Chapter 13
IER5 Is a p53-Regulated Activator of HSF1 That Contributes to Promotion of Cancer

Tatsuya Kawase, Yu Chen, and Rieko Ohki

Abstract The *p53* gene is one of the most frequently mutated genes in human cancer and functions as a tumor suppressor and transcriptional factor that regulates various genes involved in cancer. One p53 target gene is *IER5,* whose function was initially unknown, but we have shown facilitates the activation of the transcriptional activator HSF1 by recruiting the PP2A phosphatase to HSF1, leading to its hypophosphorylation and activation. HSF1 is the master transcriptional regulator of the *HSP* genes, which encode molecular chaperones essential for cellular homeostasis. HSP also exhibit anti-apoptotic functions by repressing pro-apoptotic factors, thereby protecting stressed cells from cell death. Although HSF1-HSP pathway is generally activated by cellular stress such as heat shock, this pathway is also hyperactivated in cancers independent of heat shock and contribute to promotion of cancer development and resistance to cancer treatments. We observed that *IER5* is overexpressed in several cancers in a p53-independent manner and contributes to tumor malignancy via activation of the HSF1-HSP pathway. We propose a model in which IER5 activates HSF1 in cancer as part of the p53-IER5-HSF1-HSP pathway, thereby providing stress resistance to cancer cells. This section briefly reviews the roles of HSF1, HSP, p53 and IER5.

Keywords Cancer · HSF1 · HSP · IER5 · p53 · Post-translational modification · PP2A

T. Kawase
Unit 1, Candidate Discovery Science Laboratory, Drug Discovery Research, Astellas Pharma Inc., Tsukuba-shi, Ibaraki, Japan

Laboratory of Fundamental Oncology, National Cancer Center Research Institute, Tokyo, Japan

Y. Chen · R. Ohki (✉)
Laboratory of Fundamental Oncology, National Cancer Center Research Institute, Tokyo, Japan
e-mail: rohki@ncc.go.jp

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Abbreviations

4C-seq	circular chromatin conformation capture sequencing
ABC	ATP binding cassette
ABCB1	ATP-binding cassette sub-family B member 1
AIF	apoptosis-inducing factor
Apaf-1	apoptosis protease activating factor 1
ASK1	apoptosis signal regulating kinase 1
BAG3	Bcl-2-associated athanogene domain 3
Bax	Bcl-2-associated X protein
CAD	caspase activated DNase
ChIP-seq	chromatin immunoprecipitation sequencing
GOF	gain-of-function
HSEs	heat shock elements
HSF1	heat shock factor 1
HSP	heat shock proteins
ICAD	inhibitor of CAD
IER5	immediate early gene response 5
LFS	Li-Fraumeni syndrome
MDM2	murine double minute 2
MDR	multidrug resistance
MDR-1	multidrug resistance protein 1
Pgp	P-glycoprotein
PP2A	protein phosphatase 2A
PTEN	phosphatase and tensin homolog

13.1 Introduction

The p53 protein was independently discovered by several groups in 1979 as a molecular partner of SV40 large T antigen (DeLeo et al. 1979; Kress et al. 1979; Lane and Crawford 1979; Linzer and Levine 1979). Until the mid-1980s, the *p53* gene had been generally regarded as an oncogene based on several experimental observations: the p53 protein was bound to the major oncogenic protein of SV40; high levels of p53 expression were detected in various cancers; and overexpression of p53 transformed normal cells into cancerous cells (Eliyahu et al. 1984; Parada et al. 1984). Later, however, Hinds and colleagues showed that this ability to transform cells in these experiments was due to a mutation in the *p53* gene (Hinds et al. 1989). In 1988, genetic analyses of colorectal cancer cells revealed a very high rate of heterozygous loss of the short (p) arm of chromosome 17, which carries the *p53* gene (Vogelstein et al. 1988). Analysis of the *p53* gene sequence derived from tumor specimens showed that it often contains point mutations (Baker et al. 1989; Takahashi et al. 1989). Furthermore, germ-line mutations of the *p53* gene were found in patients with Li-Fraumeni syndrome, which is associated with a broad

spectrum of cancers including osteosarcomas, breast cancer, soft tissue sarcoma and leukemia (Malkin 2011; Malkin et al. 1990). Since then, *p53* has been regarded as a tumor suppressor gene. The p53 has two divergent functions in normal cells, both as a protector and as a killer (Aylon and Oren 2007; Harris and Levine 2005; Vousden and Prives 2009). p53 is a transcription factor that induces its target genes in a manner dependent on the type, intensity and tissue context of cell damage. Under conditions of mild stress, p53 induces genes involved in cell cycle arrest, DNA repair and antioxidation to protect and restore cells that are not damaged beyond repair. However, under severe stress conditions, p53 induces genes involved in cellular senescence or apoptosis to eliminate cells that are beyond repair and thus have the potential to become cancerous. Interestingly, it has recently been suggested that the protective function of wild-type p53 could also play a role in promoting cancer development or progression. For example, among the p53 target genes are several that adapt cells to metabolic changes such as nutrient deprivation and ROS. This function could enable cancer cells to survive under harsh conditions and thereby contribute to cancer progression (Vousden and Prives 2009).

The transactivation function of p53 is essential to its ability to suppress cancer since most of the mutant p53s found in human cancers have lost this transactivation function (Levine 1997). Therefore, identification of p53 target genes is an important goal of p53 research. Analysis of p53 target genes, such as *MDM2* and *PTEN*, have further led to identification of novel cancer biology and anti-cancer drug targets. *MDM2* encodes a negative regulator of p53 and functions as an oncogene when overexpressed in cancers such as osteosarcoma (Momand et al. 1992; Oliner et al. 1992; Barak et al. 1993). Suppression of Mdm2 expression induces the accumulation/activation of p53 and thereby inhibits the proliferation of cancers in which Mdm2 is overexpressed. Based on this, inhibitors of Mdm2 have been developed and tested in clinical trials as anti-cancer drugs (Fesik 2005). PTEN, a PIP_3 phosphatase, has also been shown to function as a tumor suppressor (Maehama and Dixon 1999; Stambolic et al. 2001). We have more recently undertaken the identification of novel p53 target genes using microarray and ChIP-seq analysis with the goal to discover novel cancer biology or anti-cancer drug targets. In particular, we have focused on target genes with previously unknown functions that we could analyze to try to understand their roles and potential as novel drug targets (Asano et al. 2016; Ezawa et al. 2016; Kawase et al. 2008, 2009; Ohki et al. 2007, 2014). One particular example is the *PHLDA3* gene, whose product we determined is a repressor of the Akt oncogene and plays a role as a novel tumor suppressor of pancreatic neuroendocrine tumors (Kawase et al. 2009; Ohki et al. 2014; Takikawa and Ohki 2017).

Another gene we have recently identified as a novel p53 target gene is *IER5* (Asano et al. 2016). Functional analysis of IER5 revealed that IER5 activates HSF1 in a heat shock-independent manner resulting in the induction of HSF1 target genes, including the *HSP* genes. In addition, we showed that transcription of *IER5* is highly activated in various cancer specimens and in cancer cell lines. Consistent with these observations, it has been also reported that HSF1 is aberrantly activated and HSP are overexpressed in various cancers (Santagata et al. 2011; Ciocca and Calderwood 2005). We would further suggest that IER5 could

contribute to the tumor-promoting effects of wild-type p53 due to the ability of IER5 to regulate the HSF1-HSP pathway, which has a cellular protective function. This section briefly reviews the roles of HSF1, HSP, p53 and the novel p53 target gene IER5.

13.1.1 HSF1 and HSP

HSF1 is an evolutionarily highly conserved transcriptional factor and a master regulator of the heat shock response (Akerfelt et al. 2010). HSF1 contains four functional domains including a DNA-binding domain, an oligomerization domain, a transactivation domain and a regulatory domain (Fig. 13.1a). Under normal conditions, HSF1 exists in the cytoplasm as an inactive monomer that is repressed by its interaction with a protein complex consisting of Hsp90 and Hsp70 (Baler et al. 1996; Zou et al. 1998). Proteotoxic stressors such as heat shock trigger the dissociation of this repressive protein complex and the release of inactive HSF1. Subsequently, HSF1 is activated through trimerization, nuclear translocation, and post-translational modifications including phosphorylation, acetylation, and sumoylation (Anckar and Sistonen 2011) (Fig. 13.1b). In particular, phosphorylation of HSF1 has been shown to be critical for modulating its activation (Fig. 13.1a). For instance, it has been reported that phosphorylation of HSF1 at S121, T142, S303, S307 and S363 inhibits HSF1 activation, whereas phosphorylation at S230, S320, S326 and S419 induces its activation (Chou et al. 2012; Chu et al. 1998; Holmberg et al. 2001; Kim et al. 2005; Soncin et al. 2003; Wang et al. 2004, 2006; Zhang et al. 2011). In the nucleus, activated trimeric HSF1 binds to specific heat shock elements (HSEs) in the proximal promoter region of its target genes, and consequently induces a number of these genes including Hsp27, Hsp70 and Hsp40 (Akerfelt et al. 2010; Anckar and Sistonen 2011).

HSP are a subset of molecular chaperons that are expressed in response to increased temperature or a variety of other cellular stresses. When activated they participate in the folding of denatured or aggregated client proteins, promote the assembly/disassembly of multiprotein complexes, regulate protein degradation and facilitate protein translocation (Richter et al. 2010). Thus, HSP are important to tissue homeostasis in normal cells. In addition, a number of studies have indicated that HSP possess multiple functions to suppress apoptosis (Takayama et al. 2003). For instance, Hsp27 interferes with apoptosis by directly binding to cytosolic cytochrome c and sequestering it from Apaf-1 (Bruey et al. 2000; Garrido et al. 1999). Hsp70 protects cells from apoptosis by interacting with p53 and other pro-apoptotic factors including Apaf-1, AIF, ASK1 and Bax (Beere et al. 2000; Park et al. 2002; Ravagnan et al. 2001; Saleh et al. 2000; Wiech et al. 2012; Yang et al. 2012). Hsp40, one co-chaperon of Hsp70, negatively regulates CAD, a factor that causes chromosomal DNA fragmentation during apoptosis, by interacting with Hsp70 and ICAD (Sakahira and Nagata 2002).

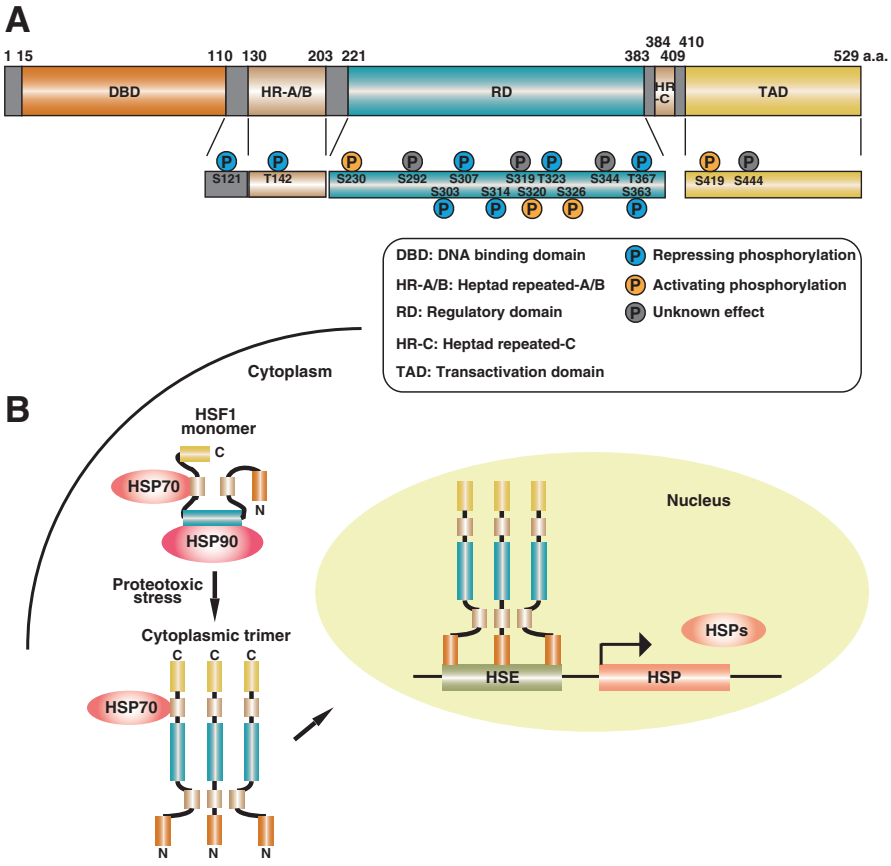

Fig. 13.1 Schematic view of HSF1 domain structure, post-translational modification and mechanism of activation. (**a**) HSF1 is comprised of four functional domains including a DNA-binding domain, an oligomerization domain (heptad repeated domain), a transactivation domain and a regulatory domain. Phosphorylation of HSF1 critically regulates HSF1 activation and inactivation. (**b**) The process of HSF1 activation

13.1.2 HSF1, HSP and Cancer

HSF1 is expressed at high levels in cancer cells and has recently been recognized as playing a significant role in cancer. HSF1 is constitutively activated in cancers even in the absence of heat-shock conditions and higher HSF1 activity has been reported to be associated with poor prognosis in breast cancer patients. However, the mechanism by which HSF1 is activated in cancer has remained unclear (Santagata et al. 2011). HSF1 has also been shown to transactivate a variety of genes involved in tumor progression and to promote tumorigenesis, a property that is critically dependent on its ability to upregulate HSF1 target genes including the HSP family of genes (Mendillo et al. 2012). As with HSF1, HSP are also highly expressed in

cancer and their overexpression is associated with poor prognosis in various types of cancer (Ciocca and Calderwood 2005). For instance, Hsp27 overexpression has been reported to be correlated with poor prognosis in gastric cancer, liver cancer, prostate cancer and osteosarcomas (Cornford et al. 2000; King et al. 2000; Takeno et al. 2001; Uozaki et al. 2000). Hsp70 overexpression is associated with poor prognosis in breast cancer, endometrial cancer, uterine cervical cancer and bladder cancer (Ciocca et al. 1993; Nanbu et al. 1998; Piura et al. 2002; Syrigos et al. 2003; Thanner et al. 2003). These data indicate that HSF1 and HSP are involved in cancer progression, and indeed their expression is regarded as useful diagnostic and prognostic markers of cancer in certain tissues.

A number of recent studies have identified HSF1 and Hsp70 as therapeutic targets for cancer treatment. For example, elimination of HSF1 has been shown to protect mice from tumors induced by mutation of the RAS oncogene or by a hot spot mutation in tumor suppressor p53 and from DEN-induced hepatocellular carcinoma formation (Dai et al. 2007; Jin et al. 2011). In addition, HSF1 knock-out or knock-down cancer cells have been shown to be more sensitive to Hsp90 inhibitors (Chen et al. 2013). As mentioned in the previous section, Hsp90 represses HSF1, and therefore, Hsp90 inhibitors confer resistance to stress in cancer cells via activation of the HSF1-HSP pathway. Simultaneous inhibition of both Hsp90 and HSF1 may be required for the effective elimination of cancer cells. On the other hand, RNAi silencing of Hsp70 has been shown to inhibit human gastric cancer growth by inducing apoptosis and enhancing the efficacy of radiotherapy (Du et al. 2009; Xiang et al. 2008). Cancer cells can acquire multidrug resistance to chemotherapy through overexpression of ABC transporters including Pgp, also known as MDR-1 or ABCB1, which results in the efflux of the therapeutic agents (Wang et al. 2017). HSF1 and Hsp70 play important roles in inducing MDR and resistance to therapies. For instance, HSF1 has been shown to induce an MDR phenotype by modulating the stability and/or the splicing of MDR-1 transcripts (Tchenio et al. 2006). In addition, Hsp70 have been reported to stabilize some mutant forms of p53, which in turn activates the MDR-1 gene, resulting in an MDR phenotype (Chin et al. 1992; Wiech et al. 2012). Thus, HSF1 and Hsp70 are thought to be promising therapeutic targets to suppress MDR and enhance the efficacy of therapeutic agents against chemoresistant cancers.

13.1.3 HSF1 Inhibitors and HSP70 Inhibitors

Since HSF1 and Hsp70 inhibitors are thought to be promising anti-cancer agents, a number of groups have tried to identify small-molecule inhibitors of HSF1 and Hsp70 (Chatterjee and Burns 2017; Dayalan Naidu and Dinkova-Kostova 2017; Evans et al. 2010). For example, Kim and colleagues showed that the natural compound cantharidin induces cancer cell death by blocking the binding of HSF1 to the promoters of HSF1 target genes, and resulting in the downregulation of Hsp70 and BAG3 expression (Kim et al. 2013). Rohinitib, also known as rocaglate, has been reported to indirectly inhibit HSF1 by interfering with ribosomal translation initiation (Dayalan Naidu and Dinkova-Kostova 2017). In addition, novel hybrid HSF1

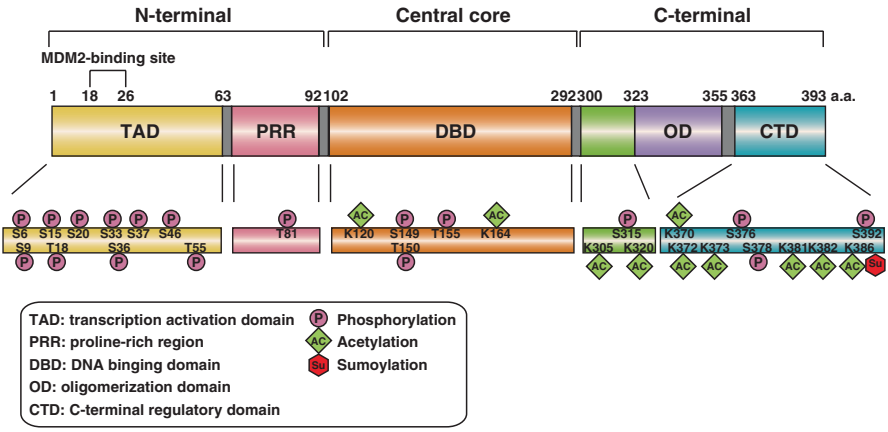

Fig. 13.2 Schematic view of the domain structure and post-translational modification of p53. The 393-amino acid p53 protein is comprised of several functional domains: a transactivation domain and proline-rich region in the N-terminus; a DNA-binding domain in the central region; and a tetramerization and regulatory domain in the C-terminus. Stabilization and activation of p53 require the post-translational modification of p53, including phosphorylation, acetylation and sumoylation

inhibitors based on rohinitib and cantharidin have been reported to have potent inhibitory activity against HSF1 (Agarwal et al. 2015). Other HSF1 inhibitors such as quercetin, KNK437, triptolide and KRIBB11 have been demonstrated to affect the transcriptional activity of HSF1 (Nagai et al. 1995; Ohnishi et al. 2004; Westerheide et al. 2006; Yoon et al. 2011). However, proof of concept that HSF1 inhibition can assist in the treatment of human cancers awaits future clinical trials. A number of HSP70 inhibitors such as MKT-077, pifithrin-μ, 15-DSG, MAL3-101 and VER155008 have been reported to inhibit Hsp70 in biological assays. In addition, a few compounds including minnelide have been tested in clinical trials (Evans et al. 2010). Minnelide, a prodrug of Triptolide, has been shown to suppress Hsp70 and inhibit in vivo growth in a xenograft model of mesothelioma (Jacobson et al. 2015). Given these results, we may expect that inhibitors of HSF1 and Hsp70 could be approved as cancer therapeutics in the future.

13.1.4 p53, a Tumor Suppressor and a Transcription Factor

The *p53* gene is the most frequently mutated tumor suppressor gene in human cancers. Both somatic and germline mutations of *p53* can lead to the development of various cancers, and the involvement of p53 in suppression of various types of cancers is one of its hallmarks. The *p53* gene encodes a transcriptional factor and its structure contains several functional domains. These include a transactivation domain and proline-rich region in the N-terminal region, a DNA-binding domain in its central region, and a tetramerization and regulatory domain in its C-terminal region (Bode and Dong 2004) (Fig. 13.2). Under normal physiological conditions, the p53 protein

has a short half-life and is maintained at low levels due to MDM2-mediated ubiquitin/proteasome degradation. However, p53 protein is stabilized and activated in response to various cellular stresses including DNA damage, hypoxia, oncogene activation and virus infection (Harris and Levine 2005). Stabilization and activation of p53 are regulated by various post-translational modifications including phosphorylation, acetylation, and sumoylation (Bode and Dong 2004; Meek and Anderson 2009) (Fig. 13.2). For example, p53 is phosphorylated by several kinases such as CHK2 at Thr18 and Ser20, which are located within the alpha-helix involved in MDM2 interaction. Phosphorylation of this region stabilizes p53 following DNA damage by disrupting the p53-MDM2 interaction (Chehab et al. 1999; Sakaguchi et al. 2000; Shieh et al. 1999; Unger et al. 1999). Additionally, phosphorylation at Ser15 has been shown to increase the association of p53 with p300/CBP and to stimulate its transactivation function (Dumaz et al. 1999; Lambert et al. 1998). Acetylation of p53 at Lys370/372/373/381/382/386 is also significant for p53 stabilization and activation as this facilitates the recruitment of CBP/p300, which interferes with MDM2-mediated p53 ubiquitination (Barlev et al. 2001; Rodriguez et al. 2000; Wei et al. 2006).

Activated p53 binds as a tetramer to p53 consensus binding sequences in the proximal promoter or intron region of its target genes. These binding sites consist of two copies of the ten base pair motif 5′-RRRCWWGYYY-3′ (R=G or A, W=T or A, Y=C or T) separated by 0–13 base pairs, (el-Deiry et al. 1992). The genes induced by p53 are involved in cell cycle arrest, DNA repair, senescence, metabolism, regulation of antioxidant generation and apoptosis, which collectively function to prevent normal cells from turning into cancerous cells. Under conditions of severe stress, p53 can induce target genes involved in apoptosis (e.g. *PUMA*, *NOXA*) or cellular senescence (e.g. *PAI-1*), which function to eliminate cells that have the potential to become cancerous (Kortlever et al. 2006; Kunz et al. 1995; Nakano and Vousden 2001; Oda et al. 2000). In addition, p53 induces genes related to cell cycle arrest (e.g. *p21WAF1/CIP1*), regulation of antioxidant generation (e.g. *TIGAR*, *Sestrins*), and DNA repair (e.g. *p53R2*) to protect and facilitate the recovery of cells from damage under conditions of mild stress (Bensaad et al. 2006; Bensaad and Vousden 2007; Budanov et al. 2004; el-Deiry et al. 1993; Tanaka et al. 2000). Together, these p53-mediated responses to various types of cellular stress suppress tumorigenesis in normal cells.

Most p53 mutations are missense mutations located in its DNA binding domain, whereas mutations found in many other tumor suppressor genes, for example *BRCA1/2* or *APC*, are mainly nonsense mutations. In particular, the residues R175, G245, R248, R249, R273 and R282 in the p53 protein are frequently mutated in cancer, and are therefore called hot spot mutations. Mutant p53 proteins found in human cancers can neither bind to the promoters of nor induce expression of the target genes normally induced by wild-type p53, indicating that the ability of p53 to prevent cancer development and progression depends on its gene-activation function. In addition, many mutant p53 proteins, including those resulting from hot spot mutations, acquire oncogenic gain-of-function properties that promote invasion, migration, angiogenesis and/or suppress apoptosis (Freed-Pastor and Prives 2012; Muller and Vousden 2014). Functional analysis of these mutant p53 proteins is an ongoing area of research that should further expand our understanding of p53 in the future.

13.1.5 p53 and Tumor Promotion

Although *p53* clearly plays an important role in tumor suppression, it has more recently been suggested that the protective functions of wild-type p53 such as DNA repair, cell cycle arrest and regulation of antioxidant generation could also contribute to tumor development in some contexts (Vousden and Prives 2009). Indeed, many cancers express wild-type p53, including sarcoma, breast cancer, cervical cancer, melanoma and neuroendocrine tumors. In sarcoma, breast cancer or cervical cancer, it has been reported that wild-type p53 is degraded or inactivated by several mechanisms. For example, p53 protein can be degraded by overexpression of MDM2 or MDMX (also known as MDM4) in sarcomas and breast cancers (Danovi et al. 2004; Oliner et al. 1992). In cervical cancers, the E6 protein derived from human papillomavirus interacts with p53 and abrogates p53 function (Scheffner et al. 1990). On the other hand, we have confirmed that p53 in neuroendocrine tumors has an apparently normal transactivation function and appears functional. To date, no negative regulators of p53 have been reported in neuroendocrine tumors. Therefore, we believe that analysis of wild-type p53 and its potential role in promoting tumor development such as in neuroendocrine tumors could be of great interest. Below we propose a model in which the p53-IER5-HSF1-HSP pathway may promote cancer development.

13.1.6 p53 and IER5

In order to identify genes involved in the regulation of tumorigenesis, and to dissect the functional relevance of p53 phosphorylation in the induction of its target genes, we undertook an exhaustive effort to identify p53 target genes that are induced in a manner dependent on p53 phosphorylation status. We found that induction of approximately 80% of the p53 target genes depends on p53 phosphorylation, illustrating the importance of this modification in p53's transactivation function. One of the genes identified by our screen was the immediate early response gene 5 (*IER5*), which can be induced by wild-type p53 but not a phosphorylation-deficient p53. To confirm that IER5 is a direct target of p53, we performed ChIP-seq analysis and identified a p53 binding site 46 kb downstream of the *IER5* gene containing sequences highly similar to the consensus p53 binding sequence. When this binding site was tested in a luciferase promoter reporter construct, we observed that p53 strongly activated the reporter. Because the p53 binding site was quite distant from the *IER5* gene promoter region, we performed circular chromatin conformation capture (4C-seq) to analyze potential three-dimensional chromatin interactions. We detected chromatin interaction between the downstream p53 binding site and the *IER5* promoter. From these analyses, we confirmed that *IER5* is a direct target of p53. In addition to our results, Wei et al. and Melo et al. have also identified p53 binding at the *IER5* gene by a comprehensive genome-wide analysis of p53 binding sites (Wei et al. 2006; Melo et al. 2013).

13.1.7 IER5 and Cancer

Although we determined that *IER5* is a p53 target gene, the molecular function of IER5 was totally unknown when we started our work. The *IER5* gene was originally characterized as an immediate-early gene induced by various growth-promoting stimuli (Williams et al. 1999). Consistent with this, we observed that *IER5* could be induced by various mitogenic stimuli such as PMA, serum and ionomycin. In addition, Yoon and his colleagues revealed that *IER5* was overexpressed in cervical cancers (Yoon et al. 2003). We also observed *IER5* upregulation and overexpression in a number of cancers using the Gene Logic SCIANTIS database and COSMIC database. These results collectively suggest that various growth-promoting and oncogenic signals may induce expression of *IER5*. Furthermore, we found so-called "super-enhancers" localized around the *IER5* gene locus, including the p53 binding site, in various cancer cell lines. It has been reported that many cancer cells acquire these super-enhancers at key driver oncogenes as well as other genes important for tumor pathogenesis. The presence of super-enhancers at the *IER5* gene locus suggests that *IER5* expression may be important for cancer cells (Hnisz et al. 2013; Loven et al. 2013). To analyze the function of IER5 in cancer cells, we first examined the effect of IER5 expression on their proliferation. First, we observed high expression of *IER5* in cells in which the *IER5* gene was associated with super-enhancers. Knock down of IER5 expression in these cancer cells resulted in a marked decrease in proliferation in suspension culture conditions and in soft agar, indicating that IER5 is required for anchorage-independent cancer cell growth. These results collectively indicate that IER5 expression is required for cancer cell proliferation.

13.1.8 IER5 and HSP

To further understand IER5 function, we undertook a comprehensive analysis of gene expression in cells expressing IER5. This analysis showed the *HSP* family genes (*HSPA1A*, *HSPA1B*, *HSPA6*, *DNAJB1*, *HSPB1* and *HSPH1*) were transcriptionally upregulated by IER5 expression. Furthermore, the *HSP* family gene promoters were strongly activated by IER5, indicating that IER5 induces transcription of *HSP* family genes. Next, using luciferase promoter reporter assays, we showed that the *HSP* family gene promoters were strongly activated by IER5. We then generated a series of serially deleted *HSPA1A* promoters and cloned these upstream of the luciferase reporter gene. We found that the region between – 310 and – 100 relative to the transcription initiation site of the *HSPA1A* gene was responsible for IER5-dependent expression (Fig. 13.3). Analysis of the sequences within this region revealed the presence of an HSF1 transcription factor binding site, i.e. a heat shock element (HSE). Given the presence of this HSE within the IER5-responsive promoter region, we expected that HSF1 would be required for induction of the *HSP*

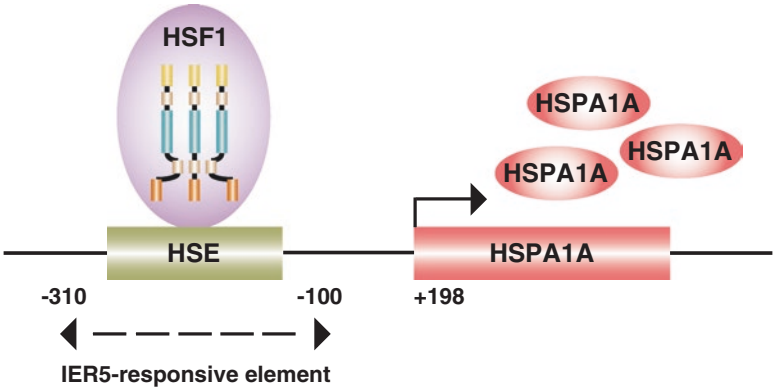

Fig. 13.3 Identification of the IER5 responsive region within the HSPA1A promoter. The region between −310 and −100 relative to the transcription initiation site of the *HSPA1A* gene mediates IER5 responsiveness. The IER5-responsive region contains an HSE (heat shock element) which is recognized by HSF1

family genes by IER5. Indeed, we found that knockdown of HSF1 suppressed IER5-mediated induction the *HSP* family genes, demonstrating that upregulation of the *HSP* family genes by IER5 is dependent on HSF1.

13.1.9 IER5 and Phosphorylation of HSF1

We found that expression of IER5 decreased the interaction between HSF1 and Hsp90 and resulted in increased HSF1 trimerization. IER5 also induced a greater accumulation of HSF1 in the nucleus. In addition, binding of HSF1 to HSE was strongly increased in cells overexpressing IER5. HSF1 trimerization, nuclear localization and acquisition of DNA binding ability are indicators of HSF1 activation, demonstrating that IER5 can activate HSF1. HSF1 activation involves various post-translational modifications, particularly phosphorylation, and we found that IER5 expression dramatically altered the post-translational modification of HSF1 (Fig. 13.4). Furthermore, LC-MS/MS analysis revealed that HSF1 phosphorylation is reduced at multiple sites in cells expressing IER5. In particular, strong reduction in phosphorylation was observed at five residues: Ser121, Ser307, Ser314, Thr323 and Thr367. It has been reported that phosphorylation at Ser121 represses HSF1 trimerization, while phosphorylation at Ser307 reduces transactivation by HSF1. These results suggested that IER5 induces HSF1 trimerization and activation through dephosphorylation of the residues involved in the repression of HSF1 activity (Wang et al. 2004, 2006). While we showed that dephosphorylation at Ser314, Thr323 and Thr367 was required for expression of *HSPA1A*, the mechanism by which this alters transactivation is unclear at present and would be an interesting subject for future study. The ability of IER5 to cause decreased phosphorylation of

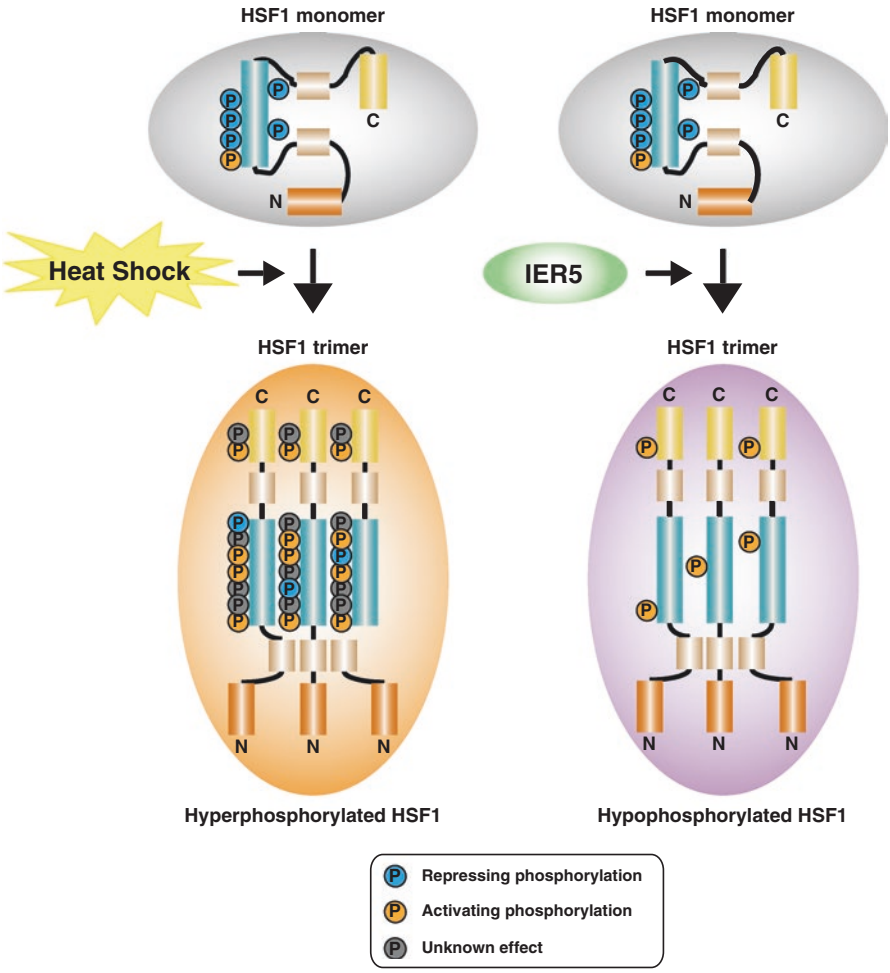

Fig. 13.4 IER5 generates a novel hypo-phosphorylated active form of HSF1. While heat shock generates a hyper-phosphorylated active form of HSF1, IER5 induces a dramatic dephosphorylation of HSF1

HSF1 suggests the potential involvement of a Ser/Thr protein phosphatase in HSF1 activation. Previous studies have reported that protein phosphatase 2A (PP2A) interacts with IER5 and the HSF family protein HSF2 (Glatter et al. 2009; Xing et al. 2007). We showed that treatment by okadaic acid, an inhibitor of PP2A, or knock down of PP2A compromised the ability of IER5 to dephosphorylate HSF1 and to induce HSP, indicating that PP2A is involved in IER5-mediated HSF1 dephosphorylation and HSF1 activation. Finally, co-immunoprecipitation experiments confirmed a physical interaction between IER5, HSF1 and PP2A. Collectively, these results demonstrate that IER5 functions as a scaffold protein to bring HSF1 and PP2A together, facilitating the dephosphorylation and activation of HSF1

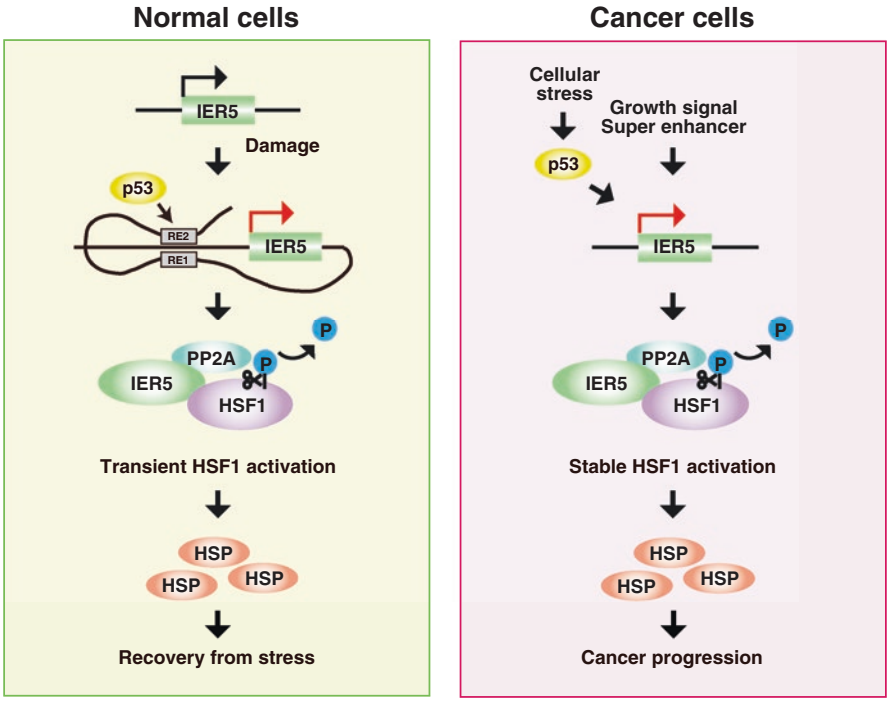

Fig. 13.5 p53/IER5/HSF1/HSP pathway in stressed cells and in cancer cells. Cellular stress transiently activates the IER5-HSF1-HSP1 pathway downstream of p53. On the other hand, IER5 is overexpressed and constitutively activates HSF1 in cancer cells. IER5 forms a complex with HSF1 and PP2A to dephosphorylate and activate HSF1

(Fig. 13.5). The involvement of IER5 and PP2A in HSF1 activation under heat stress conditions was also reported, confirming the importance of this pathway in HSF1 activation (Ishikawa et al. 2015).

13.1.10 p53/IER5/HSF1 Axis

To analyze the role of the IER5-HSF1 pathway in cancer, we introduced into cancer cells a constitutively active form of HSF1 (caHSF1), which has the ability to induce HSF1 target genes independent of an HSF1 activating signal (Uchiyama et al. 2007). In these cells, the HSP family of genes and their protein products were constitutively expressed independent of IER5. Knock down of IER5 expression resulted in poor cell proliferation in the parental cancer cells, but not in caHSF1-expressing cells, which showed anchorage-independent cancer cell growth. This indicates that the IER5-HSF1 pathway is required for anchorage-independent proliferation of cancer cells. Furthermore, a search of a publicly available cancer microarray

database (PrognoScan; Mizuno et al. 2009) showed that higher *IER5* expression is associated with poorer prognosis in various cancer patients. Additionally, higher *HSPA6* expression, the gene most highly induced by IER5, was also related to poorer prognosis and there was a positive correlation between *IER5* and *HSPA6* expression in these cancers. These data collectively suggest that the IER5-HSF1-HSP pathway may be involved in cancer progression.

Since *IER5* is a p53 target gene and its expression is induced under conditions of cellular stresses in a p53-dependent manner, we asked whether *HSP* family gene expression can be induced by DNA damage. Adriamycin treatment resulted in the coincidental upregulation of *IER5*, *HSPA6* and *HSPA1A* mRNAs in several cell lines containing wild-type p53. In addition, upregulation of HSPA1A protein by DNA damage was decreased when p53 or IER5 expression was knocked down. These results show that HSP family proteins are induced by p53 and IER5 following DNA damage, and collectively suggest a role of p53/IER5/HSF1/HSP in the recovery and protection of both normal cells and cancer cells having wild-type p53 (Fig. 13.5).

13.2 Conclusions

We have shown that IER5 is a mediator of wild-type p53 activation of the HSF1-HSP pathway. However, it has also been reported that HSF1 can be activated by mutant p53 (Li et al. 2014). Mutant p53 induces the phosphorylation of HSF1 at Ser326 via activation of MAPK and PI3K cascades, leading to HSF1 induction of Hsp70 expression. This, in turn, stabilizes the mutant p53, forming a positive feed-forward loop. It is interesting therefore that both wild-type and mutant p53 are linked to activation of the HSF1-HSP pathway, illustrating the importance and complexity of the HSF1-HSP pathways in the regulation of cancer. Extensive phosphorylation and activation of HSF1 following heat stress has been well documented. For example, heat stress induces phosphorylation of multiple residues, including Ser230, Ser320 and Ser326, and enhances the transcriptional activity of HSF1. Contrary to our expectations, we demonstrated that IER5 promotes the dephosphorylation of HSF1 at multiple residues including Ser121, Ser307, Ser314, Thr323 and Thr367, and this dephosphorylation critically mediates the activation of HSF1 by IER5. While the repressive role of phosphorylation at Ser121 and Ser307 has been reported previously, the precise roles of Ser314, Thr323 and Thr367 in the repression of HSF1 activity requires further investigation. Recently, a number of studies have implicated HSF1 in tumorigenesis. Mendillo and his colleagues have shown that HSF1 activation in cancer cells can occur in the absence of heat stresses, and that HSF1 induces the expression of different sets of genes from under heat stress conditions (Mendillo et al. 2012). However, the precise mechanisms by which HSF1 is activated in the absence of heat stress remain unclear. Since HSF1 activity is normally regulated by various post-translational modifications, overexpression of the HSF1 mRNA or protein alone would not be expected to cause enhanced HSF1

activity or tumorigenesis. We reported that the *IER5* gene is associated with super-enhancers in cancer cells and that high IER5 expression is correlated with poorer prognosis of cancer patients. Furthermore, we demonstrated a high correlation between mRNA expression of *IER5* and the HSF1 target gene *HSPA6* in several cancers. In addition, in cancer cells that express high amounts of *IER5*, knockdown of *IER5* by RNAi results in the suppression of HSF1 activity and cell proliferation. These findings collectively indicate that IER5 is responsible for HSF1 activation and cell proliferation in at least some cancers. While our results demonstrate the tumor-promoting potential of IER5, HSF1 and the HSP family of genes are well known to maintain protein homeostasis and modulate the survival of normal cells. Therefore, in normal cells, IER5 may function downstream of p53 to promote the recovery and survival in response to stress conditions via the activation of HSF1 and induction of HSP family genes. It is well known that approximately half of all human cancers carry a p53 mutation, illustrating the importance of p53 function in tumor suppression. However, the remaining half of all cancers retain wild-type p53, and we imagine that some cancer cells may actually select for wild-type p53 as induction of IER5 and the downstream activation of HSF1 may help to protect these cells from stress conditions, thus promoting cancer. It therefore will be of great interest and importance to examine the involvement of the p53/IER5/HSF1 pathway in the promotion of cancers retaining wild-type p53 in mouse models and human cancer specimens in future studies.

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