, and serine-82 with aspartic acid-15, aspartic acid-78, and aspartic acid-82) demonstrated a distinct overexpression of all of the three proteins in OVCAR-3 cells over a period of 72 h (Figures 5(a) and 5(b)).

Subsequent analysis of secreted HSP27 proteins indicated no significant differences in protein secretion comparing HSP27 wt (24 h: 1.38 ± 0.25, P = 0.0093; 48 h: 1.99 ± 0.46, P = 0.0014; 72 h: 2.41 ± 0.86, P = 0.0064), the phosphomutants HSP27-3A (24 h: 1.50 ± 0.38, P = 0.0177; 48 h: 1.99 ± 0.68, P = 0.0115; 72 h: 2.35 ± 0.90, P = 0.0096), and HSP27-3D (24 h: 1.52 ± 0.30, P = 0.0043; 48 h: 1.41 ± 0.31, P = 0.0182; 72 h: 1.93 ± 0.60, P = 0.0085) (Figure 5(c)). Again, genetically engineered overexpression of HSP27 led to an increase of extracellular protein as shown before (Figure 2); however, differences in secretion linked to nonphosphorylatable HSP27 protein (HSP27-3A), physiologically phosphorylated HSP27 protein (HSP27 wt), and phosphomimicking HSP27 protein by charge and steric characteristics of the aspartic acid residues (HSP27-3D) have not been detected.

3.5. HSP27 Secretion Is Performed by Exosome Particles. An alternative pathway for protein liberation is represented by shedding of membranous microvesicles. Within this group, there is one type of vesicles, exosomes, which are typically characterized as carrier vesicles primarily for proteins [26]. In case of SK-OV-3 cells a 37°C/39°C/37°C heat shock cycle induced the HSP27 expression intracellularly accompanied by enhanced HSP27 levels in extracellular exosomes (Figure 6). HSP27 protein analysis demonstrated a 3.47-fold (P = 0.0135) induction of HSP27 expression in cell lysates and a 10.25 ± 4.47-fold (P = 0.0061) increase in HSP27 incorporation into extracellular exosomes (Figure 6(a)) as shown by Western blot detection of HSP27 normalized to GAPDH loading control (Figure 6(b)). Thus, these data not only confirmed the concentration-dependent correlation of HSP27 expression and secretion but also demonstrated a concentration-dependent incorporation of HSP27 protein into exosomal particles.

4. Discussion

Secretion of HSP27 has been detected in a few cell types including granulocytes, macrophages, platelets, and cells of the cardiovascular system [15, 19, 27, 28], as well as in malignant cells of lung, liver, breast, and ovary [12, 14, 29, 30]. Beyond that, the understanding towards cellular functionality of secreted HSP27 is fairly limited so far. In comparison to the well-characterized cell survival properties of intracellular HSP27 in mammalian cells, secreted HSP27 may harbor new functions, for example, in angiogenesis, inflammation, and vital signal transduction pathways [16–19, 28].

In the present study we could demonstrate that HSP27 secretion depends, amongst other factors, on intracellular HSP27 concentrations. The experimental induction of HSP27 expression by an overexpression plasmid as well as by heat shock led to elevated intracellular levels of the protein followed by an enhanced liberation of cell-free HSP27. Analysis
of basal HSP27 expression and secretion in both cell lines, however, referred to further and, more likely, to individual mechanisms driving HSP27’s secretion. Even though intracellular protein levels of HSP27 are very similar in OVCAR-3 and SK-OV-3 cells (Figure I(a)), HSP27 secretion by OVCAR-3 cells was significantly higher compared to SK-OV-3 cells (Figure I(c)). Moreover, significantly elevated and comparable overexpression of HSP27 over a period of 72 h as
Figure 4: Intracellular expression of HSP27 and secretion of IL-6 and HSP27 in OVCAR-3 and SK-OV-3 cells in the presence of brefeldin A. (a, b, and c) OVCAR-3 cells were incubated with 80 nM brefeldin A and IL-6 secretion (a), intracellular HSP27 (b), and secreted HSP27 (c) were determined by ELISA (a and c) and Western blotting (b), respectively, at indicated time points. (d, e, and f) SK-OV-3 cells were incubated with 80 nM brefeldin A and IL-6 secretion (d), intracellular HSP27 (e), and secreted HSP27 (f) were determined by ELISA (d and f) and Western blotting (e), respectively, at indicated time points. Intracellular HSP27 signal was normalized to β-actin. All values were standardized to vehicle incubated controls (control = 1.0). Graphs of samples were calculated as the mean ± SD. P values were determined by Student’s t-test with $P \leq 0.05$ (∗), $P \leq 0.01$ (∗∗), and $P \leq 0.001$ (∗∗∗) given as significant.
shown in Figure 2(a) demonstrated a time-dependent accumulation of cell-free HSP27 in the cell culture supernatant. It took 72 h to increase extracellular HSP27 protein significantly during overexpression experiments (Figure 2(c)). Taken together, HSP27 secretion by OC cells seems to be controlled by several mechanisms including the intracellular protein concentration. Furthermore, heat shock experiments with incubations for 24 h (heat shock cycle) and for 4 h (unique heat shock) at 39°C resulted in discrepancies in regard to the HSP27 secretion. While intracellular levels of the heat shock protein rose by both protocols, only cell culture supernatants of heat shock cycle treated cells
Figure 6: Load of HSP27 in exosomes from heat shock-treated SK-OV-3 cells. (a) SK-OV-3 cells were treated with a 37°C/39°C/37°C heat shock cycle for 24 h each temperature (heat shock) and compared to cells incubated by 37°C for 72 h (control). Relative intracellular HSP27 expression in cell lysates and relative HSP27 load in prepared exosomes was determined by Western blotting. HSP27 signal was normalized to GAPDH. (b) Representative Western blots of experiments as described in Figure 3(a). P values were determined by Student’s t-test with $P \leq 0.05$ (∗) and $P \leq 0.01$ (∗∗) given as significant.
that collagen induces intracellular HSP27 phosphorylation in platelets and that phosphorylated HSP27 molecules are primarily secreted into the extracellular space [28]. Secreted HSP27 in plasma, however, is predominantly unphosphorylated [15], which may subsequently occur after release by cell surface and/or soluble phosphatases [40]. Thus, control of HSP27 oligomerization and thereby determination of HSP27 complexes' architecture and size possibly play pivotal role in HSP27 secretion regulation. However, overexpression experiments carried out with nonphosphorylatable HSP27-3A and phosphomimicking HSP27-3D mutants in this study negated regulation of HSP27 secretion by phosphorylation in case of OC cells.

While IL-6 secretion was significantly blocked in the presence of the endoplasmic reticulum secretory pathway inhibitor brefeldin A, HSP27 expression and secretion were not affected. Surprisingly, for periods of brefeldin A incubation longer than 5 h, IL-6 levels in SK-OV-3 cell culture supernatants increased. This effect may reflect differences in OVCA-R-3 and SK-OV-3 cell response to the cytotoxic agent brefeldin A. HSP27 secretion, however, could not be inhibited by brefeldin A, pointing to a differing secretion pathway for HSP27 release.

Protein secretion may also occur by direct translocation through the cytoplasmic membrane or by uptake into different types of export vesicles including exosomes [41]. In prostate cancer cells, HSP27 protein is unspecifically incorporated into exosomes [22], and in OC cells, Dolo et al. identified a protein release by cellular vesicle shedding [42]. The data presented here explicitly demonstrated the incorporation of HSP27 into exosomes, thereby liberating the protein from OC cells by bypassing the classical endoplasmic reticulum secretory pathway. From literature data, extracellular HSP27 appears to play a role in angiogenesis and inflammation, as well as in general signaling processes during tumor progression [16, 17, 19, 43]. Apart from that, HSP27's properties in the tumor microenvironment are widely unclear and thus representing the focus of recent research.

5. Conclusion

The tumor progression factor HSP27 is liberated from OC cells by exosomes. Protein's secretion appears independently of the phosphostatus as well as independently of the classical endoplasmic secretion pathway. Notably, HSP27 liberation is correlated with the intracellular HSP27 levels. This should be taken into consideration when anticancer treatment may induce HSP27 [3] and subsequently HSP27-mediated extracellular effects.

Regardless of the so far unknown functionality of extracellular HSP27 in OC cells and adjacent tumor tissues, secreted HSP27 may become a promising biomarker for OC. Zhao and coworkers compared 24 blood samples from healthy women to blood samples from 48 patients with epithelial OC. An association of elevated blood levels of secreted HSP27 with metastatic OC was found [12], but because of a low case number the study was of limited power. Nevertheless, secreted HSP27 may become more important as a cancer progression-related factor that controls tumor microenvironment activities. Thus, secreted HSP27 could be potentially useful as noninvasive biomarker for diagnosis, prognosis, and treatment response not only in OC therapy.

Competing Interests

The authors declare that they have no competing interests.

Authors’ Contributions

Matthias B. Stope, Dominique Koensgen, Martin Burchardt, and Alexander Mustea conceived the study, Matthias B. Stope, Gerd Klinkmann, and Karoline Diesing carried out experimental work, Matthias B. Stope, Dominique Koensgen, and Alexander Mustea analyzed and interpreted the results and carried out the statistical analysis, and Matthias B. Stope, Dominique Koensgen, Martin Burchardt, and Alexander Mustea wrote the manuscript. All authors read and approved the final manuscript.

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References

[1] J. Sehouli, A. M. Alvarez, S. Manouchehrpour et al., "A phase II trial of pemetrexed in combination with carboplatin in patients with recurrent ovarian or primary peritoneal cancer," Gynecologic Oncology, vol. 124, no. 2, pp. 205–209, 2012.
[2] M. Cohen, M. Dromard, and P. Petignat, "Heat shock proteins in ovarian cancer: a potential target for therapy," Gynecologic Oncology, vol. 119, no. 1, pp. 164–166, 2010.
[3] M. B. Stope, D. Koensgen, M. Burchardt, N. Concin, M. Zygmunt, and A. Mustea, "Jump in the fire - heat shock proteins and their impact on ovarian cancer therapy," Critical Reviews in Oncology/Hematology, vol. 97, pp. 152–156, 2016.
[4] P. Rocchi, E. Beraldi, S. Ettinger et al., “Increased Hsp27 after androgen ablation facilitates androgen-independent progression in prostate cancer via signal transducers and activators of transcription 3-mediated suppression of apoptosis,” Cancer Research, vol. 65, no. 23, pp. 11083–11093, 2005.
[5] D. R. Ciocca and S. K. Calderwood, “Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications,” Cell Stress and Chaperones, vol. 10, no. 2, pp. 86–103, 2005.
[6] B. M. Doshi, L. E. Hightower, and J. Lee, "The role of Hsp27 and actin in the regulation of movement in human cancer cells responding to heat shock," Cell Stress and Chaperones, vol. 14, no. 5, pp. 445–457, 2009.
[7] N. Ahmed, M. Ratnayake, B. Savoldo et al., "Regression of experimental mediuloblastoma following transfer of HER2-specific T cells," Cancer Research, vol. 67, no. 12, pp. 5957–5964, 2007.
[8] C. M. Joffroy, M. B. Buck, M. B. Stope, S. L. Popp, K. Pfizenmaier, and C. Knabbe, "Antiestrogens induce transforming growth factor β-mediated immunosuppression in breast cancer," Cancer Research, vol. 70, no. 4, pp. 1314–1322, 2010.
Disease Markers

[9] M. B. Stope, M. Weiss, M. Preuss et al., “Immediate and transient phosphorylation of the heat shock protein 27 initiates chemoresistance in prostate cancer cells,” Oncology Reports, vol. 32, no. 6, pp. 2380–2386, 2014.

[10] S. P. Langdon, G. J. Rabiazs, G. L. Hirst et al., “Expression of the heat shock protein HSP27 in human ovarian cancer,” Clinical Cancer Research, vol. 1, no. 12, pp. 1603–1609, 1995.

[11] M. Zhao, F. Shen, Y. X. Yin, Y. Y. Yang, D. J. Xiang, and Q. Chen, “Increased expression of heat shock protein 27 correlates with peritoneal metastasis in epithelial ovarian cancer,” Reproductive Sciences, vol. 19, no. 7, pp. 748–753, 2012.

[12] M. Zhao, J. X. Ding, K. Zeng et al., “Heat shock protein 27: a potential biomarker of peritoneal metastasis in epithelial ovarian cancer?” Tumor Biology, vol. 35, no. 2, pp. 1051–1056, 2014.

[13] T. F. Song, Z. F. Zhang, L. Liu, T. Yang, J. Jiang, and P. L. Li, “Small interfering RNA-mediated silencing of heat shock protein 27 (HSP27) increases chemosensitivity to paclitaxel by increasing production of reactive oxygen species in human ovarian cancer cells (HO8910),” Journal of International Medical Research, vol. 37, no. 5, pp. 1375–1389, 2009.

[14] J.-T. Feng, Y.-K. Liu, H.-Y. Songet al., “Heat-shock protein 27: a potential biomarker for hematopoietic carcinoma identified by serum proteome analysis,” Proteomics, vol. 5, no. 17, pp. 4581–4588, 2005.

[15] I. Kardys, N. Rifiati, O. Meilhac et al., “Plasma concentration of heat shock protein 27 and risk of cardiovascular disease: A Prospective, Nested Case-control Study,” Clinical Chemistry, vol. 54, no. 1, pp. 139–146, 2008.

[16] D. Thuringer, G. Jego, G. Wettstein et al., “Extracellular HSP27 mediates angiogenesis through Toll-like receptor 3,” FASEB Journal, vol. 27, no. 10, pp. 4169–4183, 2013.

[17] Y.-J. Lee, H.-J. Lee, S.-H. Choi et al., “Soluble HSPB1 regulates VEGF-mediated angiogenesis through their direct interaction,” Angiogenesis, vol. 15, no. 2, pp. 229–242, 2012.

[18] S. Salari, T. Seibert, Y.-X. Chen et al., “Extracellular HSP27 acts as a signaling molecule to activate NF-kB in macrophages,” Cell Stress and Chaperones, vol. 18, no. 1, pp. 53–63, 2013.

[19] K. Rayner, Y.-X. Chen, M. McNulty et al., “Extracellular release of the atheroprotective heat shock protein 27 is mediated by estrogen and competitively inhibits acLDL binding to scavenger receptor-A,” Circulation Research, vol. 103, no. 2, pp. 133–141, 2008.

[20] A. Olejek, A. Damasiewicz-Bodzek, P. Bodzek et al., “Concentrations of antibodies against heat shock protein 27 in the sera of women with ovarian carcinoma,” International Journal of Gynecological Cancer, vol. 19, no. 9, pp. 1516–1520, 2009.

[21] M. B. Stope, T. Schubert, D. Staaret al., “Effect of the heat shock protein HSP27 on androgen receptor expression and function in prostate cancer cells,” World Journal of Urology, vol. 30, no. 3, pp. 327–331, 2012.

[22] L. Rauschenberger, D. Staaret al., “Exosomal particles secreted by prostate cancer cells are potent mRNA and protein vehicles for the interference of tumor and tumor environment,” Prostate, vol. 76, no. 4, pp. 409–424, 2016.

[23] M. Pavelka, J. Neumüller, and A. Ellinger, “Retrograde traffic in the biosynthetic-secretory route,” Histochemistry and Cell Biology, vol. 129, no. 3, pp. 277–288, 2008.

[24] T. Fujiwara, K. Oda, S. Yokota, A. Takatsuki, and Y. Ikehara, “Brefeldin A causes disassembly of the Golgi complex and accumulation of secretory proteins in the endoplasmic reticulum,” Journal of Biological Chemistry, vol. 265, no. 34, pp. 18545–18552, 1988.

[25] K. Kato, K. Hasegawa, S. Goto, and Y. Inaguma, “Dissociation as a result of phosphorylation of an aggregated form of the small stress protein, hsp27,” The Journal of Biological Chemistry, vol. 269, no. 15, pp. 11274–11278, 1994.

[26] S. Mathivanan and R. J. Simpson, “ExoCarta: a compendium of exosomal proteins and RNA,” Proteomics, vol. 9, no. 21, pp. 4997–5000, 2009.

[27] J. Skrzeczynska-Moncznik, M. Bzowska, A. Nogiec et al., “Rapid externalization of 27-kDa heat shock protein (HSP27) and atypical cell death in neutrophils treated with the sphingolipid analog drug FTY720,” Journal of Leukocyte Biology, vol. 98, no. 4, pp. 591–599, 2015.

[28] H. Tokuda, G. Kuroyanagi, M. Tsujimoto et al., “Release of phosphorylated HSP27 (HSPB1) from platelets is accompanied with the acceleration of aggregation in diabetic patients,” PLOS ONE, vol. 10, no. 6, Article ID e0128977, 2015.

[29] M. Zimmermann, T. Mueller, B. Dieplinger et al., “Circulating heat shock protein 27 as a biomarker for the differentiation of patients with lung cancer and healthy controls—a clinical comparison of different enzyme linked immunosorbent assays,” Clinical Laboratory, vol. 60, no. 6, pp. 999–1006, 2014.

[30] M. A. Fanelli, F. D. Cuello-Carrión, J. Dekker, J. Schoemaker, and D. R. Ciocca, “Serological detection of heat shock protein hsp27 in normal and breast cancer patients,” Cancer Epidemiology Biomarkers and Prevention, vol. 7, no. 9, pp. 791–795, 1998.

[31] N. B. Gusev, N. V. Bogatcheva, and S. B. Marston, “Structure and properties of small heat shock proteins (sHsp) and their interaction with cytoskeleton proteins,” Biochemistry (Moscow), vol. 67, no. 5, pp. 511–519, 2002.

[32] A. J. Schuerwegh, W. I. Stevens, C. H. Bridts, and L. S. De Clerck, “Evaluation of monensin and brefeldin a for flow cytometric determination of interleukin-1 beta, interleukin-6, and tumor necrosis factor-alpha in monocyes,” Cytometry, vol. 46, no. 3, pp. 172–176, 2001.

[33] J. W. Wong, B. Shi, B. Farboud et al., “Ultraviolet B-mediated phosphorylation of the small heat shock protein HSP27 in human keratinocytes,” Journal of Investigative Dermatology, vol. 115, no. 3, pp. 427–434, 2000.

[34] K. Kato, S. Adachi, R. Matsushima-Nishiwaki et al., “Regulation by heat shock protein 27 of osteocalcin synthesis in osteoblasts,” Endocrinology, vol. 152, no. 5, pp. 1872–1882, 2011.

[35] M. J. Rane, Y. Pan, S. Singh et al., “Heat shock protein 27 controls apoptosis by regulating Akt activation,” Journal of Biological Chemistry, vol. 278, no. 30, pp. 27828–27835, 2003.

[36] M. Gaestel, W. Schroder, R. Benndorf et al., “Identification of the phosphorylation sites of the murine small heat shock protein hsp25,” The Journal of Biological Chemistry, vol. 266, no. 22, pp. 14721–14724, 1991.

[37] A. Zoubidei, A. Zardan, E. Beraldin et al., “Cooperative interactions between androgen receptor (AR) and heat-shock protein 27 facilitate AR transcriptional activity,” Cancer Research, vol. 67, no. 21, pp. 10455–10465, 2007.

[38] C. Zheng, Z. Lin, Z. J. Zhao, Y. Yang, H. Niu, and X. Shen, “MAPK-activated protein kinase-2 (MK2)-mediated formation and phosphorylation-regulated dissociation of the signal complex consisting of p38, MK2, Akt, and Hsp27,” Journal of Biological Chemistry, vol. 281, no. 48, pp. 37215–37226, 2006.

[39] S. Pavan, D. Musiani, E. Torchio et al., “HSP27 is required for invasion and metastasis triggered by hepatocyte growth factor,”
D. W. Moss, "Physicochemical and pathophysiologic factors in the release of membrane-bound alkaline phosphatase from cells," *Clinica Chimica Acta*, vol. 257, no. 1, pp. 133–140, 1997.

K. Prydz, H. Tveit, A. Vedeler, and J. Saraste, "Arrivals and departures at the plasma membrane: direct and indirect transport routes," *Cell and Tissue Research*, vol. 352, no. 1, pp. 5–20, 2013.

V. Dolo, S. D'Ascenzo, S. Violini et al., "Matrix-degrading proteinases are shed in membrane vesicles by ovarian cancer cells in vivo and in vitro," *Clinical and Experimental Metastasis*, vol. 17, no. 2, pp. 131–140, 1999.

S. K. Calderwood, S. S. Mambula, P. J. Gray Jr., and J. R. Theriault, "Extracellular heat shock proteins in cell signaling," *FEBS Letters*, vol. 581, no. 19, pp. 3689–3694, 2007.