Suppression of HSP70 Expression by Quercetin and Its Therapeutic Potential Against Cancer

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Abstract Heat shock response is one of several survival pathways that protects cells against harsh conditions. This response mechanism, which is evolutionarily protected in all organisms, enhances the expression of heat shock proteins (HSP) that show protective properties for cells under stress conditions. High expression of many HSP is observed in cancer, and their functions aides the advancement of disease. It is known that overexpression of HSP70, a member of HSP family, in cancerous cells has been closely associated with tumor cell proliferation, apoptosis inhibition, enhanced migration and metastasis and drug resistance promotion. Therefore, targeting HSP70 in cancer treatment is very important. One of the best-studied inhibitors known for HSP70 is quercetin that is widely distributed flavonoid in the plant kingdom. Several *in vivo* and *in vitro* studies have reported the efficacy of quercetin in reducing elevated HSP70 levels in cancer therapy. It has become a focal point as an anticancer agent because of the induction of apoptosis in many different cancer cells. In this chapter, we reviewed the role of HSP70 in different cancer types and the suppressive effect of quercetin on expression of HSP70 family members. Moreover, we emphasized molecular mechanisms targeted by quercetin in cancer and its relationship to Hsp70.

Keywords Apoptosis · Cancer · HSP70 · Quercetin · Stress proteins · Therapeutic target

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Abbreviations

Akts (or PKB)	protein kinase B
AMPK	AMP activated protein kinase
CaMKII	calcium/calmodulin-dependent protein kinase II
Cdk	cyclin-dependent kinases
Chk2	checkpoint kinase 2
CK2	casein kinase 2
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
Hsc	heat shock cognate
HSE	heat shock element
HSF	heat shock factor
HSP	heat shock protein
IL-6	interleukin-6
JAK	Janus kinase
JNK	C-Jun N-terminal kinase
MAPK	mitogen-activated protein kinase
MMP	matrix metalloproteinase
PI3K	phosphatidylinositol 3-kinase
pRb	retinoblastoma protein
ROS	reactive oxygen species
RSK2	ribosomal protein S6 kinase 2
S6K1	ribosomal protein S6 kinase beta-1
shRNA	short hairpin RNA
siRNA	small interfering RNA
STAT3	signal transducer and activator of transcription 3
VEGF	vascular endothelial growth factor

Introduction

Heat shock proteins (HSP, also called stress proteins) are a group of highly evolutionary conserved proteins whose expression are induced under many stress conditions such as heat shock, oxidative/ischemic stress, toxins, heavy metals, radiation, environmental pollutants and chemotherapy. They are also stimulated by the release of cytokines in the cell. Mammalian HSP are classified mainly into six families by their molecular weight: HSP100, HSP90, HSP70, HSP60, HSP40, and small HSP (Benjamin and McMillan 1998; Snoeckx et al. 2001). Many of them act as molecular chaperones and are responsible for maintaining protein homeostasis in normal cells under non-stressful conditions. However, high expression of many HSP is observed during the many diseases including cancer, and their functions aides the advancement of disease. In cancer which is one of the leading causes of death

worldwide, induction of elevated HSP expression has crucial roles in tumor onset and progression processes such as rapid cell proliferation, evading apoptosis, and metastasis. Because of these properties, HSP have become one of the major therapeutic targets in cancer therapy (McConnell and McAlpine 2013; Lianos et al. 2015; Önay-Uçar 2015; Giri et al. 2017). In particular, increased expression of members of the HSP70 family in high grade malignant tumors has been reported. HSP70 has been shown to be highly expressed in many cancers including bladder, breast, colorectal, endometrial, gastric, lung, oral, uterine cervical and prostate cancer. Decreasing HSP70 levels in cancer cells will be beneficial, because increased HSP70 expression in cancerous cells is associated with cell proliferation, metastasis and poor prognosis (Ciocca and Calderwood 2005; Rohde et al. 2005; Evans et al. 2010; Murphy 2013; Alexiou et al. 2014).

Considering the possible therapeutic potential of suppressing of HSP70 expression in cancerous cells, a large number of studies in recent years have focused on quercetin, one of the most common bioflavonoids in the plant kingdom. Quercetin is well-known for its physiological functions and its role in the elimination of cancerous cells. It has important roles in the inhibition of increased HSP and in the activation of cell death pathways in cancer (Khan et al. 2016). In recent years, the number of studies on the activation of cell death pathways by quercetin-mediated suppression of HSP70s expression has increased. In this chapter, we reviewed the status of elevated HSP70 in various human cancers, and emphasized the effects of quercetin on the suppression of HSP70 expression and its therapeutic potential against cancer.

HSP70 and Cancer

The heat shock protein 70 (HSP70) family, one of the HSP families, consists of ATP dependent chaperones with molecular weight of approximately 70 kDa (in range 66–78 kDa). Their structure and function are evolutionary conserved and can be found in all organisms. Nowadays, there are 13 known homologous members of the human HSP70 family encoded by the HSPA gene family. They are transcriptionally regulated by four members of Heat Shock Factors (HSF): HSF1, HSF2, HSF3, and HSF4. The major transcriptional regulator HSF1 is induced during stress and is bound to the promoter of HSP70 to increase the transcription. The gene products differ by amino acid sequence, expression level, and cellular localization. Generally, these proteins, which exhibiting 52–99% amino acid sequence homology, function in protein homeostasis. It is known that HSP70 family members which play a role in correct protein folding and survival of the cells under stress conditions (Kampinga et al. 2009; Zuiderweg et al. 2013; Sherman and Gabai 2015; Giri et al. 2017).

The HSP70 family members are considerable important chaperones in cancer. There are eight well-studied members related to cancer. Six of them are mainly found in cytosol: Hsp70–1a and Hsp70-1b, collectively called Hsp70-1 (also known as Hsp70 or Hsp72), Hsp70-1t (also called Hsp70-hom), Hsp70-2, Hsp70-6 and

heat shock cognate 70 (Hsc70 also called Hsp70-8 or Hsp73). One of them localizes to the endoplasmic reticulum (Hsp70-5, also known as BiP or Grp78) and one to the mitochondria (Hsp70-9, also called mtHsp70, Grp75 or mortalin) (Rohde et al. 2005; Daugaard et al. 2007a; Murphy 2013). They play a key role in the regulation of malignancy. Tumor cells that overexpress HSP70 are found to be associated with tumor cell proliferation, metastasis, resistance to therapies and poor prognosis in many human cancers (Rohde et al. 2005; Powers et al. 2008; Evans et al. 2010; Murphy 2013; Rodina et al. 2014). It is also known that the chaperones of the HSP70 family have a well-documented antiapoptotic function. Several studies in different tumor models indicated that overexpressed HSP70 blocks apoptosis by interacting with components of the apoptotic pathways, and by interfering in the apoptotic signaling such as apoptosome, caspases, and cathepsins (Mosser and Morimoto 2004; Garrido et al. 2006; Evans et al. 2010; Kumar et al. 2016).

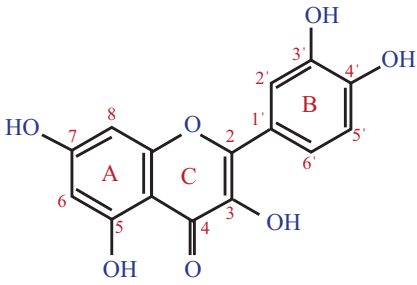
The most published data in human cancers is on Hsp70. The two best studied members of the HSP70 family are ubiquitously expressed Hsc70 and Hsp70 (Hsp70-1). There is less information about the expression levels of other HSP70 family members. Hsc70 is the only cytosolic HSP70 protein expressed constitutively under normal physiologic conditions and found abundantly in all major intracellular compartments. It is required for normal cell growth (Liu et al. 2012). In addition, Hsc70 overexpressed in colon and esophageal cancers is known (Kubota et al. 2010; Moghanibashi et al. 2013). Hsc70 has been also known to regulate functions of tumor-associated genes and proteins (Wu et al. 2017). Whereas Hsp70 is generally expressed at basal levels in normal cells under non-stressful conditions, it is the major stress inducible protein and distributed predominantly in the cytoplasm, nucleus, and plasma membrane of various malignant tumor cells. Its essential role is to maintain cell survival under stressful conditions. It is known that high Hsp70 levels are correlated with an aggressive phenotype and poor prognosis in therapeutic responses in bladder, brain, breast, colorectal, endometrial, gastric, oral, uterine cervical and prostate cancers (Ciocca and Calderwood 2005; Evans et al. 2010; Giri et al. 2017).

Downregulation of Hsp70 expression is associated with reduced tumorigenicity, and cytotoxic to transformed cells but undetectable in non-transformed cells. For this reason, Hsp70 knockdown sensitizes or kills cancer cells rather than normal cells (Schmitt et al. 2006; Kumar et al. 2016). Nylandsted and co-workers (2000) have shown that elevated expression of Hsp70 is necessary for the survival of tumorigenic breast cancer cells, and that the reduction of Hsp70 levels activates the tumor-specific death program. In a study of MCF-10A cells exposed to NZ28 which is an inhibitor of heat shock response, the researchers developed this inhibitor to enhance main effect of HSF1 on tumor development via upregulation of Hsp70. Knockout of Hsp70 is sufficient to similarly prevent cancer development in the Her2-positive breast cancer (Meng et al. 2010). It has been also found that inducible Hsp70 is effective in resistance of cancer cells to gamma radiation (Lee et al. 2001). Increased radiosensitivity was observed in a study on Hsp70 knockout mice (Hunt et al. 2004). Hsp70 overexpression in breast cancer cells has been found to be associated with lymph node metastasis (Kluger et al. 2005). Similarly, it is shown that

there is a correlation between vascular invasion and Hsp70 overexpression in gastric cancers (Canöz et al. 2002). Studies on patients with brain tumors have showed the presence of high expression of Hsp70 in gliomas, such as meningiomas and medulloblastomas (Alexiou et al. 2013, 2014). In a study performed on patients with prostate cancer, the Hsp70 expression of the patients was found to be higher than the control group, and Hsp70 was reported to be a potential biomarker for prostate cancer (Abe et al. 2004). Silencing of Hsp70 by short hairpin (sh)RNA in cervical and bladder cancer cells has been shown to suppress invasion and migration (Teng et al. 2012). Also, in the same study it has been demonstrated that Hsp70 interacted with Wiskott-Aldrich syndrome protein family 3 (WASF3), which is involved in prostate cancer invasion and metastasis. In bladder cancer cells, it has been found that the p63α protein -is a member of the p53 family- promotes the invasion and Hsp70 transcription, which has led to a new perspective in the understanding of Hsp70 function in high-invasive bladder cancer (Jin et al. 2017). In addition to studies on tissue Hsp70, studies on serum Hsp70 have also been carried out. Serum Hsp70s, which can be released by exocytotic traffic or cell disruption, have begun to gain importance in many studies. It has been found significantly high level of serum Hsp70 of patients with small cell lung cancer compared to healthy controls (Balázs et al. 2017). Similar findings observed in studies with colorectal cancer (Kocsis et al. 2010), chronic myeloid leukemia (Yeh et al. 2009), and head and neck squamous cell carcinoma (Ghermann et al. 2014).

There are few studies in the literature on other members of the HSP70 family associated with cancer except Hsc70 and Hsp70 proteins. Rohde et al. (2005) have reported that depletion of Hsp70 leaded to cancer cell detachment in many of human cancer cells. This data has shown that Hsp70 plays a role in the regulation of cancer cell adhesion. They have also reported for the first time that expression level of Hsp70-2, which is a member of HSP70 family, increases in breast, cervix, prostate and colon and liver cancers. In their study, it is shown that not only Hsp70-1 (Hsp70) but also Hsp70-2 are required for the survival of cancer cells. In another study on Hsp70-2, this protein depletion was found to induce lysosomal membrane permeabilization and cathepsin-dependent cell death in a variety of human cancers (Daugaard et al. 2007b). The level of Hsp70-5 (also known as BiP or Grp78), a member of the HSP70 family localized to the ER lumen, has been shown to be highly elevated associated with poor differentiation in many cancer types, such as breast cancer (Gazit et al. 1999; Fernandez et al. 2000), gastric cancer (Song et al. 2001), hepatocellular cancer (Shuda et al. 2003; Chen et al. 2014), lung cancer (Koomägi et al. 1999) and prostate cancer (Arap et al. 2004; Misra et al. 2005). For cancer cells subject to ER stress, Hsp70-5 acts as a survival factor, and plays an important role in drug resistance in addition to preventing apoptosis and autophagy (Lee 2007; Wu et al. 2017). Another member of the HSP70 family localized in the mitochondria, Hsp70-9 (also known as mtHsp70, Grp75 or mortalin) functions in human carcinogenesis, promotes proliferation and survival of cancer cells (Deocaris et al. 2013; Starenki et al. 2015). Elevated levels of this protein expression have been reported in human brain tumors (Takano et al. 1997), ovarian cancer (Hu et al. 2016), colorectal adenocarcinomas (Dundas et al. 2005), hepatocellular carcinoma (Yi et al. 2008) and medullary

Fig. 1 Structure of quercetin



thyroid carcinomas (Starenki et al. 2015). Hsp70-9 is known to play a role in cancer formation by activating the MAPK/MEK/ERK pathway and binding to p53 in the cytoplasm to prevent translocation to the nucleus (Hu et al. 2016). Hsp70-6, another member of the HSP70 family, is stimulated at high rates by stress while the expression of it is very low under normal physiological conditions, and it is thought to have an anti-apoptotic function (Regeling et al. 2016; Wu et al. 2017).

In summary, all these studies support members of the HSP70 family as an attractive target in cancer therapy. The studies have shown that suppression of HSP70 expressions in tumor cells would become beneficial for treatment of cancers and the development of new approaches against cancer. Acting HSP70 as an apoptosis inhibitor in tumor cells supports HSP70 to be a potential target for apoptosis-based cancer therapy. However, other molecules that interact with HSP70, and mechanisms of the HSP70 family have not been fully elucidated. New studies are needed to understand how all members work.

Quercetin and Cancer

Quercetin (3,3′,4′,5,7-pentahydroxyflavone), an important member of the flavonoid family, is a highly abundant polyphenolic compound found in various vegetables and fruits, such as apples, berries, broccoli, cabbage, dill, grapes, lemons, onions, tomatoes, and in beverages such as tea, coffee and red wine. The daily intake of quercetin is estimated to range between 3 and 31 mg (Duthie et al. 2000; Khan et al. 2016). Quercetin has been shown to possess a wide range of biological and pharmacological activities, including antioxidant, anticarcinogenic, antiproliferative, antiinflammatory, antiviral and antiallergic properties (Harwood et al. 2007; Gupta et al. 2010; Vargas and Burd 2010; Gibellini et al. 2011; Khan et al. 2016). Compared to other flavonoids, quercetin has a very high antiradical property. Quercetin reveals this property through its three active functional groups in its structure as presented in Fig. 1. These are the o-dihydroxy (catechol) structure at the 3′- and 4′-position of the B ring, the 2,3-double bond in the conjugation with a 4-oxo group, and the hydroxyl groups in the 3- and 5-position (Bors et al. 1990; Silva et al. 2002; Wang et al. 2006).

Additionally, studies on quercetin have shown that it has antioxidant or prooxidant effects depending on its concentration. While quercetin has an antioxidant effect at low doses, it has the opposite effect (pro-oxidant effect) at high doses. The antioxidant and chemopreventive effects of quercetin are seen at 1–40 μM cellular concentrations of it. However, it has been reported that quercetin could act like a ROS (Reactive Oxygen Species) at concentrations higher than 40 μM after tumor formation, and thereby it could still be useful as an antitumoral agent by increasing the oxidative stress and cytotoxicity in tumor cells (Metodiewa et al. 1999; Awad et al. 2000; Vargas and Burd 2010).

Several literature studies report on cancer preventive and therapeutic effects of quercetin in different cell lines. It demonstrates anti-cancer properties by regulating various cell signaling mechanisms, by binding to cellular receptors and proteins, and by inhibiting various proteins that are effective in carcinogenesis. It is well known for its proapoptotic effect in various tumor cells (Murakami et al. 2008; Khan et al. 2016). Quercetin is also effective in the inhibition of Hsp, and quercetin-mediated Hsp inhibition has an important role in stimulation of cell death. It is known that quercetin inhibits HSF1 activation that induces Hsp70 expression (Vargas and Burd 2010; Kumar et al. 2016). Several *in vivo* and *in vitro* studies have demonstrated that quercetin has protective and preventive effects against cancers such as brain, breast, cervix, colorectum, lung and prostate (Table 1). Jakubowicz-Gil et al. (2002) have shown that quercetin reduces Hsp27 and Hsp72 expression and increases the number of apoptotic cells in human cervix and glioma cell lines. 15 μg/mL of quercetin was found to increase apoptosis by ~16% in human cervical carcinoma cell line (HeLa cells). In a study with MOG-G-CCM cells, human brain astrocytoma cells, co-administration of temozolomide with quercetin has been shown to be a useful, potent and promising combination for glioma treatment. In the study, it was shown that quercetin significantly reduced Hsp27 expression at low concentrations and that the combination of 100 μM temozolomide and 30 μM quercetin was highly effective in inducing apoptosis (Jakubowicz-Gil et al. 2010). In a similar study, co-administration of 50 μM quercetin with 100 μM temozolomide has been shown to significantly reduce the expression of Hsp27 and Hsp72 (Jakubowicz-Gil et al. 2013b). In a study performed on U-251 MG and U87 MG cells, it was determined that administration of quercetin at a concentration of 30 μM to cells reduced Hsp27 expression at a significant level (Li et al. 2016). *In vivo* studies in various animal models also show that quercetin reduces tumor growth and tumor volume.

In a study on colon carcinogenesis by Dihal et al. (2008), it was observed that the potential oncogenic MAPK signal decreased in F344 rats fed with 10 g quercetin/kg for 11 weeks. In another study by Jones et al. (2004), it has been shown that quercetin suppresses Hsp70 expression in PC-3 prostate cancer cells. Furthermore, apoptosis has been shown to have a negative correlation with the HSP70 expression. Hsp70 expression was increased in multiple pancreatic cancer cells compared with normal pancreatic ductal cells. Downregulation of HSP70 with siRNA in pancreatic cancer cells causes caspase dependent apoptotic cell death. Depletion of Hsp70 with quercetin decreased cell viability and induced apoptosis in cancer cells but not in normal pancreatic ductal cells. Quercetin treatment decreased tumor size and Hsp70

Table 1 *In vivo* and *in vitro* studies on the anti-cancer effects of quercetin

Cancer type	Animal model or cell line	Findings	References
Brain	U138MG cells	Inhibited proliferation, induced apoptosis (caspase-3/7 activation)	Braganhol et al. (2006)
	A172 cells	Inhibited proliferation, induced apoptosis (caspase-3 activation)	Kim et al. (2008)
	U87-MG and U251 cells	Reduced expression of Survivin and XIAP, induced apoptosis (TRAIL-Induced Apoptosis)	Siegelin et al. (2009)
		Reduced expression of Hsp27	Li et al. (2016)
	MOGGCCM cells	Reduced expression of Hsp27, induced apoptosis and autophagy	Jakubowicz-Gil et al. (2010)
	T98G cells	Reduced expression of Hsp27 and Hsp72, induced apoptosis (caspases 3/8/9 activation)	Jakubowicz-Gil et al. (2013a, b)
		Inhibited proliferation, induced apoptosis	Bądziul et al. (2014a)
Breast	MDA-MB-231 cells	Inhibited proliferation	Conklin et al. (2007)
	MCF-7 cells	Inhibited proliferation, induced apoptosis (Bcl-2 and Bax regulation)	Duo et al. (2012)
		Inhibited cell growth (G2/M arrest), induced apoptosis	Choi et al. (2001)
	4 T1 cells	Inhibited proliferation (Regulation of Wnt signaling activity)	Kim et al. (2013a)
Cervix	HeLa cells	Reduced expression of Hsp27 and Hsp72, induced apoptosis	Jakubowicz-Gil et al. (2002, 2005)
		Reduced expression of Hsp70, induced apoptosis (caspases 3 activation)	Jung et al. (2010)
		Reduced expression of Hsp72, induced apoptosis (caspases 3 activation)	Bądziul et al. (2014b)
Colorectum	F344 rats	Decreased oncogenic MAPK signal	Dihal et al. (2008)
	HCT116 cells	Inhibited proliferation	Shan et al. (2009)
	Mutant Apc mice	Loss of Hsp70, reduced tumor size, increased tumor cell death, increased apoptosis	Tao et al. (2016)
Leukemia	U937 cells	Reduced expression of Hsp70, induced apoptosis	Storniolo et al. (2015)
Lung	A549 cells	Reduced expression of Hsp70, induced apoptosis	Niu et al. (2006)
		Inhibited cell growth (G2/M arrest)	Yeh et al. (2011)
	A549 and H460 cells	Reduced cell viability, suppressed HSP70 expression, increased apoptosis (caspase-3 and caspase-9 activation)	Lee et al. (2015)

(continued)

Table 1 (continued)

Cancer type	Animal model or cell line	Findings	References
Pancreas	MiaPaCa-2 and Panc-1 cells	Reduced expression of Hsp70, induced apoptosis	Aghdassi et al. (2007)
	Nude mice	Decreased tumor size and Hsp70 levels	
Prostate	PC-3 cells	Reduced expression of Hsp70, induced apoptosis	Jones et al. (2004)
		Inhibited proliferation, reduced expression of matrix metalloproteinases 2 and 9 proteins	Vijayababu et al. (2006)
	PC-3 and LNCaP cells	Inhibited proliferation, reduced expression of Hsp90	Aalinkeel et al. (2008)
		Inhibited proliferation	Bhat et al. (2014)

levels in mice (Aghdassi et al. 2007). It has been found that reduced expression of HSP70 by both siRNA or by quercetin causes an enhancement in cytosolic calcium levels. Reduced expression of HSP70 in pancreatic cancer cells leads to release of lysosomal enzymes into the cytosol due to lysosomal membrane permeabilization. Lysosomal enzymes activate cell death pathways in the cytosol (Dudeja et al. 2009). Niu et al. (2006) have reported a significant increase in Hsp70 level after heat shock at 41 °C for 1 h in the study conducted in A549 cells, but the increase in Hsp70 was reported to be inhibited by quercetin treatment for 6 h before heat shock treatment. In addition to a significant decrease in Hsp70 levels, apoptosis has also been found to be induced. As a result, many studies have indicated that quercetin is an effective agent against cancer therapy by causing inhibition of cell growth, induction of apoptosis and suppression of many HSP expression in different cancer cells.

Molecular Mecanishms Targeted by Quercetin in Cancer and Its Relation to HSP70

As quercetin is a lipophilic molecule, it can trigger many intracellular pathways by crossing cellular membranes. Numerous studies have indicated that quercetin inhibits cell proliferation by inducing various apoptotic pathways and/or arresting cell cycle at different checkpoints. It has been shown that quercetin treatment regulates the expression of the cyclin-dependent kinases (Cdks), thus causing the cell cycle arrest at the G0/G1, G1/S and G2/M checkpoints in different cell types. p21, p27, p53, and Chk2 (Checkpoint kinase 2) upregulation, Cdk1 and cyclin B1 downregulation, and pRb (retinoblastoma protein) phosphorylation have been observed in the arrest of the cell cycle by quercetin (Yoshida et al. 1990; Csokay et al. 1997; Ong et al. 2004; Mu et al. 2007; Jeong et al. 2009; Vidya-Priyadarsin et al. 2010; Yeh et al. 2011; Atashpour et al. 2015; Srivastava et al. 2016; Nguyen et al. 2017). In a variety of studies quercetin has been shown to work as an activator in apoptotic pathways in

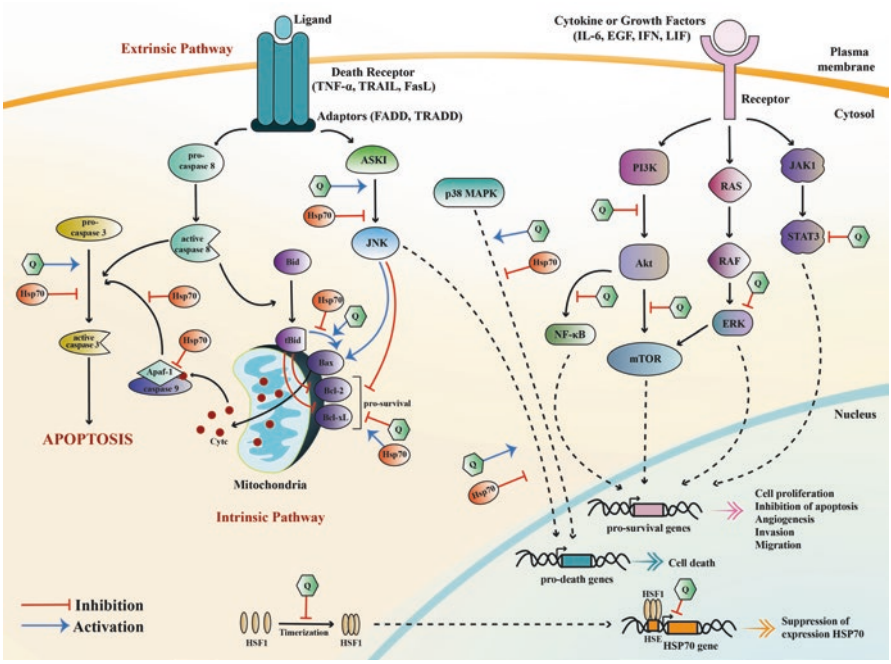


Fig. 2 Molecular mechanisms targeted by quercetin in cancer and its relation to Hsp70 (Q: Quercetin)

which overexpressed HSP70 acts as an inhibitor (Kashyap et al. 2016). It is known that quercetin is able to trigger both intrinsic (mitochondrial) and extrinsic (TNF-α, TRAIL, and Fas/FasL) apoptotic pathway in cancer cells (Elmore 2007; Kashyap et al. 2016; Khan et al. 2016). The apoptotic pathways targeted by quercetin in cancer and the relationship with Hsp70 are illustrated in Fig. 2. Increased expression of Hsp70 can be triggered by many factors such as HSF1, loss of p53 function and high expression of protooncogenes like HER2 and c-Myc (Balázs et al. 2017). It has been shown that overexpressed Hsp70 blocks the TNF-induced apoptosis (Jäättelä and Wissing 1993), inhibits caspase-3 induced apoptosis (Lee et al. 2005), blocks recruitment of procaspase-9 to the apoptosome and formation of the active apoptosome by binding to apoptotic protease-activating factor 1 (Apaf-1) (Beere et al. 2000; Saleh et al. 2000). Evidences have emphasized that quercetin induce apoptosis by direct activation of the caspase cascade in a variety of human cancer cell lines. In addition to apoptosis induction, it has been also stated that the expression of HSP70 is suppressed (Jung et al. 2010; Jakubowicz-Gil et al. 2013a, b; Bądziul et al. 2014b). Studies have also revealed that exposure of cancer cells to quercetin leads to an increment in the expression of Bax and Cyt-c (Cytochrome c), which are pro-apoptotic proteins (Chien et al. 2009; Zhang et al. 2013). Many studies have shown that quercetin suppress Bcl-xL and Bcl-2 anti-apoptotic proteins, and inhibit cellular-signaling proteins such as NF-κB and Cox-2 (Cyclooxygenase-2) (Banerjee et al. 2002; Cheong et al. 2004; Vijayababu et al. 2005; Kim et al. 2013b; Han et al. 2015).

Moreover, in the apoptotic pathway, Hsp70 also interacts with p38 mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK) (Gabai et al. 1997; Lee et al. 2005). It has been shown that activation of JNK pathway and overexpression of Hsp70 in cancer cells show negative correlation, and JNK pathway in the presence of high Hsp70 levels is inactive (Li et al. 2007). Quercetin has been shown to activate JNK pathway, and increase levels of the JNK and p53 (Lee and Yoo 2013). It is known that cellular signal transduction pathways such as ERKs, Akts (also known as PKB, Protein Kinase B) and MAPKs, which affect cell survival modulate by quercetin (Kashyap et al. 2016). IL-6/JAK/STAT3 (Interleukin-6/Janus kinase/Signal Transducer and Activator of Transcription 3) and Akt/mTOR signaling pathways have been also shown to be downregulated by quercetin during cancer treatment (Mukherjee and Khuda-Bukhsh 2015; Chen et al. 2016). In addition, quercetin-mediated downregulation of Mcl-1 (myeloid cell leukemia-1), MMP-2 (matrix metalloproteinase-2), MMP-9 (matrix metalloproteinase-9), and VEGF (vascular endothelial growth factor) which are genes targeted by the STAT3 signal pathway have also been demonstrated (Cao et al. 2014). Quercetin has been shown to inhibit large survival signal pathways ERK and phosphatidylinositol 3-kinase (PI3K)/Akt in human hepatoma cells (Granado-Serrano et al. 2006). It has been also found that quercetin suppresses ERK signaling pathway resulting in the inhibition of angiogenesis in cancerous cells (Li et al. 2015). In a study on myeloma cells, the expression of HSF1 and inducible Hsp70 have been found to decrease by small interfering (si)RNA mediated inhibition of PI3K/Akt pathway (Chatterjee et al. 2013).

Transcription of HSP70 is generally regulated by HSF1. Activated HSF1 induces HSP70 expression. In this case it may be possible to block Hsp70 expression by inhibition of HSF1. Quercetin is well known inhibitor of HSP induction. The induction and regulation of HSP are highly complex processes and, in summary, involves the following steps: Initial release of heat shock transcription factor 1 (HSF1) from a chaperone complex including Hsp90 and Hsp70, trimerization, translocation to the nucleus, binding to heat shock element (HSE). The transcriptional activation of HSF1 localized as inactive monomers in the cytoplasm occurs by multiple phosphorylation by kinases (Önay-Uçar 2015). It is known that quercetin reduces HSP70 expression by suppressing HSF1 phosphorylation and transcriptional activity. It is one of the first inhibitors to be shown to be effective on expression of Hsp70 in this way. Quercetin has been also shown that inhibits a number of kinases, including AMPK (AMP-activated protein kinase), CK2 (Casein Kinase 2), CaMKII (Calcium/Calmodulin-Dependent Protein Kinase II), RSK2 (Ribosomal protein S6 kinase 2,) and S6 K1 (Ribosomal protein S6 kinase beta-1). Various studies have emphasized that quercetin suppress Hsp70 induction by blocking phosphorylation of HSF1 by CK2 and/or CaMKII kinases. There are also studies that demonstrate the effect of quercetin on HSF1 binding to HSE (Davies et al. 2000; Zorzi and Bonvini 2011). In a study investigating the effect of quercetin on HSP expression,150 μM quercetin has been shown to reduce Hsp70 expression by inhibiting both CK2 and CaMKII activity in Jurkat cells. Quercetin derivatives that poorly inhibit these kinases

have been also shown to be weak inhibitors of Hsp70 induction, indicating that the activity of quercetin is due to its ability to inhibit both kinases. In addition to study, very low level of HSF1/HSE complex formed has been detected in quercetin treated cells 1 h before heat shock (Wang et al. 2009).

As a consequence, many studies have indicated that quercetin shows a negative correlation with elevated HSP70, which acts as an antiapoptotic. Evidence suggests that quercetin acts as an inhibitor of cell proliferation by inducing apoptosis and/or arresting cell cycle in cancerous cells, indicating that the activity of quercetin may be due to its ability to suppress the expression of HSP, especially Hsp70.

Conclusions

Today, the researchers working on cancer therapy have focused on downregulation of elevated HSP. It is known that HSP70 family plays a key role in the regulation of malignancy and have significant roles in various cancer types. High expression of HSP70 family members is related to tumor progression, aggressive phenotype, and a poor prognosis in therapeutic responses (Evans et al. 2010; Murphy 2013, Sherman and Gabai 2015; Giri et al. 2017). Especially, many studies have shown the prognostic value of the Hsp70 protein, a member of HSP70, is noteworthy in several human cancer (Nylandsted et al. 2000; Canöz et al. 2002; Abe et al. 2004; Hunt et al. 2004; Kluger et al. 2005; Yeh et al. 2009; Kocsis et al. 2010; Meng et al. 2010; Teng et al. 2012; Alexiou et al. 2013, 2014; Ghermann et al. 2014; Balázs et al. 2017; Jin et al. 2017). Therefore, the inhibition of expression of HSP70 family members, particularly Hsp70, and induction of apoptosis have become a novel strategy against cancer (Evans et al. 2010; Murphy 2013; Giri et al. 2017). Quercetin, a plant flavonoid, has long been known to suppress the expression of stress-induced Hsp70 (Jakubowicz-Gil et al. 2002, 2013b; Jones et al. 2004; Niu et al. 2006; Aghdassi et al. 2007, Bądziul et al. 2014b; Lee et al. 2015; Storniolo et al. 2015; Tao et al. 2016). Studies concerned with the role of quercetin in suppressing effect on expression of HSP70 family members have shown that this agent acts as an effective inhibitor of HSP70 expression in many cancer cell line. The number of studies investigating the effect of quercetin on the other members according to Hsp70 is relatively small, but nowadays studies are to ongoing (Vargas and Burd 2010; Khan et al. 2016; Kumar et al. 2016). As described in this chapter, all these findings indicate that quercetin alone or in combination with chemotherapeutic drugs causes suppression of HSP70 expression in different cancer cells, which makes this agent may be an effective adjuvant for cancer therapy.

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