Chapter 3
Heat Shock Proteins and Cancer: Plant Based Therapy

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Abstract Cancer is one of the major causes of mortality in the world. Each year approximately 13 million people suffer from cancer disease, and approximately 60 % of them die because of cancer. Besides most of the patients response harmful side effects of chemo- and radiotherapies. Therefore the establishment of new therapeutic strategies for the treatment of cancers will be required. A number of studies have shown that some HSP are induced in specific tumor cells. For example, increased levels of HSP105, HSP90, HSP70, HSP60, HSP27 have been detected in colon cancer, lung cancer, hepatocellular carcinoma, colorectal cancer, and gliomas, respectively. Elevated HSP levels in tumor cells are suggested to be responsible for increased chemotherapy resistance and poor prognosis. Suppression of HSP expressions in cancer cells is a new strategy for the treatment. It is well known that some plant extracts and their flavonoids significantly decrease HSP expression, and induce apoptosis of cancer cells. In addition, using of the HSP inhibitors in association with classical chemotherapy increases the sensitivity of cancer cells to the cytotoxic drugs. Therefore, some plants and their biologically active natural compounds have been investigated for their possible contribution to cancer therapy. The current chapter reviews the role of HSP in different cancer types and suppressing HSP with some natural products.

Keywords Cancer • Plant • Heat shock proteins

Abbreviations

17-AAG	Tanespmycin
17-DMAG	Retaspmycin
CDDP	Cisplatin
CRC	Colorectal carcinoma

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A.A.A. Asea et al. (eds.), *Heat Shock Protein-Based Therapies*,
Heat Shock Proteins 9, DOI 10.1007/978-3-319-17211-8_3

Dox	Doxorubicin
EGCG	Epigallocatechin-3-gallate
ERK	Extracellular signal-regulated kinase
GA	Geldanamycin
Grp	Glucose-regulated protein
HBV-related HCC	Hepatit B virus-related hepatocellular carcinoma
HSE	Heat shock element
HSF	Heat shock factor
HSP	Heat shock protein
JNK/SAPK	Jun-amino-terminal kinase/stress-activated protein kinase
PA	Peptide aptamer
PDTC	Pyrrolidine dithiocarbamate
PEITC	Phenethyl isothiocyanate
PES	2-phenylethynesulfonamide
Phen	1,10-phenanthroline
PTMs	Posttranslational modifications
RCC	Renal cell carcinoma
RP101	Brivudine
siRNA	Small interfering RNA
TF	Theaflavins
TR	Thearubigins
ZER	Zerumbone

3.1 Introduction

One of the major causes of mortality in the world is cancer. According to known statistical and epidemiological data, in 2008 approximately 7.6 million people have died from cancer in all over the world and it is estimated that by 2030 there will be 22 million new cancer cases every year (http://www.cancerresearchuk.org/cancer-info/cancerstats/world/; [1]). Although the most common cancer treatments such as surgery, radiotherapy, and chemotherapy are used for cancer therapy, these treatments are not enough to cure all cancer types. When it is considered that the cancer cases will increase in the future, the establishment of new therapeutic strategies for the management of the cancers will be essential.

Heat shock proteins (HSP, also called stress proteins) are one of the largest components of the cytoplasmic network. The evolutionary conserved HSP are classified into different families by their molecular weight: HSP100, HSP90, HSP70, HSP60, HSP40, and small HSP [2, 3]. These proteins are responsible for maintaining protein homeostasis in the cell. They generate dynamic complexes by forming non-covalent bonds with each other, other proteins and all of the cytoplasmic network components, and play an important role in preventing damage occurring in various proteins [4]. Heat shock proteins can be overexpressed in all organisms to protect themselves from environmental stresses such as heat, oxidative stress, and ischemia. They mediate the refolding or degradation of stress-damaged

proteins, thus protect the cells from potential deleterious effects and promote the cell recovery [5]. Besides, HSP have a lot of functions in the cell, they play some roles in cell division, apoptosis inhibition, and metastasis of cancer cells [6, 7]. They also play the regulatory roles in cell viability and death. Some stress-induced HSP, especially HSP27 and HSP70, protect the cells against apoptosis and necrosis [8, 9].

HSP also play important roles in the development of various diseases, especially in cancer and neurodegenerative diseases. A lot of studies indicated that the expressions of some HSP are elevated in many cancer types. Therefore some researchers have reported that these proteins are used as a biomarker in some cancer types [10–15]. It is revealed that HSP105, HSP90, HSP70, HSP60 and HSP27 are increased in colon cancer, lung cancer, hepatocellular carcinoma, colorectal carcinoma, and gastric cancer, respectively [11–13, 16, 17]. It is also known that the elevated HSP in tumor cells are suggested to be responsible for increased therapy resistance and poor prognosis [16, 18, 19]. A lot of studies have indicated association between HSP and chemotherapeutic drug resistance in cancer cells [10, 20–24].

As a result, a new strategy for the cancer treatment has been put forward to reduce the HSP expressions, and to decrease the resistance of chemotherapy and radiotherapy in cancer cells. Many studies have been performed to prove the accuracy of this new strategy and "a new HSP target". Recent studies showed that HSP inhibition by using antisense oligonucleotides or inhibitors has revealed successful results in clinical trials related to cancer treatment [25–30]. Besides, several plant extracts and some natural compounds have been used to suppress HSP expression in cancer cells for a long time [31–37]. In this chapter, HSP suppression using plant extracts and natural compounds will be discussed in detailed.

3.2 The Role of HSP in Cancer

Recent studies indicated that different HSP have been altered in different cancer types. The studies related to association between overexpressed HSP and cancer behaviour are still going on. The list of elevated HSP levels in different cancer types is shown in Table 3.1. Kai and his coworkers showed that HSP105 was increased in colorectal cancer and pancreatic adenocarcinoma patients [11]. Similar study on pancreatic adenocarcinoma patients revealed that HSP105 was remarkably increased in carcinogenic tissue versus normal tissue [38]. In a clinical study of prostate cancer patients, it has been found that HSP70 expression is increased in cancer patients in comparison to normal individuals [47]. HSP27, which is found abundantly in human serum, is suggested as a potential diagnostic marker in breast cancer [14]. Overexpressed HSP27 has been found in metastatic hepatocarcinoma tissues when compared to non-metastatic tissue [66]. Similar results were also obtained in gastric cancer [57]. HSP27 was found to be upregulated in colorectal carcinoma (CRC) versus normal cells [54]. A number of reports have been revealed that HSP27 has been upregulated in primary nervous system tumors, human astrocytoma, glioma and brain tumour [60, 62–64].

Table 3.1 Elevated heat shock proteins (HSP) in cancer

HSP	Cancer type	Findings	References
HSP105	Colorectal cancer and other several cancer types	HSP105 is overexpressed	Kai et al. [11]
	Pancreatic ductal and colon adenocarcinoma	HSP105 is overexpressed	Nakatsura et al. [38]
HSP90	Bladder carcinoma	HSP27, HSP60, HSP70 and HSP90 are overexpressed	Ischia and So [15]
	Breast cancer	HSP90 is overexpressed	Yano et al. [39], Pick et al. [40]
	Hepatocellular carcinoma	HSP27, HSP70, HSP90, Grp78, and Grp94 are increased in HBV-related HCC	Lim et al. [41]
	Leukemia	Upregulated HSP90 reflected aggressiveness of the cancer	Zackova et al. [42]
	Lung cancer	HSP90 is overexpressed	Didelot et al. [16]
HSP70	Breast cancer	HSP70 is upregulated	Ciocca et al. [43]
	Cervical squamous cell carcinoma	HSP70 can be use as diagnostic marker	Garg et al. [44]
	Hepatocellular carcinoma	Increased HSP70 is sensitive marker in early HCC	Chuma et al. [45], Takashima et al. [17]
	Intrahepatic cholangiocarcinoma	HSP70 is overexpressed	Lagana et al. [46]
	Prostate cancer	HSP70 is overexpressed	Abe et al. [47]
HSP60	Colorectal cancer	HSP60 is overexpressed	Cappello et al. [12]
	Lung cancer	HSP60 is overexpressed	Rubporn et al. [48]
	Prostate cancer	HSP60 and HSP10 are overexpressed	Cornford et al. [49], Cappello et al. [50]
HSP27	Breast cancer	HSP27 is upregulated	Conroy et al. [51], So et al. [14], Zhu et al. [52]
	Colorectal cancer	HSP27 is overexpressed	Yu et al. [53], Pei et al. [54]
	Endometrial cancer	HSP27 is upregulated	Geisler et al. [55]
	Gastric cancer	HSP27 is overexpressed	Kapranos et al. [56], Ryu et al. [13], Chen et al. [57]

	Gliomas and other brain tumours	HSP27 is overexpressed	Hitotsumatsu et al. [58], Hermisson et al. [59], Zhang et al. [60], Graner and Bigner [61], Cao et al. [62, 63], Liang et al. [64]
	Head and neck squamous cell carcinoma	HSP27 is overexpressed in metastatic lymph nodes	Zhu et al. [52]
	Hepatocellular carcinoma	HSP27, HSP70 and Grp78 are increased	Luk et al. [65], Song et al. [66], Chen et al. [67]
	Intrahepatic cholangiocarcinoma	HSP27 is related to aggressive tumor behaviour	Romani et al. [68]
	Acute myeloid and lymphoblastic leukemia	HSP27 is overexpressed	Yang et al. [69]
	Ovarian cancer	HSP27 level is increased	Arts et al. [70], Ciocca and Calderwood [23]
	Prostate cancer	HSP27 is overexpressed	Cornford et al. [49], Garrido et al. [71], Zhang et al. [72], Parcellier et al. [73], So et al. [14], Andrieu et al. [24])
	Renal cell carcinoma	HSP27 expression is significantly increased	Erkizan et al. [74]

Several studies in different tumour models exhibited that there is an association between HSP expression and multidrug resistance [75] and increased tumorogenesis-related apoptosis [76]. Studies have revealed that the upregulated HSP cause to increased resistant against anticancer drugs, such as Cisplatin (CDDP), Doxorubicin (Dox), Vincristine, Paclitaxel etc. in cancer cells [10, 77–82]. Besides, some studies have demonstrated that overexpression of HSP eliminates the lethal effect of gamma radiation in cancer cells [21, 82, 83].

Especially, HSP27 and HSP70 are found abundantly in malignant cancer cells, and they cause chemotherapy resistance [84]. In human ovarian tumor cell line, it has been shown that HSP27 and HSP70 are linked with the resistance to Cisplatin (CDDP). CDDP is a compound containing platinum and a potential anti-cancer agent. It has been widely used in the treatment of several malignant tumors such as testicular, head and neck, esophageal, lung, ovaries, and bladder cancers etc. The long-lasting CDDP treatment is limited, because of the risk of developing the resistant cells. Then these resistant cells become malignant. According to the western blot analysis increased expression levels of HSP27 and HSP70 are critical for the resistance mechanism of CDDP [22]. Upregulated HSP70 and HSP90 are also enhanced drug sensitivity in ovarian cancer [85].

In a study with 300 breast cancer patients, an association between tumour aggressiveness and HSP27 localization has been found [86]. The increased levels of HSP27 and HSP70 in breast cancer indicate that the cancer cells' resistance against to chemotherapy especially to Dox and apoptosis is increased [10, 23, 26, 78, 87, 88]. A recent report has indicated a relationship between HSP27 and Herceptin sensitivity in breast cancer cells. Overexpressed HSP27 has reduced Herceptin susceptibility in these cells [81]. Upregulated HSP70 is also essential for survival of tumorogenic breast cancer cells, and the decreasing of HSP70 activates tumor-specific cell death program (apoptosis) [20]. Especially, overexpressed HSP70 has been revealed to connect with weak prognosis and treatment resistance of breast cancer, servical cancer and hepatocellular carcinoma cells [6, 17, 45]. It has been shown that the overexpression of this protein in breast cancer is an indicator of failed treatment [43].

Additionally, it is known that HSP27 and HSP72 expressions are upregulated in prostate cancer [49, 89]. Recent immunohistochemical studies have shown that there is a correlation between HSP27 expression and prostate cancer aggressiveness, progression, and the development of the phenotype that does not respond to the hormone therapy [14]. HSP27 expression is induced to respond to the hormone or chemotherapy, thus it suppresses therapy with induced-apoptosis [79, 90]. The overexpressions of HSP27 and HSP70 in human prostate cancer have been shown to provide resistance to apoptosis and chemotherapy [24, 91]. Garrido and Parcellier have also found that increased HSP27 levels protect prostate cancer cells by increasing tumor proliferation and decreasing apoptosis, thereby facilitating tumor progression [71, 73]. Similar results were obtained for HSP72 in prostate cancer. Prolonged downregulation of HSP72 in PC-3 cells enhanced the sensitivity of cells to radiotherapy, and chemotherapy agents such as CDDP, vinblastin and taxol [89].

HSP27 has also been determined in several brain tumors, and there is a correlation between its expression and the degree of tumour malignancy [58, 92]. In neuronal cells in vivo overexpression of HSP27 exhibits neuroprotective properties by HSP27-mediated inhibition of apoptosis [93]. Induced HSP27 is also important for ranking histologically, which is related with weak prognosis in hepatocellular carcinoma [6]. The overexpressed HSP27 inhibits etoposide-induced apoptosis in human leukemic cells [94].

Besides, some studies have proved that overexpressed HSP70 is prior condition for the survival of various cancer types and suppressed HSP70 in tumour cells has been caused to cell death [20]. It is known that some inducible HSP (especially HSP27 and HSP70) protect the cells against apoptosis [84] and necrosis [19, 79, 95–99]. Recent studies have indicated that these proteins inhibit apoptosis via preventing caspases in different stages [100, 101]. The antiapoptotic effects of HSP27 and HSP70 have clarified by associating cytochrome C release from mitochondria, formation of apoptosome, and caspase [76, 101–103].

Considering all these studies, decreasing the HSP level in cancer cells would be beneficial for the treatment of cancers and the development of new therapeutic approaches targeted to HSP.

3.3 Why Are HSP Induced?

There are a variety of physiological, pathological and environmental factors such as growth factors, cell differentiation, tissue development, viral, bacterial, parasitic infections, ischemia, heat shock, heavy metals, ethanol, antibiotics etc. that induce HSP expression in the cells [104].

The transcription of *hsp* genes is provided through the interaction of heat shock transcription factor (HSF) and heat shock element (HSE). It has been determined four different HSFs up to now: HSF1, HSF2, HSF3 and HSF4 [3]. All HSFs are induced during development and adaptation of the cells, but only HSF1 regulates the HSP synthesis. HSF1 is activated when the cell exposed to stress, and regulates the expression of *hsp* genes [3, 103, 105].

The mechanism of *hsp* gene activation is still remaining not fully understood, but it is well known that the HSE and HSF1 carry impontant role in this mechanism. Although under normal circumstances HSFs are found in the cytoplasm as inactive monomers, which are linked to HSP70 and HSP90, they can only bind to DNA under stress conditions [106]. Under stress conditions HSF is subjected to a number of posttranslational modifications (PTMs), and converted to three phosphated form (homotrimer), and then transferred from cytoplasma to nucleus for binding to the DNA. After binding of HSF to promoter regions of *hsp* genes called as HSE, the gene transcription is get started in nucleus [103, 107, 108].

Several factors can lead to HSF activation. The most important clue for HSF activation is connection balance between the HSP molecules (like HSP70) and HSF

and stress caused- unfolded proteins. Any increase in the unfolded proteins entity, this balance changes the direction of the unfolded protein-HSP balance, so the HSF monomers are released from this complex [3].

HSF1 is a constitutively expressed protein, which is located in the cell nucleus and cytosol. Its molecular weight is 75 kDa, and which is found in a complex of approximately 200 kDa as inactive monomers [109]. Both DNA-binding and transcription activities of HSF1 monomers are suppressed with negative control, these monomers bind normally to HSP70 and HSP90 in the cell [105]. In physiological stress conditions such as high temperature and ischemia, HSF1 is separated from HSP via activation of kinases. This HSF1 monomers are hyperphosphorilated by ERK1, JNK/SAPK and p38 protein kinase [110] and they form a homotrimer approximately 700 kDa [105]. This active trimer is transferred from cytosol to the nucleus and binds to *hsp* genes [104]. The HSF1-DNA binding is closely associated with HSE, which is found in upstream promoter region of *hsp* genes. When the homotrimer is phosphorilated again by kinases, the *hsp* gene transcription is triggered in the nucleus. Following the transcription, mRNA of HSP is moved to cytosol and the synthesis is completed there. At the end of these events, HSF1 is retransferred to the cytosol, and created a complex with newly synthesised HSP for interrupting of HSP synthesis (Fig. 3.1).

In disease conditions, it is thought that the induction of HSF1 reduces protein damages by increasing *hsp* gene expression. Cell culture studies have revealed that the treatments of hypoxia, ethanol and sodium arsenite increase HSF1-DNA binding

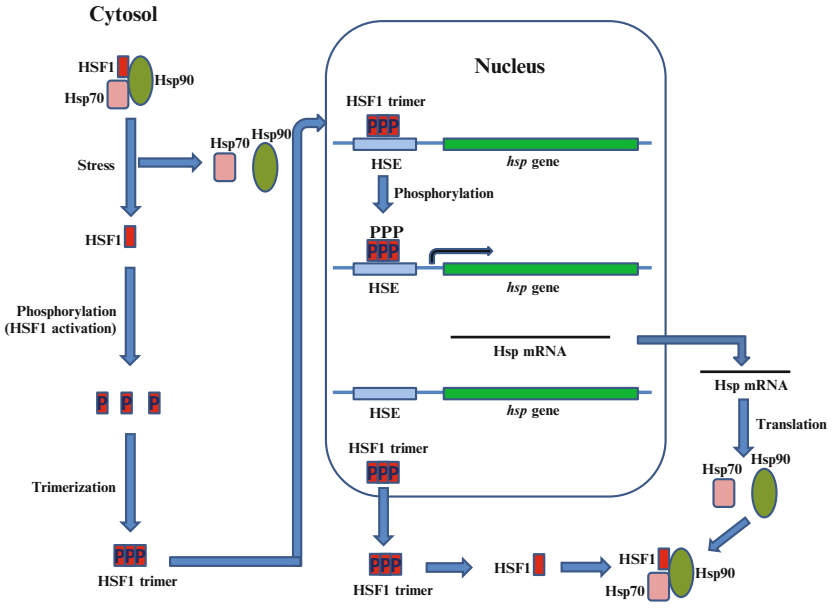

Fig. 3.1 The regulation mechanism of stress-induced *hsp* gene expression

and HSP70 level within the cell. High temperature weakens the association between HSF1 and HSP90, since the stress caused-denaturated proteins compete with HSFs to bind HSP90 [111]. The increasing of inducible HSP in cell suppresses the HSF1 activation via a regulatory mechanism [112].

The studies showed that overexpressed *hsp27* gene provides temporary resistance against lethal heat shock and increases the stability of actin filaments in the cell [113–115]. HSP27 also immobilizes the mitochondria [9]. Overexpressed HSP70 in pulmonary endothelial cells induced endotoxins and besides reduced apoptosis [116]. This suppression is obtained with interrupting procaspase-3 transformation to active caspase-3 [117]. Proapoptotic signals, such as Fas, decrease the HSF1-DNA connection in heat shock conditions, because HSF1 is not hyperphosphorylated [3]. It has been shown that HSP inhibit apoptosis via prohibiting to the different phases caspases [9, 100]. HSP27 plays a role in cellular redox state [96] and in preventing cellular damage as a result of declining of ATP [95]. The antiapoptotic effects of HSP27 and HSP70 are associated with the release of cytochrome C from mitochondria, caspase activation, and the formation of apoptosome [100, 101].

3.4 HSP Inhibition and Clinical Trials

Nowadays, many researchers focus on HSP inhibition as one of the important pharmacological approaches in cancer therapy. For that purpose, different methods such as antisense oligonucleotide, some natural agents, siRNA (small interfering RNA) applications etc. are used to suppress HSP in the cancer therapy [30]. Basically, there are three different strategies for suppressing of HSP: (1) Direct inhibitors, (2) Peptide aptamers binding to HSP, (3) Antisense oligonucleotides [30].

Because of the relationship between upregulated HSP and the treatment effectiveness, the HSP suppression is among the strategic targets in cancer treatment. The HSP inhibitors, which have been proposed for the cancer treatments, have been used either alone or with chemotherapic agents. According to the US National Istitutes of Health, today in all over the world, 168 studies are carried out with the title of “HSP”, and 75 clinical trials of them are related with “HSP inhibitors” for the treatment of different cancer types (http://clinicaltrials.gov/). These active clinical trials are generally related with HSP90 suppression. The importance of HSP90 is associated with the number of successful studies [7, 25, 26, 29, 118].

It is well known that HSP90 is responsible for maintaining the correct folding and stability of over 100 client proteins in cancer survival [119]. The inhibition of HSP90 causes to cell death (apoptosis). The natural products geldanamycin (GA), and semi-synthetic derivatives tanespimycin (17-AAG) and retaspimycin (17-DMAG) are known as HSP90 inhibitors and they evaluated alone or in combination with other drugs for the treatment of breast cancer in Phase 1 and Phase 2 clinical trials [25, 26, 29]. The other natural products such as radicicol

analogues, cyclopropradicicol and radicicol oximes are also used in preclinical trials to inhibit HSP90 [118, 119]. A recent study showed that the use of VER-155008 and 17-DMAG inhibitors to suppress HSP70 and HSP90 significantly increased antiproliferative and proapoptotic effects in acute myeloid leukemia [120].

The previously mentioned different strategies are also used to suppress the HSP27 expression in several clinical studies. These experiments revealed that quercetin and brivudine (also called RP101) directly inhibited HSP27 expression in several cancer cells [27, 79], the use of antisense oligonucleotides reduced HSP27 expression in cancer cells [121]. Besides, when peptide aptamers PA11 and PA50 are used for inhibiting of HSP27, chemo/radio-therapy efficacy is increased in HeLa cells [28].

Antisense oligonucleotides induce apoptosis of tumor cells by suppressing HSP [34]. When HSP27 expression is reduced about 40 % in HeLa cells by using antisense technology, these cells become more sensitive to apoptotic inducers [122]. Similarly, HSP27 supression decreases the potential of creating tumor from the prostate cancer cells, and increases the sensitivity of cells to anticancer drugs such as paclitaxel [79]. In a pancreatic cancer study, upregulated HSP27 caused to resistance of gemcitabine. When HSP27 expression was suppressed with siRNA, the cells became more sensitive to gemcitabine [123].

Recently, the silencing of HSP27 and HSP90 are one of the new targets to sensitize prostate cancer cells to chemotherapy and radiotherapy [118, 124]. Some experiments indicated that suppressed HSP70 by using quercetin, antisense oligonucleotide or siRNA increased apoptosis in prostate cancer cells [14, 34]. HSP27 antisense oligodeoxynucleotides and siRNA that target the human translation initiation site were reported to potentely inhibit HSP27 expression in human prostate PC-3 cells with increased caspase-3 cleavage, apoptosis and 87 % suppression of cell growth [79, 125]. Besides, targeting HSP27 by the second-generation antisense oligodeoxynucleotides (OGX-427) inhibited HSP27 expression and enhanced drug sensitivity in several xenograft models [79, 126].

Several studies emphasized that some synthetic antioxidants reduced increasing of HSP expression in the cells. Gorman and his colleagues have shown that certain antioxidant compounds (such as pyrrolidine dithiocarbamate (PDTC) and 1,10- phenanthroline (Phen)) prevented *hsp* gene induction and inhibited HSP27 and HSP70 in HL-60 cell line. The combination of antioxidant treatment led to cell death, which were exposed to heat stress [31]. There are also some evidences that several chemicals, such as benzylidene lactam, triptolide, emunine etc., inhibit *hsp* gene expressions by interacting with HSFs [108]. It is known that 2-phenylethynesulfonamide (PES, pifitrin-μ) is a specific inhibitor of stress-inducible HSP70, the usage of PES induces cell death in primary effusion lymphoma [127].

3.5 Plant-Based HSP Inhibitions

Nowadays in addition to synthetic drugs, a wide range of natural products are used in the treatment of cancer as supportive (supplemental) products. The use of medicinal plants in the treatment of cancer has a long history. They have been used since ancient times for the treatment of several diseases. Currently, more than 60 % of the anticancer agents is obtained from natural sources, such as plants, water organisms and microorganisms [128]. According to the clinical trials web page, at least 106 studies related to plant-based cancer therapy are being conducted (http://clinicaltrials.gov/).

In the literature, a lot of studies have been found related to HSP inhibitions by using plant extracts or natural products in different cancer cells (see Table 3.2). For example, Morino and his coworkers have pointed out that some flavonoids reduced the expressions of HSP27, HSP40, HSP60 and HSP70 in different tumor cell lines [144]. Similarly, in 2002, Rusak and his coworkers have revealed that quercetin, kaempferol, taxifolin, and isorhamnetin flavonoids are significantly decreased HSP27 and HSP70 gene expressions in heat-stressed leukemia cells [132]. Proteomic-based results indicated that resveratrol caused suppression of HSP27 and thus induced apoptosis in breast cancer (MCF-7) cells. Besides, inhibition of HSP27 expression by specific siRNA transfection also enhanced the chemotherapeutic effects of Dox in this cell [37].

It is well known that several natural compounds inhibit HSP expression in cancer cells. Boesenbergin A is a natural compound isolated from *Boesenbergia rotunda* and has apoptotic effect on the cancer cells. Boesenbergin A treatment is caused significantly suppressing of HSP70 in human T4-lymphoblastoid cells [36]. Phenethyl isothiocyanate (PEITC), a natural compound found in some plants, significantly reduced HSP27, HSP70, HSP90 and HSF-1 expressions in MCF-7 and MDA-MB-231 breast cancer cell lines [134]. Triptolide from *Triptergium wilfordii* suppressed HSP70 expression via inhibiting heat shock response in HeLa cells [141]. Zerumbone (ZER), an antioxidant isolated from *Zingiber zerumbet Smith* inhibited HSP27 expression in lung adenocarcinoma cells and also increased radiosensitization of this cell [143]. The plant polyphenols also show similar effects on cancer cells. For example, epigallocatechin-3-gallate (EGCG), one of the major polyphenols in green tea, specifically suppressed the expressions of HSP90 and HSP70 in MCF-7 human breast cancer cells by inhibiting the promoter activity of HSP90 [130]. EGCG has also been shown to induce apoptosis in human urinary bladder carcinoma cell line (TSGH-8301) by suppressing of HSP27 [131]. In an other study high-dose green tea polyphenols caused to downregulation of HSP27 and HSP90 mRNAs in mouse kidney and liver [145]. Black tea polyphenols, theaflavins (TF) and thearubigins (TR), downregulated the HSP90 expression and induced apoptosis in human leukemic U937 and K562 cells [140]. Lycopene and its derivative apo-14′-lycopenoic acid inhibited HSP70 and HSP90 expression in acute monocytic leukemia cells [133]. Deguelin isolated from *Mundulea sericea* induced

Table 3.2 Downregulated HSP by use of natural compound/plant extract in cancer

Plant extract/natural compound	Cancer cell/type	Findings	References
Boesenbergin A	T4 Lymphoblastoid cells	HSP70 ↓	Ng et al. [36]
Cimicifuga foetida extract	Breast cancer cells (MCF-7 cell line)	HSP27 ↓	Soler et al. [129]
Deguelin	Head and neck squamous cell carcinoma	HSP90 ↓, apoptosis ↑, autophagy ↑	Yang et al. [69]
EGCG	Breast cancer cells (MCF-7 cell line)	HSP70 ↓, HSP90 ↓	Tran et al. [130]
	Urinary bladder carcinoma	HSP27↓, apoptosis ↑	Chen et al. [131]
Kaempferol	Leukemia cell line (HL-60)	HSP27 ↓, HSP70↓	Rusak et al. [132]
Lycopene	Acute monocytic leukemia cell	HSP70 ↓, HSP90 ↓	Catalano et al. [133]
PEITC	Breast cancer cell	HSP27↓, HSP70 ↓, HSP90 ↓, HSF-1 ↓	Sarkars et al. [134]
Quercetin	Breast cancer	Dox efficacy↑	Staedler et al. [135]
	HeLa cell line	HSP27 ↓, HSP70↓, CDDP-induced apoptosis ↑	Jakubowicz-Gil et al. [136, 137]
	Leukemia cell line (HL-60)	HSP27 ↓, HSP70↓	Rusak et al. [132]
	Lung cancer cell line (A549)	HSP27↓, CDDP and gemcitabine efficacy ↑	
	Neuroblastoma and Ewing's sarcoma	HSP27↓, Dox efficacy ↑	Zanini et al. [138]
	Prostate cancer	HSP70↓	Kagaya et al. [32], Asea et al. [33], Jones et al. [34]
Resveratrol	Breast cancer cells (MCF-7 cell line)	HSP27↓, apoptosis ↑, Dox efficacy↑	Diaz-Chavez et al. [37]
Taxifolin, isorhamnetin	Leukemia cell line (HL-60)	HSP70↓	Rusak et al. [132]
Taxol	Ovarian and uterine cancer cells	HSP27↓, etoposide, colcemid and vincristine efficacy↑	Tanaka et al. [139]
Theaflavin and thearubigin	Leukemia cell lines (U937 and K562)	HSP90 ↓, apoptosis ↑	Halder et al. [140]
Triptolide	HeLa cell line	HSP70↓	Westerheide et al. [141]
Viscum album extract	Glioma cell line	HSP27 ↓, 14-3-3 β ↓, ζ ↓, γ ↓, apoptosis ↑	Önay-Uçar et al. [35]
Withaferin A	Pancreatic cancer	HSP90 ↓	Yu et al. [142]
Zerumbone	Lung adenocarcinoma cells	HSP27↓, radiosensitization ↑	Choi et al. [143]

↑: upregulation, ↓: downregulation

apoptosis and autophagy in head and neck squamous cell carcinoma ans is proposed as a potential HSP90 inhibitor [69]. Taxol has been suggested to overcome drug resistance to etoposide, colcemid and vincristine in ovarian and uterine cancer cells in vitro by inhibiting HSP27 expression [139]. These examples can be multiplied.

There are strong evidences related to suppressed HSP expression by using some plant extracts [35, 129]. Various *Viscum album* (mistletoe) extracts are widely used as complemantary cancer therapies in Europe [146, 147]. We checked antioxidant activity of the methanolic extract of *Viscum album* [148]. Our further studies revealed that *Viscum album* methanolic extract decreased the expression level of HSP27 and some 14-3-3 isoforms in glioma cells, pretreated with the extract before heat shock, and increased apoptosis via caspase-3 activation [35]. 14-3-3 proteins are considered as HSP, because the expression of some isoforms are induce via a process mediated by heat shock transcription factor [149]. In another study, it was reported that *Cimicifuga foetida* extract reduced HSP27 expression in MCF-7 cells [129].

There are a lot of studies explained that how quercetin affects HSP induction in the cells. Plant-derived flavonoid quercetin is an antioxidant molecule and regarded as an HSP inhibitor [150]. It suppressed heat shock induced-HSP70 expression in prostate cancer cells [32–34]. Quercetin also repressed heat shock induced-HSP27 and HSP70 expressions in HeLa cells [136]. This flavonoid reduced *hsp* gene expression at trancription level via preventing between HSF and HSE linkage [32, 33, 136, 151, 152] and inhibited heat shock response by preventing the formation of HSF trimers [153]. Quercetin acts on early steps of HSP synthesis, by blocking the additional modifications necessary for activation of HSFs, like posttranslational phosphorylation or by causing conformational changes of the factor, and by inhibiting its interaction with other DNA-binding proteins in the promoter region [136, 154]. Quercetin reduced the intracellular HSF1 level, especially constitutive phosphorylated forms [153], and thus connection to DNA [155, 156]. Quercetin inhibits not only HSF1 activation, but also many protein kinase activities [108].

Antioxidant compounds, such as quercetin and other bioflavonoids are useful for not only establishing positive and negative regulatory mechanisms for HSP expression but also for the clinical improvement of hyperthermic therapy of tumors [152]. In addition, many studies have demonstrated that some flavonoids exhibited a synergistic antitumour effect with chemotherapeutics [137, 157]. Quercetin sensitises HeLa cells to cisplatin and increases the level of apotosis. The significant decrease in HSP27 and HSP72 expression after the treatment correlates with the highest sensitivity of HeLa cells to cisplatin-induced apoptosis [137]. Additionally, it is well known that while the quercetin enhanced Dox efficacy in highly invasive breast cancer, it helped to reduce the cytotoxic side effects of Dox in non-tumoral cells [135]. The heat shock-induced stress proteins increased Dox resistance, but quercetin treatment caused a decrease in HSP expression and as a result the cells become more sensitive to drug in neuroblastoma and Ewing's sarcoma cells [138]. Besides, the quercetin caused to the suppression of HSP27 in lung cancer cells (A549). Using it combined with CDDP or gemcitabine, leaded to reduction of

the survival rate of lung cancer stem cells [158]. These findings indicate that other natural antioxidant compounds may also have potential for suppressing HSP expression.

3.6 Conclusion

Today, the researchers working on cancer therapy focused on HSP suppression, as it is well know that the HSP levels are elevated in many cancer types. Overexpressed HSP causes inhibition of programmed cell death, and increases resistance to the chemotherapeutic drugs [16, 19, 52]. Therefore, the inhibition of HSP has become an interesting strategy in cancer therapy. A lot of studies have also emphasized that HSP inhibition is gaining importance in cancer treatment [15, 25, 28, 82, 118, 119, 159]. Although some HSP inhibitors are used in several clinical trials, new agents that target HSP inhibition should be investigated for the treatment of cancer.

As described in this chapter, the suppressive effect of some plant extract or natural products on HSP expression may provide the development of new approach in cancer therapy. Especially downregulation of HSP can enhance the impact of chemotherapy or may reduce the side effects of applied drugs through medicating with low doses of chemotherapy agents to the patients. Considering all these studies, it is understood that the rate of success in the cancer treatment may have been boosted via new drug development, which has targetted to inhibition of HSP expression. Thus, the cancer cells may have been sensitized against the chemotherapeutic or radiotherapeutic agents. In summary, all data indicate that suppressing HSP by natural products may be a promissing wat to enhance apoptosis, and improve treatment efficacy, alongside with minimizing of toxic side effects in the cells. Future studies targeting these proteins for development of chemosensitizers may help to achieve more effective cancer treatment methods in combinational therapy.

Acknowledgements The author thanks to Prof. Dr. Nazlı Arda (Istanbul University) for helpful advice, and Dr. Murat Pekmez (Istanbul University) and Dr. Farinaz Jafari Ghods (Istanbul University) for suggestions. This study was supported by the Research Fund of Istanbul University (Project no. T-746/13092005 and 4120).

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