Chapter 1
HSP27 as a Therapeutic Target of Novel Inhibitors and Dietary Phytochemicals in Cancer

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Abstract Heat shock proteins (HSP) are a family of evolutionary conserved proteins induced by cellular stressors. HSP are essential players in the development and progression of cancer inducing resistance to conventional treatment in a wide range of human tumors. Overexpression of HSP27, a member of HSP family, is associated with apoptosis inhibition, enhanced migration and metastasis and several clinical features of cancer including drug resistance promotion. At present, HSP27 could be a promising strategy to enhance sensitivity of tumors to cancer treatment. A plethora of novel compounds present in the diet, including flavonoids, can efficiently inhibit the growth of tumor cells by acting as natural "chemopreventers". Many works reported the efficient targeting of HSP27 by using small inhibitors and dietary natural compounds in order to enhance the effectiveness of cancer therapies. In this chapter, we reviewed the current status of treatments based in dietary components targeting HSP27 as a novel strategy to circumvent chemotherapy cytotoxicity in cancer patients. Moreover we addressed the current status of HSP27 overexpression in many types of human cancers and highlighted the prominent role of targeting HSP27 as a novel therapeutic strategy in cancer.

Keywords HSP27 • Cancer • Dietary phytochemicals • Therapeutic target • Chemotherapy

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Abbreviations

5-FU	5-fluorouracil
AA	Anaplastic astrocytoma
AR	Androgen receptor
Asp17	Asparagine 17
CRPC	Castration-refractory prostate cancer
CSCs	Cancer stem cells
DRSP	Drug-resistant sphere
ESI MS/MS	Electrospray ionization/multi-stage mass spectrometry
GBM	Glioblastoma multiforme
HCC	Hepatocellular carcinoma
HER2	Human epidermal receptor 2
HSF1	Heat shock factor protein 1
HSP27	Heat shock protein 27
NSCLC	Non-small cell lung cancer
OSCC	Oral squamous cell carcinoma
Phe55	Phenylalanine 55
p-HSP27	Phosphorylated HSP27
siRNA	Small interfering RNA
TDP	1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone
TRAIL	Tumor necrosis factor-alpha-related apoptosis-inducing ligand

1.1 Introduction

Heat shock proteins (HSP) are a family of evolutionary conserved proteins induced by cellular stressors. They were serendipity discovered five decades ago in the common fruit fly Drosophila melanogaster [1]. Ferruccio Ritossa published the first observation that cells could mount a specific transcriptional activity when exposed to elevated temperatures that was dubbed as the heat shock response. This discovery led to the identification of the so-called HSP, which impact many areas of current biology and medicine, as well as to the understanding of its involvement in human diseases [2]. Differential expression of individual HSP occurs in a broad range of neoplastic processes. In cancer, the HSP facilitate rapid cell division, metastasis, and the evasion of apoptosis induced by cytotoxic anti-neoplastic drugs [3]. Therefore, HSP are essential players in the development and progression of cancer and they are prime therapeutic targets. In particular, many tumors including breast, prostate, ovarian and renal cancer as well as osteosarcoma and leukemias have been shown to unusually express high levels of HSP27 [4]. Activation of HSF1 during cell stress induces the up-regulation of HSP27 in many cancer types [5] and is associated with drug resistance promotion, apoptosis inhibition, enhanced migration and metastasis, more aggressive tumors behavior, and poor patient outcomes [6, 7]. In this chapter,

we reviewed the current status of HSP27 overexpression in many types of human cancers and highlighted the prominent role of small inhibitors and dietary natural compounds targeting HSP27 as a novel therapeutic strategy in cancer.

1.2 Chemopreventive Dietary Phytochemicals in Cancer

Chemotherapy constitutes the standard regimen used for treatment of many types of human cancers. However, non-desirable side effects in normal cells related to these cytotoxic treatments limits its rationale use. Therefore, novel natural compounds and complementary medications including herbal molecules have become more frequently used for the prevention and treatment of cancer [8]. A plethora of novel compounds present in the diet, including flavonoids, can efficiently inhibit the growth of tumor cells by acting as natural "chemopreventers". The potent antioxidant activity of these compounds has recently received a great interest, because oxidative stress participates in the initiation and progression of cancer. Phytochemicals are capable of preventing oxidative damage to DNA, thus a wide use of natural food-derived molecules is receiving greater attention as potential anti-carcinogens. Epidemiological evidences from observational and prospective studies, mainly in Asian and European populations indicate that dietary compounds may substantially alter the natural history of carcinogenesis. A large body of epidemiologic data supports the fact that diet and nutrition play a key role in carcinogenesis. In fact, an inverse correlation between a high consumption of fruits and vegetables and the incidence of some cancers has been widely documented in diverse studies [9]. In this section, we reviewed the current status of treatments based in dietary components targeting HSP27 as a novel strategy to circumvent chemotherapy cytotoxicity in cancer patients.

1.2.1 HSP27 Targeting by Resveratrol Sensitize Breast Cancer Cells to Doxorubicin Therapy

Breast cancer represents the neoplasia with the highest incidence and mortality that affects women worldwide [10]. Breast carcinomas represent a heterogeneous group of tumors that are diverse in behavior, outcome, and response to therapy. Despite advances in screening, diagnosis, and therapies, the percentage of patients with complete pathological response to standard chemotherapy-based treatments is very low. The use of chemopreventive natural phytochemicals represents a promising strategy in the search for novel therapeutic agents in breast cancer. Resveratrol (3,4′,5-trans-trihydroxystilbilene) is a dietary polyphenol found in fruits, vegetables and medicinal plants that exhibits chemopreventive and anti-tumor effects in a wide spectrum of human cancers. Using a proteomic approach based on two-dimensional

electrophoresis and ESIMS/MS tandem mass spectrometry, Diaz Chavez et al. reported that resveratrol (250 μM) treatment for 48 h significantly modulated the expression of 16 proteins in MCF-7 breast cancer cells (fold change >1.5; p value <0.05). Particularly, resveratrol treatment at 100, 200, 250 μM resulted in an efficient down-regulation of HSP27 protein [11]. It has been well established that HSP27 is frequently overexpressed in human cancer cells resulting in apoptosis inhibition and resistance to anti-neoplastic therapy [12]. Interestingly, resveratrol was able to induce apoptosis in MCF-7 cells. Cell death was associated with a significant increase in mitochondrial permeability transition, cytochrome c release in cytoplasm, and caspases-3 and -9 independent cell death. These data suggested that resveratrol might exert its effects in cell death and chemotherapy resistance by targeting HSP27. The potential chemosensitizing effect of resveratrol was demonstrated by treating MCF-7 cells with increasing concentrations of the polyphenol in combination with doxorubicin therapy. Combined therapy caused a potent effect in MCF-7 cells viability. Moreover, targeted abrogation of HSP27 expression using specific short-harping RNAs exerted a synergistic effect in cytotoxicity caused by doxorubicin. These data indicate that resveratrol inhibits HSP27 expression resulting in a cooperative effect on doxorubicin-induced cell death. The potential modulation of HSP27 using natural alternative agents, as resveratrol, may be an effective adjuvant in breast cancer therapy.

1.2.2 Ginko biloba *EGb761 Inhibits Migration by Targeting HSP27 in Non-small Cell Lung Cancer*

Lung cancer remains as one of the most aggressive cancer types with nearly 1.6 million new cases worldwide each year [13]. Non-small cell lung cancer (NSCLC) is the most common subtype of lung cancer, comprising three major histological subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Overall 5-year survival rates for lung cancer are consistently low (7.5–16 %). Approximately 40 % of patients with advanced unresectable disease at the time of diagnosis have a poor prognosis. The treatment choice for unresectable stage III NSCLC is platinum-based chemotherapy regimen and thoracic radiation. Considering that the limited therapeutic options for NSCLC, novel therapeutic targets and drugs are urgently needed. The extracts from the leaves of *Ginkgo biloba* have been widely used for centuries due to their antioxidant properties. A standard *G. biloba* extract, EGb761 (commercial name), contains 22–27 % flavonoids and 5–7 % terpenoids that constitute the most active molecules. At cellular level EGb761 may scavenge free radicals and neutralize ferry ion-induced peroxidants. In addition, EGb761 displays beneficial effects by blocking exacerbated cell proliferation, angiogenesis, and apoptosis in cancer cells [14, 15]. Importantly, phase II combined treatment involving 5-fluorouracil (5-FU) and EGb761 has been tested in patients with pancreatic or colorectal cancer, and has shown promising results [16, 17]. Recently,

Tsai et al., analyzed the cellular effects of EGb761 in NSCLC cell lines, and the HSP27 expression in patients with NSCLC in relationship with clinical outcomes. In NSCLC tumors HSP27 is overexpressed in comparison to normal lung tissue and is a poor prognostic indicator of NSCLC. Kaplan–Meier survival curve showed that NSCLC patients with low HSP27 expression had significantly better survival time than those with a high expression. EGb761 did not have cytotoxic neither apoptotic effects in A549/H441 cell lines. Interestingly, EGb761 inhibited HSP27 expression and migration of NSCLC cell lines. Moreover, HSP27 abrogation by using specific HSP27-siRNA significantly inhibited the migratory ability of A549/H441 cell lines. Mechanistic studies showed that EGb761 treatment activated the AKT and p38 pathways but not affected the PI3K, ERK, and JNK pathways [18]. These data showed that EGb761 could decrease the migration of A549/H441 by inhibiting HSP27 expression most likely through AKT and p38 MAPK pathways activation.

1.2.3 Targeting of HSP27 by Quercetin in Oral Cancer Cells

Oral squamous cell carcinoma (OSCC) is one of the most common and lethal head and neck malignancies worldwide [13, 19]. Treatment failure in oral squamous cell carcinoma (OSCC) leading to local recurrence(s) and metastases is mainly due to drug resistance. Cancer stem cells (CSCs) are thought be responsible for the development of drug resistance. However, the correlations between CSCs, drug resistance, and new strategy against drug resistance in OSCC remain elusive. Quercetin is a phytochemical found in apples, onions, teas, red wines, and many other foods. It can inhibit the growth and proliferation of cancer cells. Quercetin cancer-preventive effects have been attributed to various mechanisms, including the induction of cell-cycle arrest and/or apoptosis, as well as its antioxidant functions [20]. Using a drug-resistant sphere (DRSP) model of SCC25 oral cancer cells, Chen et al. [21] showed that quercetin suppresses drug-resistant spheres via the p38 MAPK-HSP27 apoptotic pathway in oral cancer cells. The molecular characterization of DRSPs revealed the upregulation of the drug-resistance-related genes ABCG2 and MDR-1 and of CSC-representative markers, suggesting that DRSPs have greater resistance to cisplatin and stronger CSC properties. Moreover, overexpression of phosphorylated (p-HSP27) via the activation of p38 MAPK signaling was found in DRSPs. Remarkably, targeting of HSP27 decreased cisplatin resistance and drug-induced apoptosis in DRSPs. Furthermore, quercetin treatment suppressed p-HSP27 expression resulting in apoptosis activation. Importantly, inhibition of tumor growth and attenuation of cisplatin resistance by quercetin was demonstrated in vivo using a xerograph model. These data showed that the p38 MAPK–HSP27 axis plays an important role in CSCs-mediated drug resistance in OSCC and that a combination of quercetin and cisplatin regimen can reduce tumor growth and decrease drug resistance in OSCC.

1.2.4 *Targeting of HSP27 Induces Apoptosis Upon Temozolomide and Quercetin Treatments in Glioma Cells*

Gliomas are the most common and devastating brain tumors. The most malignant group is represented by glioblastoma multiforme (GBM, WHO grade IV) and anaplastic astrocytoma (AA, WHO grade III) [22]. Prognosis for patients is poor even with aggressive treatment including surgical resection, chemotherapy, and radiation. Unfortunately, the average patient survival remains less than 2 years. Temozolomide is an alkylating drug used for treatment of grade IV astrocytoma, relapsed grade III anaplastic astrocytoma and melanoma [23]. In an interesting study, Jakubowicz-Gil et al. showed that silencing of HSP27 or HSP72 expression using specific siRNAs in GBM T98G and anaplastic astrocytoma MOGGCCM cells, increases their sensitivity to apoptosis induction upon temozolomide and/or quercetin treatment. After subsequent quercetin and/or temozolomide treatment, the level of HSP within the cells remained unchanged, indicating that the effect in cell death was specific for HSP27 and HSP72 downregulation. Notably, no autophagy neither necrosis was detected upon drugs treatments in T98G and MOGGCCM HSP27 or HSP72-deficient cells. Cell death was correlated with a decreased mitochondrial membrane potential, release of cytochrome c in the cytoplasm, and activation of caspase 3 and caspase 9 indicative of internal pathway involvement [24]. These findings indicate that molecular chaperones HSP27 and HSP72 are responsible, at least in part, for glioma cells resistance to programmed cell death. Thus, targeting of HSP using temozolomide and quercetin seems to be a potent and promising combination that might be useful in glioma therapy.

1.2.5 *Targeting of HSP27 by Xanthones from* Garcinia oblongifoliain *in Hepatocellular Carcinoma*

Hepatocellular carcinoma (HCC) is one of the most aggressive malignancies and leading cause of cancer-related death worldwide which accounts for approximately 500,000 deaths annually [25]. HCC is characterized by poor prognosis and recurrence after liver resection. With advances in the knowledge of molecular mechanisms of carcinogenesis, a lot of new diagnostic and therapeutic molecular targets may provide novel therapies in HCC [26]. Some of these novel therapeutic modalities are represented by dietary phytochemicals. In an recent study, Fu et al., showed that 1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone (TDP), isolated from the traditional Chinese medicinal herb, *Garcinia oblongifolia*, effectively inhibited cell growth and induced the caspase-dependent mitochondrial apoptosis in HCC. Using a proteomic approach based on two-dimensional gel electrophoresis and mass spectrometry identified the molecular targets of TDP in HCC cells. Particularly, HSP27 protein was one of the most significantly

down-regulated proteins by TDP treatment. Functional studies showed that HSP27 mediated cell death induced by TDP in vitro. Furthermore, a nude mice model also demonstrated the suppressive effect of TDP on HCC [27]. These data suggests that TDP induces apoptosis by repressing HSP27 expression, which was associated with the caspase-dependent pathway. Therefore, TDP may be a potential novel anti-cancer drug, especially to cancers with an aberrant high expression of HSP27. In other related study, it was demonstrated that TDP effectively stimulated HSP27 to form aggregates ex vitro, leading to suppression of its chaperone activity [28]. Mechanistic investigations shown that complexes were degraded by the ubiquitin-proteasome pathway. Moreover, TDP directly interacted with Asp17 and Phe55 in chain C of HSP27. In conclusion, this report indicates that HSP27 is a direct target of TDP in its anti-cancer activity, which provides strong support for a clinical application.

1.3 HSP27 as a Therapeutic Target in Human Cancers

HSP have been found overexpressed in a wide range of human tumors inducing resistance to conventional treatment; therefore they represent hopeful therapeutic targets in cancer [29, 30]. Many studies showed that high expression of HSP27 in cancer has been associated with metastasis [31–33] and has been described as differential prognostic marker [34–36]. HSP27 studies in animal models shown increased tumourigenic potential of rodent cells transplanted in syngeneic hosts [33, 37, 38]. Tumorigenic potential of HSP27 is explained at least in part, through their cytoprotective activity. In addition, the overexpression of HSP27 seems to be correlated with the induction of chemo- and radio-resistance in cancer cells. Studies in vivo e in vitro in cancer models shown that HSP27 is a potential gene target because its inhibition enhances the effectiveness of anti-cancer chemotherapies [39]. In this section, we reviewed the studies using inhibitors of HSP27 as a strategy to improve the chemotherapy or antibodies based treatments in many types of cancers

1.3.1 HSP27 Targeting in Breast Cancer

Clinical-pathological studies in breast cancer reveals that overexpressed HSP27 correlates with small tumor size, low proliferation index and short survival for patients node-negative [40]. In vivo analysis shown that HSP27 overexpression increases the metastatic potential of human breast cancer cells inoculated into athymic nude mice [41]. Moreover, in breast tumors the expression of estrogen receptors is correlated with up-regulated HSP27 expression, now it know that the HSP27 gene contains an imperfect estrogen-responsive element and can be induced by estrogen treatment in breast cancer cells [42]. Despite the chemo-protective effects of HSP, studies attempting to correlate HSP27 protein level in

breast cancers after therapy with tumor progression and clinical outcome have not been entirely clear. HSP27 overexpression confers resistance to doxorubicin and paclitaxel through inhibits apoptosis in human breast cancer cells [43, 44]. However, increased levels of HSP27 expression were not significantly associated with response to tamoxifen, time to treatment failure, or survival in an estrogen receptor-positive breast cancer population [45]. Studies in vitro demonstrated that downregulation of HSP27 in breast cancer cells expressing the human epidermal receptor 2 (HER2) resulted in increased responsiveness to Herceptine (trastuzumab) therapy, a monoclonal antibody specific to HER2, widely used against HER2-overexpressing metastatic breast cancers. Kang, et al. showed that HSP27 could enhance HER2 protein stability, which results in low susceptibility to treatment with Herceptine [46]. Moreover, the authors demonstrated by co-immunoprecipitation analysis that HSP27 can bind to HER2, which could be a mechanisms for obstructed the downregulation of HER2 by trastuzumab.

1.3.2 HSP27 Inhibition in Prostate Cancer

In patients with prostate cancer the overexpression of HSP27 is associated with poor clinical outcome following hormonal therapy [47]. Some studies showed that expression of HSP27 is upregulated by hormonal ablation and chemotherapy and is associated with castration-refractory prostate cancer (CRPC). Rocchi et al. showed that HSP27 expression was low or absent in prostate tumor tissues from patients untreated, but starts increasing 4 weeks after androgen ablation, to become uniformly highly expressed in CRPC [48]. As HER2 in breast cancer, in prostate tumors HSP27 regulate the expression of androgen receptor (AR) [49]. Therefore HSP27 is an independent predictor of clinical outcome because a low expression of HSP27 is associated with a delay in prostate tumor progression. Experimentally, HSP27 antisense oligonucleotides can enhance apoptosis and delay tumor progression in prostate cancer. Many studies shown that the use of HSP27 antisense oligonucleotides is an effective strategy for successful conventional therapy in some cancers including prostate, bladder and pancreas [50].

1.3.3 HSP27 in Leukemia and Lymphomas

HSP27 protein expression was evaluated in 98 adult patients with newly diagnosed acute myeloma leukemia for to identify prognostic factors alternative or additional to drug-resistance and apoptosis proteins. The expression of HSP27 was analyzed by immunocytochemistry and western blot and the results showed that it was expressed in a median of 15 % of total patients. In the multivariate analysis, HSP27 correlated with poor-risk cytogenetic [51]. Expression of HSP was associated with major adverse prognostic factors in acute myeloid leukemia. In pediatric patients,

HSP27 is highly expressed in bone marrow mononuclear cells of newly diagnosed acute myeloid leukemia-M4/M5 subtypes and leukemia cell lines. Knockdown of HSP27 expression allow apoptosis induced by anthracycline and cytarabine-based induction chemotherapy regimen. Downregulation of HSP27 increased the chemosensitivity of leukemia cells and the cytotoxicity anticancer drug-induced [52]. HSP27 confers resistance to bortezomib, a proteasome inhibitor that is currently used in clinical for the treatment of lymphoma cells. Blocking HSP27 using an antisense strategy enhanced the cells sensitivity allow apoptosis induced by bortezomib [53]. The cytoprotective effect from HSP27 in cancer has been studied in lymphomas and multiple myelomas. HSP27 is overexpressed in dexamethasone resistant cell lines. Specific down-regulation of HSP27 demonstrated that it could sensitize dexamethasone-resistant multiple myeloma cell lines and primary patient cells to treatment with this drug. Downregulation of HSP27 by siRNA restored the sensitivity to dexamethasone treatment by enhancing apoptotic response by triggering the release of mitochondrial protein Smac, followed by activation of caspase-9 and caspase-3 [54]. Therefore HSP27 may play a role in the inhibition to apoptotic pathways activation in order to decrease the cytotoxic action of chemotherapy drugs.

1.3.4 HSP27 Targeting in Lung Cancer Cells

Tumor necrosis factor-alpha-related apoptosis-inducing ligand (TRAIL) has recently emerged as a cancer therapeutic agent however most tumor cells, including A549, are resistant to TRAIL treatment. Zhuang et al. investigate the effect of HSP27 abrogation using specific siRNA on drug sensitization of A549 cells to TRAIL treatment. Results showed that combination of HSP27 siRNA with TRAIL induced caspases activation and apoptosis in TRAIL resistant A549 cells. Combined treatment with HSP27 siRNA and TRAIL also increased JNK and p53 expression and activity [55]. Collectively, these findings indicate that targeting of HSP27 can sensitized the cells to TRAIL-induced apoptosis.

1.3.5 Targeting HSP27 in Cervical Cancer

A novel mechanism underlying the TRAIL sensitizing activity of the small molecule LY303511, an inactive analog of the phosphoinositide 3-kinase inhibitor LY294002, was reported in HeLa cells that are refractory to TRAIL-induced apoptosis. On the basis of the fact that LY303511 was derived from LY294002, itself derived from quercetin, and earlier findings indicating that quercetin and LY294002 affected HSP27 expression, authors investigated whether LY303511 sensitized cancer cells to TRAIL via a conserved inhibitory effect on HSP27. Data showed that upon treatment with LY303511, HSP27 was progressively sequestered in the nucleus,

thus reducing its protective effect in the cytosol during apoptosis. Remarkably, LY303511-induced nuclear translocation of HSP27 was linked to its sustained phosphorylation via activation of p38 kinase and MAPKAP kinase 2 and the inhibition of PP2A. Furthermore, genetic manipulation of HSP27 expression affected the TRAIL sensitizing activity of LY303511, which further corroborated the HSP27 targeting activity of LY303511. These data suggest a novel mechanism of small molecule sensitization to TRAIL through targeting of HSP27 functions, which could have therapeutic implications for overcoming chemotherapy resistancein HeLa tumor cells [56].

1.4 Conclusion

HSP27 expression is enhanced in many tumor cells, and it is involved in tumor progression and the development of treatment resistance in various tumors. Therefore, it is tempting to conclude that using conventional therapy for cancer treatment together with an inhibitor of HSP27 would have additive or synergistic anti-cancer effects. The phytochemicals derived from dietary compound represents potent novel inhibitors of the expression or activation of HSP27, which have prominent roles by affecting the hallmarks of cancer (Fig. 1.1). Moreover, phytochemical intervention

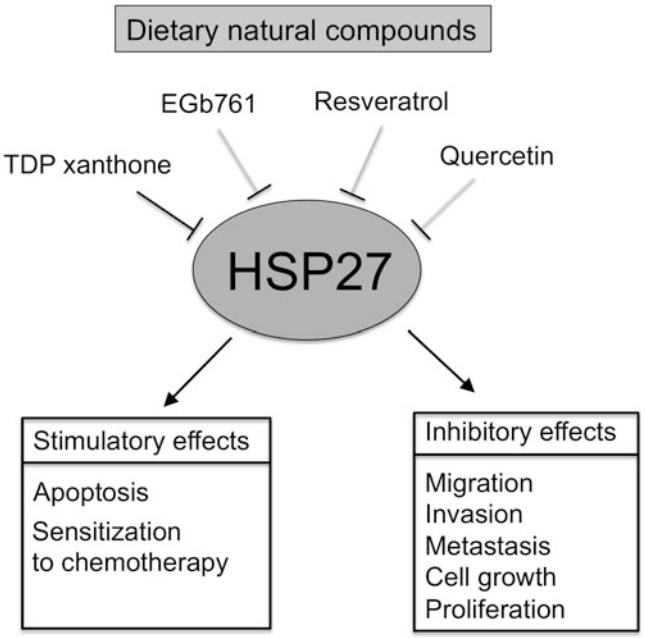

Fig. 1.1 Dietary compounds targeting HSP27 expression. HSP27 inhibition could induce stimulatory and inhibitory effects on the hallmarks of cancer

in combination with conventional chemotherapy induced strong drug sensitivity, mainly allowing apoptosis, at less in part, through to HSP27 inhibition. Therefore phytochemicals in combination with standard chemotherapy could represent a novel strategy for cancer treatment in tumors with overexpression of HSP27 chaperone.

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