GADD45 in Stress Signaling, Cell Cycle Control, and Apoptosis

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Abstract

GADD45 is a gene family consisting of *GADD45A*, *GADD45B*, and *GADD45G* that is often induced by DNA damage and other stress signals associated with growth arrest and apoptosis. Many of these roles are carried out via signaling mediated by p38 mitogen-activated protein kinases (MAPKs). The GADD45 proteins can contribute to p38 activation either by activation of upstream kinase(s) or by direct interaction, as well as suppression of p38 activity in certain cases. In vivo, there are important tissue and cell type specific differences in the roles for GADD45 in MAPK signaling. In addition to being p53-regulated, GADD45A has also been found to contribute to p53 activation via p38. Like other stress and signaling proteins, GADD45 proteins show complex regulation and numerous effectors. More recently, aberrant GADD45 expression has been found in several human cancers, but the mechanisms behind these findings largely remain to be understood.

Keywords

GADD45 · Cell cycle · Apoptosis · MAPK · UV radiation · p53 · Foxo3a · Oxidative stress · BRCA1 · NFkappaB · Tumorigenesis

1.1 Overview

GADD45 was first identified based on increased mRNA levels following stress-induced growth arrest and was therefore given the acronym *G*rowth *A*rrest and *D*NA *D*amage (*GADD*) as its name (Fornace et al. 1989). *GADD45*, now designated *GADD45A*, shows no sequence homology with the other original members of the *GADD* gene group (Kastan et al. 1992; Zhan et al. 1994), and was subsequently found to be a member of a highly conserved three-gene family consisting of *GADD45A* (GADD45A, DDIT1, GADD45α), *GADD45B* (GADD45β, MYD118), and *GADD45G* (GADD45γ, cytokine responsive 6 or CR6). The *GADD* genes were first cloned from Chinese hamster ovary (CHO) cells, which were

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subsequently found to be p53-deficient, as a subset of transcripts that were consistently upregulated after exposure to ultraviolet (UV) radiation and in many cases to other DNA-damaging agents, including methyl methanesulfonate (MMS), hydrogen peroxide, and N-acetoxy-2-acetylaminofluorene, as well as to other growth cessation signals, such as medium depletion/starvation or hydroxyurea (Fornace et al. 1988). *GADD45A* was the 45th member of this collection of over a hundred cDNA clones (Fornace et al. 1988). *GADD45A* is responsive to a myriad of agents implicated in DNA damage, apoptosis, cell cycle checkpoint control, cell injury, and other growth regulatory processes. The GADD45 proteins have likewise been implicated in a wide variety of cellular processes often associated with stress signaling and with other growth regulatory pathways (Gao et al. 2009; Zhang et al. 2014). Many GADD45 binding proteins have been identified using methods such as two-hybrid (Vinayagam et al. 2011) and affinity chromatography (Gao et al. 2013). Some of the prominent interactions of the GADD45 proteins are summarized in Fig. 1.1, which highlights regulatory pathways and downstream targets. As shown in this figure, GADD45 has a broad scope of potential roles in many cellular processes that will be covered in this and subsequent chapters, with emphasis in this chapter on growth control and apoptosis.

Among the radiation-response genes, *GADD45A* was unique at the time because it could be induced in an ATM-dependent and protein kinase C-independent manner following human cell exposure to ionizing radiation (IR) (Papathanasiou et al. 1991). This IR-responsiveness was subsequently found to be p53-regulated (Kastan et al. 1992); indeed, *GADD45A* was the first stress gene discovered that was regulated by p53 at the transcriptional level (Hollander and Fornace 2002). *GADD45B* was originally cloned as a gene expressed after terminal differentiation and growth arrest of M1D+ myeloid precursor cells induced by IL-6 (Selvakumaran et al. 1994). *GADD45G* was originally cloned as an early IL-2 response gene in T cells (Zhang et al. 1999). All three members show responsiveness to a variety of environmental cues associated with growth control. These three proteins are highly conserved among Metazoa although insects have only a single *GADD45* gene that is most similar to *GADD45G*, indicating this may be the ancestral gene. The proteins are all small (18 kDa), highly negatively charged (in the top two percentile of proteins in the ratio of negative charge to amino acids) (Zhan et al. 1994), and localized to the nucleus (Cretu et al. 2009). GADD45A is the best-characterized isoform and will be a major focus of this review although the other family members have important characteristics that will also be discussed.

Like most signaling proteins, the GADD45 proteins are small, highly regulated at both the transcriptional and post-transcriptional levels, and have multiple roles in mediating stress signaling and growth regulation. In addition to repair and apoptosis, cell injury, particularly in response to genotoxic stress, is known to trigger growth delays in prokaryotes and eukaryotes (Friedberg 2006). GADD45 proteins have been shown to play important roles in these processes. There is also a remarkable overlap between responses to genotoxic stress and aberrant growth signaling by oncogenes, referred to as oncogenic stress, which triggers a variety of responses involving GADD45. Many of these genotoxic and oncogenic stress responses are highlighted in Fig. 1.1. While they are discussed individually in more detail below, this overview diagram exemplifies the complexity of GADD45 regulation and function in these processes.

The stress mitogen-activated protein kinases (MAPK), namely the JNK and particularly the p38 MAPK, have complex regulatory roles involving GADD45. Other growth-arrest associated regulatory factors such as p53, BRCA1, FOXOA3, C/EBP, and ATF participate in transcriptional regulation of *GADD45A* and to some extent of the less-studied *GADD45B* and *GADD45G* genes, which in multiple cases can have roles distinct from GADD45A. The GADD45 proteins are involved, directly or as part of regulatory pathways, in cell cycle checkpoints and stimulation of DNA repair. They interact with a wide variety of cellular proteins and protein com-

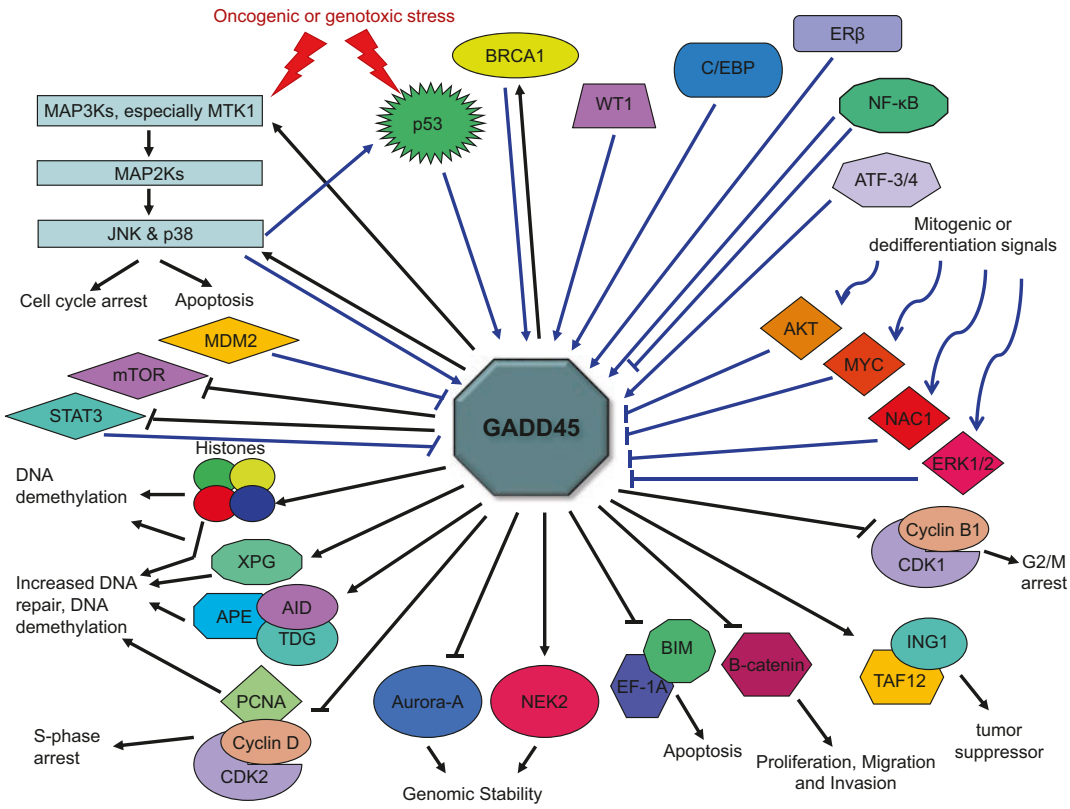

Fig. 1.1 Schematic representation of upstream regulators of *GADD45* and its downstream effects. Blue lines indicate upstream regulators, while black lines indicate downstream effects. Arrows indicate positive regulation, while blocked lines indicate negative regulation. Note that the interactions shown are primarily for GADD45A but may also occur for GADD45B and GADD45G. For example, all three proteins upregulate MTK1, but TGFβ (not shown in figure) is known to induce only GADD45B. Additionally, all GADD45 proteins are able to interact with each other and form homo- or hetero-dimers, which are crucial for GADD45 functions. Note that this is by no means a complete picture of all GADD45 interactions, but rather an overview of key interactions in stress signaling, cell cycle control, and apoptosis. For a discussion of GADD45 interactions involving methylation, please refer to Chap. 4: *GADD45 in DNA Demethylation and DNA Repair*

plexes, including cyclin-dependent kinase 1 (CDK1), for which it is a strong inhibitor of CDK1-Cyclin B1 activity both in vivo and in vitro and a component of certain G2 checkpoint events (Wang et al. 1999; Zhan et al. 1999; Vairapandi et al. 2002). Interestingly, like some other highly acidic proteins such as SET1, the GADD45 proteins bind directly to nucleosome histones and modify DNA accessibility, particularly on damaged chromatin (Carrier et al. 1999), which is one role reported for GADD45 in DNA repair (Smith et al. 2000). As shown in Fig. 1.1, the GADD45 proteins interact with and/or influence a variety of proteins involved in DNA repair, including APE (Jung et al. 2007), XPG (Barreto et al. 2007), PCNA (Smith et al. 1994), and p53. GADD45A in particular has been shown to play a role in heterochromatin relaxation (Chen et al. 2016). It has also been shown to bind to R-loops to promote DNA demethylation (Arab et al. 2019). These interactions will be discussed further in Chap. 4: *GADD45 in DNA Demethylation and DNA Repair*.

Although most of the interactions shown in Fig. 1.1 were initially discovered in cell culture systems, multiple functions of GADD45 have since been demonstrated using genetic approaches, both in vivo with mouse models and

in vitro with primary cells such as mouse embryo fibroblasts (MEFs). Among these findings, a consistent feature has been the prominent role of p38 MAPK signaling in vivo. For example, GADD45A-null mice lack the normal p53-mediated sunburn response in skin. As discussed in more detail later in this chapter, this is due to the requirement for p38 in p53 activation after stresses such as UV radiation (Hildesheim and Fornace 2004). Detailed studies in vivo and in MEF showed that GADD45 proteins can contribute to p38 activation either directly (Bulavin et al. 2003) or via MTK1, a MAPK kinase kinase (MAP3K) (Takekawa and Saito 1998) which is encoded by the *MAP3K4* gene. Additionally, p38 can directly phosphorylate regulatory sites in p53, such as Ser 46 (implicated in proapoptotic signaling) (Bulavin et al. 1999), and thus upregulate downstream effectors including GADD45A, which will then contribute to p38 activation. Thus, p38-p53-GADD45A defines a stress-activated regulatory loop, as shown in Fig. 1.2. While this positive feedback loop is transient during genotoxic-stress-induced growth arrest, it is necessary for oncogene-induced permanent growth arrest, i.e., premature senescence (Bulavin et al. 2003). Consistent with these findings, GADD45A-null mice show increased carcinogenesis after genotoxic stresses such as IR (Hollander et al. 1999) or UV radiation (Hildesheim et al. 2002).

1.2 GADD45 Regulation in Growth Arrest and Apoptosis

As outlined in Fig. 1.1 and Table 1.1, *GADD45* is regulated in response to genotoxic stress and other growth-arrest signals at both the transcriptional and post-transcriptional levels (Gao et al. 2009). *GADD45* plays an important role in stress-induced growth arrest, such that it is one of only a few genes that is upregulated consistently after IR in numerous conventional and gene expression profiling studies of p53 wild-type (wt) cells

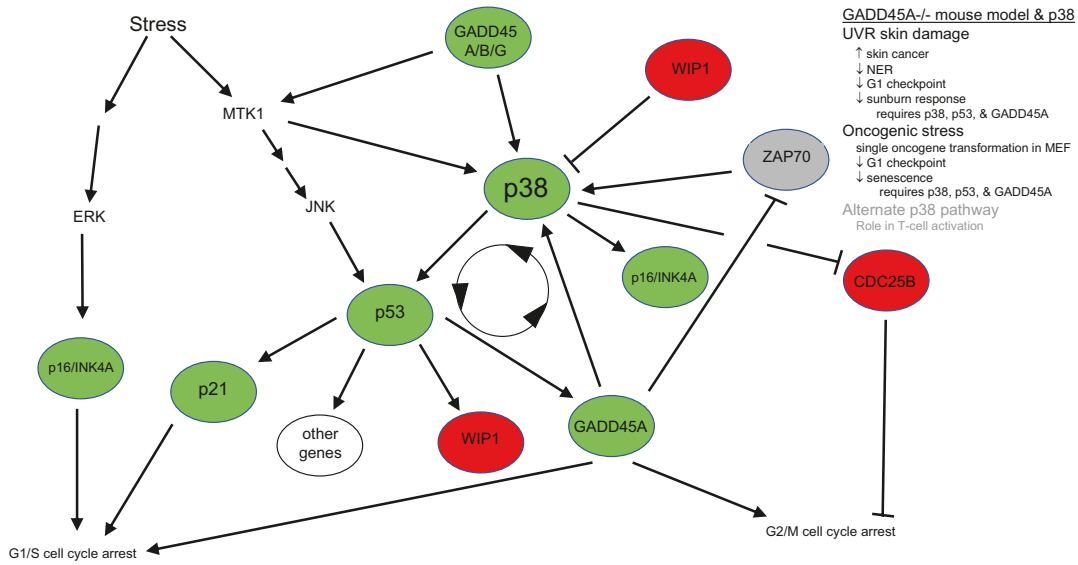

Fig. 1.2 Central role for p38 signaling in the GADD45A phenotype. Arrows indicate positive regulation, while blocked lines indicate negative regulation. Green circles indicate proteins typically considered tumor suppressors, while red circles indicate potential oncogenes. As described in the text, p38, p53, and GADD45A can function in a positive feedback loop (indicated by black circle with arrows) to maintain p53 signaling and growth arrest. GADD45 proteins are positive effectors for p38 activation after many stresses. As discussed in Chap. 5, GADD45A has a prominent role in the alternative p38 activation pathway in T cells and immunity by modulating ZAP70 activity

Table 1.1 GADD45 effectors with roles in growth control and apoptosis. Note that this is not a complete summary of all GADD45 interactions, but rather a list of key protein interactors involved in growth control and apoptosis. Protein interactors involved in methylation, such as ING1, will be discussed further in Chap. 4: *GADD45 in DNA Demethylation and DNA Repair*

p38	Cell cycle arrest, apoptosis, induction of senescence, negative regulation of T cell activation, full activation of innate immune cells
MTK1	Activation that signals to p38 and JNK branches of MAPK pathways
p53	p53 activation via p38 signaling, required for sunburn response in skin
CDK1	Inhibits CDK1/CLNB1 activity and contributes to G2 checkpoint activation
CDKN1A (p21)	Positive role in chondrocyte senescence (GADD45B); negative regulation of p21 in keratinocytes allowing nucleotide excision repair
APC	Destruction of ß-catenin via p38 signaling
ß-catenin	Inhibition of its pro-invasion program, increased ß-catenin plasma membrane localization and cell–cell adhesion
JNK	Cell cycle arrest and apoptosis; can be mediated by MTK1 signaling
EF-1A	Release of BIM, apoptosis
PCNA	S-phase arrest; DNA repair and demethylation
Aurora-A	Maintenance of genomic stability
NEK2	Maintenance of genomic stability
mTOR	Suppression of tumor angiogenesis by inhibition of mTOR signaling
STAT3	Inhibition of STAT3 promotion of tumor angiogenesis
ING1	Tumor suppression via DNA methylation

(Snyder and Morgan 2004). For example, in the NCI60 cell screen panel, only p53 wt human tumor lines showed appreciable *GADD45A* induction (Weinstein et al. 1997). Although ubiquitous, basal *GADD45* expression is usually very low and varies through the cell cycle, with highest levels during G_1 and lowest levels during S phase (Kearsey et al. 1995).

As highlighted in Fig. 1.1, GADD45A expression is induced by MAPK signaling via p38 and JNK kinases. These kinases activate c-Jun, which, similarly to p53, binds to the third intron of *GADD45A* and promotes transcription; this finding is not surprising since AP-1-binding sites have been identified both in the promoter region and the third intron of *GADD45A* (Gao et al. 2009). It is of interest that transient ERK signaling induces *GADD45A* expression, whereas sustained signaling represses it (Gao et al. 2009); this *GADD45A* induction might be due to transient activation of other MAPK pathways through crosstalk. Sustained or oncogene-driven ERK signaling also promotes upregulation of p16 (Bulavin et al. 2003), which plays an important role in G1/S cell cycle arrest, as shown in Fig. 1.2. Estrogen receptor β (ERβ) can bind to the *GADD45A* promoter in a ligand-independent manner and recruits c-Jun and NCOA2 to stimulate transcription and subsequent G_2/M arrest (Paruthiyil et al. 2011). Indeed, in a panel of human breast cancer samples, GADD45A expression was found to depend on estrogen receptor expression (Tront et al. 2013). BRCA1, a breast (and other) cancer tumor suppressor, has also been implicated in *GADD45A* gene regulation with binding sites in both the first and third exon of this gene (Harkin et al. 1999; Pietrasik et al. 2020).

1.2.1 Transcriptional Regulation of GADD45

At the transcriptional level, there are several tumor suppressor genes that induce *GADD45A* expression. As mentioned earlier, one well-characterized mechanism of *GADD45A* induction involves the binding of p53 to a conserved site within the third intron of the *GADD45A* gene (Kastan et al. 1992). This binding is induced by genotoxic stress but is necessary only in the case of IR exposure and not in the *GADD45A* response to UV radiation or MMS although loss of p53 does attenuate subsequent *GADD45A* induction. WT1, a transcription factor that is mutated in various tumors and congenital defects, can bind to the *GADD45A* promoter and induce transcription in a p53-dependent manner (You et al. 2019) but in the absence of direct p53-DNA binding in the response to non-ionizing radiation (Zhan

et al. 1998). BRCA1 induces *GADD45A* expression indirectly by interacting with the transcription factors OCT-1 and NF-YA. The CCAAT/enhancer-binding protein-α (C/EBPα) and other C/EBP proteins can induce *GADD45G* expression as well (Gao et al. 2009; Jung et al. 2000).

GADD45A has been identified as a direct target gene of FOXO3A, a tumor suppressor that is a member of the mammalian family of forkhead transcription factors. FOXO3A binds to *GADD45A* at the promoter region and promotes transcription in response to treatment with phosphoinositol-3 kinase inhibitor (Tran et al. 2002) or oxidative stress (Sengupta et al. 2011). However, FOXO3A has been observed to suppress the induction of GADD45B (Lee et al. 2008), suggesting a different possible role of GADD45B in response to stress (Tran et al. 2002). As shown in Fig. 1.1, activating transcription factor-4 (ATF-4) has a central role in cellular stress responses and induces *GADD45A* transcription in response to arsenite exposure, leucine deprivation, inhibition of the proteasome, and endoplasmic reticulum stress; GADD45A protein levels rise after arsenite exposure or proteasome inhibition, showing a sophisticated regulation of *GADD45A*, which responds differentially to various cellular stressors (Gao et al. 2009; Chang et al. 2007; Song et al. 2006). The TNF superfamily ligand APRIL also induces *GADD45* transcription. Binding of APRIL to the receptor BCMA triggers JNK2 phosphorylation, FOXO3A activation, and *GADD45* transcription, inhibiting cell proliferation in hepatocellular carcinoma cells through cell cycle arrest at the G_2/M checkpoint (Notas et al. 2012).

The interaction of *GADD45* with BRCA1, a key breast cancer tumor suppressor, plays an important role in cell cycle control and DNA repair (Pietrasik et al. 2020). BRCA1 has been shown to induce *GADD45* transcription after γ-radiation treatment of cells (Li et al. 2000; Park et al. 2008). Similarly, overexpression of BRCA1 resulted in increased GADD45 expression and also stimulation of nucleotide excision repair (NER) in a GADD45-dependent manner (Hartman and Ford 2002). Since BRCA1-deficient cells are hypersensitive to cisplatin, this suggests a defect in NER of cisplatin adducts (Husain et al. 1998). Additionally, in response to hypoxic shock or anisomycin treatment, ATF2 binds to BRCA1, NF-1, and OCT-1 to stimulate transcription of *GADD45A* (Maekawa et al. 2008), such that BRCA1 indirectly and directly (Park et al. 2008) activates transcription of *GADD45A*. The importance of BRCA1 in the DNA damage response (DDR) is well known (Wu et al. 2010), and these findings highlight the importance of GADD45 as a downstream effector of BRCA1. This will be discussed further in Chap. 10: *GADD45 in Breast Cancer*.

As shown in Fig. 1.1, there are also several growth stimulatory factors that are involved in negative regulation of *GADD45A*. Transcriptional repression by c-MYC and AKT proto-oncogenes expression highlights the frequent association of *GADD45* with cell growth suppression (Gao et al. 2009; Bulavin and Fornace 2004; Brown-Clay and Fornace Jr 2018). MYC regulates *GADD45A* gene expression by inhibiting FOXO3A-dependent transcription of *GADD45A* (Amente et al. 2011). AKT inhibition of *GADD45A* is also mediated by FOXO3A inactivation (Amente et al. 2011).

More recently, clinical studies have demonstrated the role of miRNA in regulating GADD45A expression. In Sertoli cells of patients with Sertoli-cell-only syndrome, miR-4270 has been found to inhibit *GADD45A* mRNA expression by binding to its 3′-UTR (Wang et al. 2020). In blood samples from patients with chronic myeloid leukemia, increased miR-362-5p levels were associated with decreased GADD45A levels (Yang et al. 2015).

1.2.2 Post-transcriptional Regulation of GADD45

Early on, it became evident that *GADD45A* regulation at the post-transcriptional level is complex and can be regulated based on the mRNA stability of *GADD45A* and other *GADD* genes (Jackman et al. 1994). In unstressed cells, AUF1 destabilized *GADD45A* mRNA and TIAR1 hindered its translation, potently inhibiting expres-

sion of the GADD45A protein. After cell exposure to MMS or UV radiation, these proteins dissociate rapidly from *GADD45A* mRNA and allow robust expression of the protein. Conversely, the mRNA stabilizing protein, nucleolin, binds *GADD45A* mRNA after cellular stimulation with arsenic chloride or NF-κB inhibition and potently increases both mRNA and protein levels (Lal and Gorospe 2006). MAPK kinases (MAP2Ks) upstream of p38 have been shown to phosphorylate three proteins involved in RNA regulation, HNRNPA0, TIAR, and PARN, resulting in stabilization of *GADD45A* mRNA (Reinhardt et al. 2010). In the same report, p38/MK2 complex was found to relocalize from the nucleus to the cytoplasm, where MK2 phosphorylated hnRNPA0, and stabilized GADD45A mRNA, while p38 was found to phosphorylate and release the translational inhibitor TIAR. At the post-translational level, arsenite stimulation of cells induces formation of an IκB-kinase-β (IKKβ)/NF-κB p50 subunit complex that reduces ubiquitinated GADD45A levels and its subsequent proteasomal degradation (Yang et al. 2009).

1.2.3 GADD45 and NF-κB

The role of NF-κB in the regulation of *GADD45* is complicated and appears to depend on cellular context. NF-κB signaling is often considered a pro-survival response and was reported to reduce GADD45A and GADD45G expression and escape from apoptosis in cancer cells (Zerbini et al. 2004). NF-κB activation of EGR-1 leads to direct EGR-1-mediated transcriptional activation of *GADD45A*. The NF-κB-activating kinases, IKKα and IKKβ, are also able to induce *GADD45* expression through a NF-κB-independent mechanism. The p65 (RelA) subunit of NF-κB binds directly to three κB elements in the *GADD45B* promoter and activates its transcription. However, NF-κB also inhibits *GADD45A* and *GADD45G* expression by activating c-MYC (Zhang et al. 2014). This differential regulation of *GADD45A* might therefore contribute to the observed pro- and anti-oncogenic actions of NF-κB although the mechanisms that govern this switch are not well understood (Yang et al. 2009). In the case of *GADD45B*- and *GADD45G*-specific mechanisms of transcriptional regulation, the p65 (RelA) subunit of NF-κB binds directly to three κB elements in the promoter of *GADD45B* and activates its transcription (Yang et al. 2009). Nucleus accumbens-1 (NAC1) is a transcription factor associated with embryonic stem cell self-renewal and pluripotency that is also upregulated in several cancer types, particularly chemoresistant, recurring ovarian carcinomas. NAC1-mediated GADD45G downregulation has been shown to contribute to paclitaxel resistance in ovarian cancer cells (Jinawath et al. 2009).

1.2.4 GADD45A Reporter as an Assessor of Genotoxicity

GADD45A mRNA and proteins are frequently induced by a plethora of stresses and types of injury, and this responsiveness can be used to monitor for such events. As discussed earlier, GADD45A regulation is complex and involves multiple regulatory factors that contribute to stress responsiveness. In addition to a classic p53-binding site in its third intron (Kastan et al. 1992) and a WT1 site in its promoter that can also contribute to p53 signaling (Zhan et al. 1998; Johnson et al. 2013), there are a variety of regulatory elements, such as OCT-1, AP-1, C/EBP, GRE, and EGR-1 in the *GADD45A* gene that can contribute to stress responsiveness (Zhang et al. 2014; Takahashi et al. 2001); for a complete listing, see https://www.genecards.org/cgi-bin/carddisp.pl?gene=GADD45A. There are numerous reports of GADD45A responsiveness to various types of injury in vivo. In TK6 cells, a human lymphoblastoid line used in many toxicology assays, *GADD45A* mRNA levels were rapidly increased following exposure to a variety of genotoxic agents such as heavy metals, resulting in the unfolded protein response (UPR), oxidative stress, medium (nutrient) depletion, and inhibition of glycolysis and certain other pathways of energy metabolism (Amundson et al. 2005; Li et al. 2017). While many such stresses can rapidly induce *GADD45A* mRNA expression, geno-

toxic stress agents typically trigger stronger responses (Li et al. 2015, 2017), such that *GADD45A* induction may have utility in monitoring for genotoxic stress that is triggered either directly by DNA damage or indirectly by agents such as topoisomerase poisons and DNA synthesis inhibitors. Importantly, there is a need for newer assays to assess for genotoxic stress because the current in vitro testing battery, especially mammalian cell assays, has high sensitivity but suffers from low specificity, leading to high rates of false or irrelevant positive findings (Li et al. 2007, 2017; Snyder and Green 2001; Kirkland et al. 2005; Goodsaid et al. 2010; Krewski et al. 2020). *GADD45A* promoter reporter constructs have been employed by a variety of laboratories to assess for genotoxicity since first reported (Todd et al. 1995). Using Green Fluorescent Protein (GFP) reporter, a study of 75 genotoxic and non-genotoxic compounds demonstrated that the assay could respond positively to various classes of genotoxic damage with high specificity and high sensitivity (Hastwell et al. 2006). This and other groups (Xin et al. 2015; Simpson et al. 2013; Luzy et al. 2013; Walmsley and Tate 2012; Röckner et al. 1989) have developed high-throughput screening approaches to apply *GADD45A* reporter constructs to assess for genotoxicity with rapid in vitro methodology.

While the *GADD45A* reporter construct approach has merit, concern may arise because a variety of non-genotoxic stress stimuli are known to induce *GADD45A* as discussed above. To complement these approaches, a variety of laboratories have proposed toxicogenomics approaches to assess for genotoxicity (Amundson et al. 2005; Li et al. 2007, 2015; Liu et al. 2019; Ellinger-Ziegelbauer et al. 2009; Cui and Paules 2010; Herwig et al. 2016; Moffat et al. 2015; Chepelev et al. 2015). Many of these reports include assessment of *GADD45A* mRNA levels. The advantage here is that bioinformatic approaches can be implemented to develop a more accurate prediction of genotoxicity rather than reliance on a single gene alone. As an example, a panel of 64 genes including *GADD45A* was developed to assess genotoxicity in TK6 cells, and prediction of genotoxicity was high using a panel of genotoxic and non-genotoxic agents (Li et al. 2015, 2017). Notably, 90% of non-genotoxic agents that were positive in the traditional mammalian cell genotoxicity assays were classified as non-genotoxic with this gene expression approach (Li et al. 2017). This toxicogenomic approach also has the capability for high-throughput screening (Li et al. 2017; Cho et al. 2019a) and offers an exciting strategy to complement classic in vitro toxicology in the assessment of genotoxicity (Krewski et al. 2020).

1.3 GADD45A Effectors in Growth Arrest and Apoptosis

GADD45A, GADD45B, and GADD45G share quite a bit in common when it comes to downstream effectors. However, the literature for GADD45A is much larger, so it will be discussed first. As can be anticipated for a protein that is predominantly stress-induced, many of the well-characterized GADD45A functions are associated with growth arrest and apoptosis. Although limited direct biochemical mechanisms have been shown for GADD45A, it has been found repeatedly to form complexes with a variety of proteins and even with chromatin. It thus seems likely that its biologic effects are due to its ability to facilitate protein–protein interactions as well as to directly affect protein conformation, as in the case of MTK1. These interactions and their effects are highlighted for selected proteins in Fig. 1.1 and Table 1.1.

1.3.1 GADD45A Effectors in Growth Arrest

As shown in Fig. 1.1, GADD45A has important roles in both S phase and G_2/M arrest (Hollander and Fornace 2002; Smith et al. 1994). *GADD45A* knockdown is associated with G_2/M checkpoint abrogation following endoplasmic reticulum stress (Lee et al. 2019). It can displace PCNA from the cyclin D1 complex, possibly inhibiting

DNA replication during S phase (Smith et al. 1994). Likewise, GADD45A can inhibit CDK1 activity by promoting dissociation of CDK1/Cyclin B1, arresting the cell cycle at the G_2/M checkpoint (Zhang et al. 2014; Wang et al. 1999; Zhan et al. 1999). GADD45A can directly inhibit purified CDK1/Cyclin B1 activity in vitro (Zhan et al. 1999). In the case of control of S phase progression, loss of GADD45A results in centrosome amplification, particularly when S phase progression is chemically inhibited; in normal cells, initiation of S phase and centrosome activity are tightly coordinated by GADD45A (Hollander and Fornace 2002). GADD45A interacts with the tumor suppressor cyclin-dependent kinase inhibitor 1a (encoded by *CDKN1A*), also known as p21, CIP1, or WAF1, such that deletion of both GADD45A and p21 is associated with attenuated S-phase arrest (Hollander et al. 2005a). The two protein products compete for interaction with PCNA, and GADD45A seems to negatively regulate CDKN1A expression in keratinocytes, allowing nucleotide excision repair (NER) after UV radiation (Gao et al. 2009).

GADD45A has been found to play a role in the inhibition of β-catenin signaling, a pro-growth pathway (Hildesheim et al. 2004, 2005). Following exposure to UV radiation, GADD45A stimulates p38 in the dephosphorylation of glycogen synthase kinase 3β (GSK3 β). This activates the adenomatous polyposis coli (APC) destruction complex, which increases β-catenin phosphorylation and degradation. GADD45A also increases p38 positive regulation of APC translocation to the nucleus, an important step in β-catenin degradation, as well as localization of β-catenin at the plasma membrane. This prevents activation of the pro-invasion transcriptional program and increases its interaction with caveolin-1, strengthening cell–cell adhesion (Gao et al. 2009). Consistent with its tumor suppressor-like properties, GADD45A inhibits tumor cell invasion and migration induced by high β-catenin levels (Hildesheim and Fornace 2004).

As mentioned above, GADD45A is often required in oncogene-induced senescence (Bulavin et al. 2003) and DNA damage-induced establishment of the senescent phenotype (Passos et al. 2010). In both cases, GADD45A signaling via p38 is essential for induction of this phenotype and for full transactivation of p53, whose activity is essential for cell entry into a senescent state. In senescent human fibroblasts, p53 preferentially occupies the promoters, resulting in a unique combination of phosphorylated p53 sites (Gao et al. 2009). The positive feedback loop between GADD45A, p38, and p53 (Fig. 1.2) is thus essential for induction and maintenance of the senescent phenotype after oncogene overexpression or severe DNA damage in fibroblasts and keratinocytes, and likely in other cell types as well. This will be discussed further in Chap. 8: *GADD45 in Senescence*. In addition to premature senescence, differentiation can be used to remove damaged or potentially tumorigenic cells from the growth compartment. GADD45A upregulation in response to genotoxic conditions is associated with increased terminal differentiation of hematopoietic stem cells (Wingert and Rieger 2016; Wingert et al. 2016).

1.3.2 GADD45A Effectors in Apoptosis

GADD45A has been repeatedly associated with apoptosis after oncogenic and genotoxic stresses. Its level rises notably in mammalian apoptotic cells, and inhibition of GADD45A expression reduces apoptosis in response to DNA damage. p38 and JNK often mediate the proapoptotic effects of GADD45A. All three GADD45 proteins bind the N-terminus of MTK1, which activates p38 and JNK signaling, inducing a conformational change that results in its autophosphorylation, activation, and a strong apoptotic response (Takekawa and Saito 1998; Mita et al. 2002). As shown in Fig. 1.2, GADD45A activation of p38 and JNK signaling, which are upstream activators of GADD45A (as well as of p53, which also induces GADD45A expression), forms the basis of a positive feedback loop that raises levels of these tumor suppressive signaling molecules in the event of genotoxic stress and unresolved DNA damage. Furthermore, GADD45A expression is necessary for sustained

p38 and JNK signaling and consequent growth arrest or apoptosis in keratinocytes after UV radiation (Hildesheim et al. 2002). The sunburn response, which has a prominent apoptotic component, requires p53, p38, and GADD45A (Hildesheim and Fornace 2004), whereas GADD45A is necessary for normal p53 activation after UV radiation of keratinocytes in vivo and in primary culture, it is not needed in dermal fibroblasts (Hildesheim et al. 2002). How p53 signaling compensates in GADD45A-null dermal fibroblasts is uncertain, but it has been shown that the other GADD45 proteins are expressed more abundantly in this cell type. This observation thus highlights the cell specificity for some in vivo roles of GADD45.

GADD45A has also been suggested to be involved in early events of the apoptotic cascade through interactions with the cytoskeleton. Elongation factor 1α (EF-1α) is a microtubule-severing protein that plays a key role in cytoskeletal stability by binding, bundling, and promoting microtubule assembly. Increased GADD45A expression results in interactions with EF-1α that inhibit microtubule bundling and destabilize the cytoskeleton (Tong et al. 2005). This causes release of BIM, a BCL-2 family proapoptotic protein, from microtubule-associated complexes and allows for BIM translocation to the mitochondria, triggering cytochrome C release into the cytoplasm and initiation of apoptosis (Gao et al. 2009).

At the same time, there are other features of GADD45A that can have an opposing effect on apoptosis potential. This is not surprising, as checkpoint activation and DNA repair can also enhance cell survival. For example, GADD45A deficiency sensitizes cells to cisplatin and UV radiation, implying subtleties to the proapoptotic effects of this protein that likely result in reduced DNA repair in the absence of GADD45A. In hematopoietic cells exposed to UV radiation, GADD45A is implicated in a NF-κB-p38 survival pathway (Cretu et al. 2009). GADD45A also protects neurons from apoptotic cell death after withdrawal of nerve growth factor in spinal cord ligation (Lin et al. 2011). The first two examples can be explained as GADD45A enhancing survival by mitigating the effects of genotoxic stress, that is, arresting cell replication and stimulating DNA repair. The last example is clearer evidence of a GADD45A pro-survival function and of pronounced tissue specificity in GADD45A action.

1.3.3 Other Notable GADD45A Effectors

GADD45A, through its involvement in cell cycle control, DNA repair, apoptosis, and p53 signaling, thus, has a key role in maintaining genomic stability. This is particularly evident in GADD45A-null cells and mice that exhibit centrosome amplification and incomplete chromosome condensation during mitosis. Mitotic abnormalities lead to defective chromosome segregation, which likely leads to the chromosome and chromatid aberrations often seen in this genotype (Hollander and Fornace 2002). The genomic instability phenotype resembles that of p53-null mice although GADD45A-null mice do not show the marked spontaneous tumorigenesis seen in p53-null mice. In the case of centrosome instability, GADD45A physically associates with Aurora-A protein kinase, whose deregulated expression produces centrosome abnormality and strongly inhibits its activity (Shao et al. 2006). Conversely, *GADD45A* and *BRCA1* are both needed for full, physiological transcriptional upregulation of NEK2 (Wang et al. 2004), the correct concentration of which is essential for timely centrosome separation (Gao et al. 2009).

GADD45A also has the ability to stimulate DNA repair, as discussed in detail in Chap. 4: *GADD45 in DNA Demethylation and DNA Repair.* In vitro and cell culture assays show that recombinant GADD45A can stimulate NER in chromatin-bound DNA (Smith et al. 1994; Tran et al. 2002), whereas loss of GADD45A expression in ex vivo assays of lymphoblasts results in substantially reduced NER (Gao et al. 2009). The ability of GADD45A to interact with acetylated or UV radiation-exposed mononucleosomes and increase local DNA accessibility might facilitate stimulation of DNA repair (Ma et al. 2009).

Also discussed in more detail in Chap. 4 is the role of GADD45A-related excision repair events in the removal of DNA methylation, which is an epigenetic marker associated with repression of transcriptional initiation. GADD45A interacts directly with the four core histones and increases DNase accessibility to DNA with hyperacetylated mononucleosomes in vitro, perhaps allowing access of demethylation and DNA repair complexes to DNA in chromatin. TATA-binding protein-associated factor 12 (TAF12) was found to recruit GADD45A and the nucleotide excision repair complex to the ribosomal DNA promoter and induce its transcription in a demethylation-dependent manner (Schmitz et al. 2009). GADD45 interacts directly with various nuclear hormone receptors, including constitutive active/androstane receptor (CAR) (Yamamoto et al. 2010), RXRα, RARα, ERα, PPARα, PPARβ, and PPARγ2, perhaps mediating or facilitating transcriptional initiation of their target genes (Ma et al. 2009). GADD45A- and GADD45B-mediated DNA demethylations are also necessary for full expression of epidermal differentiation-inducing genes during calcium-triggered differentiation of epidermal stem cells (Sen et al. 2010).

Although p38 is typically discussed in the context of growth arrest, it also has key stimulatory roles in lymphocytes. GADD45A has been shown to have an important regulatory role in the case of T cell activation via p38 signaling (Salvador et al. 2005a, b; Ashwell 2006). Surprisingly, GADD45A is a negative regulator of p38 signaling during T cell activation and subsequent proliferation, as discussed in Chap. 5: *GADD45 in Immunity*. Briefly, p38 is activated by an alternate pathway involving autophosphorylation of p38 at Tyr323, and it is this pathway that is inhibited by GADD45A (Ashwell 2006). Interestingly, inhibition of the p38 alternative activation pathway in infiltrating T cells inhibits pancreatic cancer progression (Alam et al. 2015). This was demonstrated with a plasma membrane–permeable GADD45A peptide, so in this case, GADD45A may well have a tumor suppressor effect by inhibiting tumor-promoting inflammation (Alam et al. 2015).

1.4 Roles for GADD45B and GADD45G

As mentioned earlier, less is known about GADD45B and GADD45G compared to GADD45A. However, GADD45B and GADD45G are clearly defined as proapoptotic, growth-arrest proteins that share several similarities with GADD45A. Both proteins inhibit CDK1 activity and have a role in S and G_2/M checkpoints. Loss of GADD45B is associated with G_2/M checkpoint arrest and premature senescence in mouse embryo fibroblasts (MEFs) (Magimaidas et al. 2016). Like GADD45A, GADD45B promotes dissociatio of CDK1/Cyclin B1 (Zhang et al. 2014). Both GADD45B and GADD45G activate MTK1 to trigger JNK signaling (Takekawa and Saito 1998; Yang et al. 2009). They also interact with p21, and GADD45B positively regulates its expression in senescing chondrocytes (Ijiri et al. 2005) although the result of this interaction is unclear in other tissues and contexts (Gao et al. 2009). GADD45B facilitates p38-mediated activation of retinoblastoma tumor suppressor protein (Rb) by enhancing their interaction after Fas stimulation in murine hepatocytes (Cho et al. 2010). It also mediates TGF-induced apoptosis in murine hepatic cells in a p38- and SMAD-dependent manner, as well as both GADD45B and GADD45G overexpression-induced apoptosis in HeLa cells. GADD45G is associated with neuronal cell death and GADD45B with the apoptotic response in neural ischemia (Cretu et al. 2009; Cho et al. 2019b). GADD45G levels are significantly lower in anaplastic thyroid cancer cells compared to primary cultured thyrocytes, and its reintroduction by viral expression has been shown to inhibit proliferation (Yang et al. 2009).

Both GADD45B and GADD45G have been suggested to have roles in the growth and development of specific tissues in the embryo, such that they are differentially expressed during embryonic development. For example, *GADD45B* is expressed in the chorion, whereas *GADD45G* is expressed in the mouse brain (Kaufmann et al. 2011). At the cellular level,

GADD45 genes are expressed in cells undergoing differentiation, including forming somites and neuronal precursors, and their expression pattern is consistent with a potential role in cell cycle arrest.

1.4.1 GADD45B and GADD45G in p38 and JNK Signaling

GADD45B has been reported to mediate TNFα-induced NF-κB suppression of JNK-induced apoptosis by directly binding to MKK7 and inhibiting its catalytic activity (Karin 2014). However, as discussed previously, the role for GADD45B in NF-κB signaling was somewhat uncertain since *GADD45B*-null mice do not show a clear phenotype, as might be expected for deletion of an upstream inhibitor of NF-κB. Still, NF-κB is frequently over-expressed in tumor cells, and suppression of JNK-induced apoptosis has been shown to be mediated by direct binding of GADD45B to MKK7. Additionally, development of a specific inhibitor that blocks GADD45B inhibition of MKK7 has been shown to trigger cell death in a panel of multiple myeloma cell lines with high constitutive levels of GADD45B (Tornatore et al. 2014a). The GADD45B-MKK7 complex has thus been suggested as a therapeutic target in the treatment of multiple myeloma (Tornatore et al. 2014b). GADD45B has also been described to suppress JNK signaling in hematopoietic cells in response to UV treatment (Yang et al. 2009). In mouse hepatocytes, stimulation of CAR also induces its interaction with GADD45B, leading to GADD45B-mediated repression of JNK signaling and subsequent cell death (Yamamoto et al. 2010). The role of GADD45B in TGFβ-mediated apoptosis was shown using a genetic approach in GADD45B-null hepatocytes, confirming the need for GADD45B in p38 activation (Yoo et al. 2003). GADD45B promotes liver regeneration in vivo (Papa et al. 2008) and protects retinal ganglion cells in response to neuronal injury, oxidative stress, TNFα, and glutamate cytotoxicity (Liu et al. 2009).

GADD45B and GADD45G show both similarities and differences to GADD45A in immune cells. Unlike GADD45A, they potentiate p38 signaling in Th1 and $CD8^+$ cytotoxic T cells in order to promote full effector function; like GADD45A, they are negative regulators of T cell activation and proliferation (Lu 2006; Ju et al. 2009). In addition, GADD45B is necessary for full expression of the Th1 lineage-inducing proteins, T-bet, and Eomes (Ju et al. 2009). The GADD45 family members thus seem to work together to promote full maturation and function of Th1 and $CD8^+$ cells, but they also prevent inappropriate overexpression, except under certain pathological conditions.

These results highlight the complex roles for the GADD45 proteins in MAPK signaling. As shown in Fig. 1.2, the GADD45 proteins clearly stimulate the stress-mediated activation of MTK1, which is upstream of p38 and JNK, as well as more directly for p38. However, GADD45B has an opposing effect on JNK signaling by inhibition of upstream MKK7, and GADD45A has a specialized role in dampening p38's role in T cell activation, as discussed in Chap. 5: *GADD45 in Immunity*. Taken together, one can conclude that the GADD45 proteins are important components of MAPK signaling and can have either stimulatory or inhibitory effects depending on the cellular context.

1.4.2 Notable Roles of GADD45G Only

With primarily genetic approaches, GADD45 has been found to have several features distinct from other GADD45 proteins. Recently, GADD45G has been suggested to play a role in cardiomyocytes following stress. GADD45G expression is elevated following myocardial infarction in murine cardiomyocytes, and it is associated with increased p38 MAPK-dependent apoptosis and heart failure (Lucas et al. 2015). Additionally, miR-128-1-5p has been shown to decrease GADD45G expression and apoptosis in cardiomyocytes following myocardial ischemia/reperfusion injury (Wan et al. 2020).

GADD45G has also been shown to have a specific role in gonad development, male fertility, and sex determination (Gierl et al. 2012; Warr et al. 2012; Johnen et al. 2013). Notably, mice deficient in GADD45G show an unexpected male-to-female sex reversal phenotype. GADD45G-deficient XY mice on a mixed 129/C57BL/6 background have varying degrees of disorders of sexual development, ranging from male infertility to complete gonadal dysgenesis (Johnen et al. 2013). On a pure C57BL/6 background, all GADD45G$^{-/-}$ XY mice were born as completely sex-reversed XY-females (Gierl et al. 2012; Warr et al. 2012; Johnen et al. 2013). The GADD45G expression pattern is not sexually dimorphic. GADD45G levels are similar in wt XY and XX gonads during the sex determination period, and peak at the time of primary sex differentiation, when SRY is also present. GADD45A and GADD45B are not expressed in purified somatic supporting precursor cells. Only GADD45G expression is induced robustly in embryonic gonads and in somatic precursor cells (Johnen et al. 2013).

In male gonads, SRY plays a key role in the male developmental pathway by promoting differentiation of a somatic supporting cell lineage into Sertoli cells. In the absence of SRY in XX gonads, SOX9 is downregulated, and a female-specific gene expression program is activated, leading to differentiation of the somatic supporting lineage into granulosa cells, which support oocyte development. Surprisingly, GADD45G, but not GADD45A or GADD45B, is necessary for activation of the male sex-determining pathway in mice, such that its absence leads to the development of female gonads. Lack of GADD45G decreases SRY expression and blocks SOX9 expression, resulting in ovary and Müllerian duct development, whereas lack of GADD45A and/or GADD45B has no effect on testis development (Johnen et al. 2013). Although it remains to be determined how GADD45G regulates SRY expression, it is proposed that GADD45G is needed to promote MAP3K4-mediated activation of p38 signaling in murine embryonic gonadal somatic cells. p38 can phosphorylate GATA4 and then phospho-GATA4 might bind and activate the *SRY* promoter to induce the male program (Gierl et al. 2012; Warr et al. 2012). In utero exposure to Di (2-ethylhexyl) phthalate (DEHP) has been shown to inhibit the GADD45G-dependent sex determination pathway in mice (Wang et al. 2015).

1.5 Involvement of GADD45 in Tumorigenesis

Loss of GADD45A has been shown to confer a tumor-prone phenotype after genotoxic stress. GADD45A has been shown to inhibit autophagy in tumors, which likely provides a nutrient advantage to tumor cells, by inhibiting BECN1-PIK3C3 interactions (Zhang et al. 2015). Studies in GADD45A-null mice illustrate that GADD45A-dependent protection against UV irradiation-induced skin tumors requires functional p38 (Hildesheim et al. 2002). Abolition of either GADD45A or p38 activity results in compromised negative regulation of β-catenin via the APC destruction complex (Gao et al. 2009). p53-signaling in the sunburn response requires GADD45A for effective p38 activation, which then signals p53 (Hildesheim et al. 2002), as shown in Fig. 1.2. GADD45A-null mice also show increased rates of IR- or dimethylbenzanthracene-induced tumors, with a shorter latency period than controls (Hollander et al. 1999, 2001). Deletion of *GADD45A* in an XPC$^{-/-}$ mouse model of lung cancer led to an increase in lung tumor malignancy, and allelic deletion of *GADD45A* is associated with multiple tumor types, including lung (Hollander et al. 2005b) and mammary tissue (Pietrasik et al. 2020). Loss of GADD45A is also associated with worse outcomes in chronic myeloid leukemia in mice (Mukherjee et al. 2017), and similar findings have been demonstrated with loss of GADD45B as well (Sha et al. 2018). Increased expression of lncRNA NEAT1 and binding to BRG1 are associated with decreased

GADD45A expression and reduced survival for gastric cancer in mice (Ma et al. 2020). Sustained ERK1/2 signaling in an acute myeloid leukemia model cell line downregulates *GADD45A*, and the reintroduction of expression induces S phase arrest and apoptosis (Cretu et al. 2009). Simultaneous H-RAS overexpression and *GADD45A* knockout are sufficient to transform cells, indicating that *GADD45A* knockout can function as one of the "two hits" in oncogenic transformation (Bulavin et al. 2003).

GADD45 has been shown to play a role in the inhibition of angiogenesis, which is an important component of tumorigenesis. GADD45A is central to suppression of tumor angiogenesis by blocking the mTOR/STAT3 pathway. Lack of GADD45A increases STAT3 phosphorylation at Ser727 and elevates STAT3 transcriptional activity. This process induces the expression and secretion of vascular endothelial growth factor (VEGF-A) and promotes formation of tumor blood vessels. Moreover, GADD45A can interact with mTOR and suppress STAT3 phosphorylation, leading to downregulated expression of VEGF-A (Yang et al. 2013).

1.5.1 GADD45 Expression in Clinical Studies

Aberrant GADD45 expression has been found in an increasing number of clinical studies. These findings are summarized in Table 1.2 and in the text below. The *GADD45A* promoter is methylated in a majority of breast cancers and a significant fraction of prostate cancers, whereas the *GADD45G* promoter is likewise hypermethylated in several human hepatocellular carcinomas, in both cases with subsequent downregulation of expression (Cretu et al. 2009). However, the pregnane X receptor can activate GADD45B/p38 MAPK signaling to induce a change in morphology and migration in a hepatocellular carcinoma cell line (Kodama and Negishi 2011). Increased GADD45A expression is associated with improved prognosis in patients with ovarian cancer (Yuan et al. 2015). Decreased expression of GADD45A and GADD45G is associated with worse prognosis in patients with gastric cardia adenocarcinoma (Guo et al. 2013a). Loss of GADD45A in acute myeloid leukemia (Wang et al. 2012; Perugini et al. 2013) similarly carries a worse prognosis. Increased GADD45B expression is associated with worse prognosis in

Table 1.2 Examples of aberrant GADD45 protein expression in various human cancers. Based on mouse model studies, reduced expression (↓) of GADD45 proteins in human cancers would be expected, but there are also a limited number of examples where increased expression (↑) has also been found

Tumor type	Expression	Prognosis
Breast	↓ GADD45A	Worse
Prostate	↓ GADD45A	Worse
Hepatocellular carcinoma	↓ GADD45G	Worse
Ovarian	↑ GADD45A	Improved
Gastric cardia adenocarcinoma	↓ GADD45A+G	Worse
Acute myeloid leukemia	↓ GADD45A	Worse
Papillary thyroid carcinoma	↑ GADD45B	Worse
Colorectal	↑ GADD45B, ↓ GADD45G	Worse
Esophageal squamous carcinoma	↓ GADD45A&G	Worse
Pancreatic	↑ GADD45A, ↓ GADD45G	Worse
Thyroid	↑ GADD45A	Worse
Lung	↓ GADD45G	Worse
Lymphoma	↓ GADD45G	Worse
Nasopharyngeal carcinoma	↓ GADD45G	Worse
Cervical carcinoma	↓ GADD45G	Worse
Pituitary adenoma	↓ GADD45G	Worse

patients with papillary thyroid carcinoma (Barros-Filho et al. 2020) and colorectal cancer (Wang et al. 2012; Zhao et al. 2018). Decreased expression of GADD45A and GADD45G is associated with worse prognosis in patients with esophageal squamous cell carcinoma (ESCC) (Ishiguro et al. 2016; Guo et al. 2013b). More recently, GADD45G has been suggested to inhibit ESCC migration and invasion through its interactions with E-cadherin (Li et al. 2020).

Although GADD45 has clear tumor suppressor features, it might also offer pro-growth advantages to certain malignant cells, in line with its roles in cell growth arrest and DNA repair. In one study, point mutations were found in exon four of the *GADD45A* gene in 14% of pancreatic cancer samples, and GADD45A expression in p53-positive tumors was associated with a lower patient survival rate (Yamasawa et al. 2002). GADD45A induction can protect melanoma cells from UV radiation-induced death (Jean et al. 2001). Lack of GADD45A induction in cervical carcinomas correlates with a good clinical response to radiotherapy (Gao et al. 2009). In addition, despite decreased FOXO3A transcriptional activity, GADD45A expression is upregulated in thyroid cancers (Karger et al. 2009).

In cancer, given the higher reported rate of promoter hypermethylation or upregulation of GADD45-repressed transcription of a multitude of different proteins, multiple *GADD45* functions could be important as alteration of a single function might be insufficient to induce or intensify the tumor phenotype. GADD45G is also deficient in several tumors. Its gene promoter region is hypermethylated and its transcription is repressed in a significant number of non-small cell lung cancers (Na et al. 2010), lymphomas, nasopharyngeal carcinomas, cervical carcinomas, esophageal carcinomas, pituitary adenomas, and gastric, colorectal, and pancreatic cancers (Yang et al. 2009; Zhang et al. 2010); however, genetic mutation and inactivation are rare. Exogenous reintroduction of GADD45G results in G_2/M arrest in a number of tumor cell lines, including prostate carcinoma and pituitary adenoma (Yang et al. 2009).

References

Alam MS, Gaida MM, Bergmann F, Lasitschka F, Giese T, Giese NA, Hackert T, Hinz U, Hussain SP, Kozlov SV, Ashwell JD (2015) Selective inhibition of the p38 alternative activation pathway in infiltrating T cells inhibits pancreatic cancer progression. Nat Med 21:1337–1343. https://doi.org/10.1038/nm.3957

Amente S, Zhang J, Lavadera ML, Lania L, Avvedimento EV, Majello B (2011) Myc and PI3K/AKT signaling cooperatively repress FOXO3a-dependent PUMA and GADD45a gene expression. Nucleic Acids Res 39:9498–9507. https://doi.org/10.1093/nar/gkr638

Amundson SA, Do KT, Vinikoor L, Koch-Paiz CA, Bittner ML, Trent JM, Meltzer P, Fornace AJ (2005) Stress-specific signatures: expression profiling of p53 wild-type and -null human cells. Oncogene 24:4572–4579. https://doi.org/10.1038/sj.onc.1208653

Arab K, Karaulanov E, Musheev M, Trnka P, Schäfer A, Grummt I, Niehrs C (2019) GADD45A binds R-loops and recruits TET1 to CpG island promoters. Nat Genet 51:217–223. https://doi.org/10.1038/s41588-018-0306-6

Ashwell JD (2006) The many paths to p38 mitogen-activated protein kinase activation in the immune system. Nat Rev Immunol 6:532–540. https://doi.org/10.1038/nri1865

Barreto G, Schäfer A, Marhold J, Stach D, Swaminathan SK, Handa V, Döderlein G, Maltry N, Wu W, Lyko F, Niehrs C (2007) Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature 445:671–675. https://doi.org/10.1038/nature05515

Barros-Filho MC, de Mello JBH, Marchi FA, Pinto CAL, da Silva IC, Damasceno PKF, Soares MBP, Kowalski LP, Rogatto SR (2020) GADD45B transcript is a prognostic marker in papillary thyroid carcinoma patients treated with total thyroidectomy and radioiodine therapy. Front Endocrinol (Lausanne) 11:269. https://doi.org/10.3389/fendo.2020.00269

Brown-Clay JD, Fornace AJ Jr (2018) Gadd45. In: Choi S (ed) Encyclopedia of signaling molecules, 2nd edn. Springer, Cham

Bulavin DV, Fornace AJ (2004) p38 MAP kinase's emerging role as a tumor suppressor. Adv Cancer Res 92:95–118. https://doi.org/10.1016/S0065-230X(04)92005-2

Bulavin DV, Saito S, Hollander MC, Sakaguchi K, Anderson CW, Appella E, Fornace AJ (1999) Phosphorylation of human p53 by p38 kinase coordinates N-terminal phosphorylation and apoptosis in response to UV radiation. EMBO J 18:6845–6854. https://doi.org/10.1093/emboj/18.23.6845

Bulavin DV, Kovalsky O, Hollander MC, Fornace AJ (2003) Loss of oncogenic H-ras-induced cell cycle arrest and p38 mitogen-activated protein kinase activation by disruption of Gadd45a. Mol Cell Biol 23:3859–3871. https://doi.org/10.1128/mcb.23.11.3859-3871.2003

Carrier F, Georgel PT, Pourquier P, Blake M, Kontny HU, Antinore MJ, Gariboldi M, Myers TG, Weinstein JN, Pommier Y, Fornace AJ (1999) Gadd45, a p53-responsive stress protein, modifies DNA accessibility on damaged chromatin. Mol Cell Biol 19:1673–1685. https://doi.org/10.1128/mcb.19.3.1673

Chang Q, Bhatia D, Zhang Y, Meighan T, Castranova V, Shi X, Chen F (2007) Incorporation of an internal ribosome entry site-dependent mechanism in arsenic-induced GADD45 alpha expression. Cancer Res 67:6146–6154. https://doi.org/10.1158/0008-5472.CAN-07-0867

Chen K, Long Q, Wang T, Zhao D, Zhou Y, Qi J, Wu Y, Li S, Chen C, Zeng X, Yang J, Zhou Z, Qin W, Liu X, Li Y, Li Y, Huang X, Qin D, Chen J, Pan G, Schöler HR, Xu G, Liu X, Pei D (2016) Gadd45a is a heterochromatin relaxer that enhances iPS cell generation. EMBO Rep 17:1641–1656. https://doi.org/10.15252/embr.201642402

Chepelev NL, Moffat ID, Labib S, Bourdon-Lacombe J, Kuo B, Buick JK, Lemieux F, Malik AI, Halappanavar S, Williams A, Yauk CL (2015) Integrating toxicogenomics into human health risk assessment: lessons learned from the benzo[a]pyrene case study. Crit Rev Toxicol 45:44–52. https://doi.org/10.3109/10408444.2014.973935

Cho HJ, Park SM, Hwang EM, Baek KE, Kim IK, Nam IK, Im MJ, Park SH, Bae S, Park JY, Yoo J (2010) Gadd45b mediates Fas-induced apoptosis by enhancing the interaction between p38 and retinoblastoma tumor suppressor. J Biol Chem 285:25500–25505. https://doi.org/10.1074/jbc.M109.091413

Cho E, Buick JK, Williams A, Chen R, Li HH, Corton JC, Fornace AJ, Aubrecht J, Yauk CL (2019a) Assessment of the performance of the TGx-DDI biomarker to detect DNA damage-inducing agents using quantitative RT-PCR in TK6 cells. Environ Mol Mutagen 60:122–133. https://doi.org/10.1002/em.22257

Cho CH, Byun HR, Jover-Mengual T, Pontarelli F, Dejesus C, Cho AR, Zukin RS, Hwang JY (2019b) Gadd45b acts as neuroprotective effector in global ischemia-induced neuronal death. Int Neurourol J 23:S11–S21. https://doi.org/10.5213/inj.1938040.020

Cretu A, Sha X, Tront J, Hoffman B, Liebermann DA (2009) Stress sensor Gadd45 genes as therapeutic targets in cancer. Cancer Ther 7:268–276

Cui Y, Paules RS (2010) Use of transcriptomics in understanding mechanisms of drug-induced toxicity. Pharmacogenomics 11:573–585. https://doi.org/10.2217/pgs.10.37

Ellinger-Ziegelbauer H, Aubrecht J, Kleinjans JC, Ahr HJ (2009) Application of toxicogenomics to study mechanisms of genotoxicity and carcinogenicity. Toxicol Lett 186:36–44. https://doi.org/10.1016/j.toxlet.2008.08.017

Fornace AJ, Alamo I, Hollander MC (1988) DNA damage-inducible transcripts in mammalian cells. Proc Natl Acad Sci U S A 85:8800–8804. https://doi.org/10.1073/pnas.85.23.8800

Fornace AJ, Nebert DW, Hollander MC, Luethy JD, Papathanasiou M, Fargnoli J, Holbrook NJ (1989) Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. Mol Cell Biol 9:4196–4203. https://doi.org/10.1128/mcb.9.10.4196

Friedberg EC (2006) DNA repair and mutagenesis. ASM Press, Washington, DC

Gao M, Guo N, Huang C, Song L (2009) Diverse roles of GADD45alpha in stress signaling. Curr Protein Pept Sci 10:388–394. https://doi.org/10.2174/138920309788922216

Gao M, Li X, Dong W, Jin R, Ma H, Yang P, Hu M, Li Y, Hao Y, Yuan S, Huang J, Song L (2013) Ribosomal protein S7 regulates arsenite-induced GADD45α expression by attenuating MDM2-mediated GADD45α ubiquitination and degradation. Nucleic Acids Res 41:5210–5222. https://doi.org/10.1093/nar/gkt223

Gierl MS, Gruhn WH, von Seggern A, Maltry N, Niehrs C (2012) GADD45G functions in male sex determination by promoting p38 signaling and Sry expression. Dev Cell 23:1032–1042. https://doi.org/10.1016/j.devcel.2012.09.014

Goodsaid FM, Amur S, Aubrecht J et al (2010) Voluntary exploratory data submissions to the US FDA and the EMA: experience and impact. Nat Rev Drug Discov 9:435–445. https://doi.org/10.1038/nrd3116

Guo W, Dong Z, Guo Y, Chen Z, Kuang G, Yang Z (2013a) Methylation-mediated repression of GADD45A and GADD45G expression in gastric cardia adenocarcinoma. Int J Cancer 133:2043–2053. https://doi.org/10.1002/ijc.28223

Guo W, Zhu T, Dong Z, Cui L, Zhang M, Kuang G (2013b) Decreased expression and aberrant methylation of Gadd45G is associated with tumor progression and poor prognosis in esophageal squamous cell carcinoma. Clin Exp Metastasis 30:977–992. https://doi.org/10.1007/s10585-013-9597-2

Harkin DP, Bean JM, Miklos D, Song YH, Truong VB, Englert C, Christians FC, Ellisen LW, Maheswaran S, Oliner JD, Haber DA (1999) Induction of GADD45 and JNK/SAPK-dependent apoptosis following inducible expression of BRCA1. Cell 97:575–586. https://doi.org/10.1016/s0092-8674(00)80769-2

Hartman AR, Ford JM (2002) BRCA1 induces DNA damage recognition factors and enhances nucleotide excision repair. Nat Genet 32:180–184. https://doi.org/10.1038/ng953

Hastwell PW, Chai LL, Roberts KJ, Webster TW, Harvey JS, Rees RW, Walmsley RM (2006) High-specificity and high-sensitivity genotoxicity assessment in a human cell line: validation of the GreenScreen HC GADD45a-GFP genotoxicity assay. Mutat Res 607:160–175. https://doi.org/10.1016/j.mrgentox.2006.04.011

Herwig R, Gmuender H, Corvi R, Bloch KM, Brandenburg A, Castell J, Ceelen L, Chesne C, Doktorova TY, Jennen D, Jennings P, Limonciel A, Lock EA, McMorrow T, Phrakonkham P, Radford R, Slattery C, Stierum R, Vilardell M, Wittenberger T, Yildirimman R, Ryan M,

Rogiers V, Kleinjans J (2016) Inter-laboratory study of human in vitro toxicogenomics-based tests as alternative methods for evaluating chemical carcinogenicity: a bioinformatics perspective. Arch Toxicol 90:2215–2229. https://doi.org/10.1007/s00204-015-1617-3

Hildesheim J, Fornace AJ (2004) The dark side of light: the damaging effects of UV rays and the protective efforts of MAP kinase signaling in the epidermis. DNA Repair (Amst) 3:567–580. https://doi.org/10.1016/j.dnarep.2004.02.012

Hildesheim J, Bulavin DV, Anver MR, Alvord WG, Hollander MC, Vardanian L, Fornace AJ (2002) Gadd45a protects against UV irradiation-induced skin tumors, and promotes apoptosis and stress signaling via MAPK and p53. Cancer Res 62:7305–7315

Hildesheim J, Belova GI, Tyner SD, Zhou X, Vardanian L, Fornace AJ (2004) Gadd45a regulates matrix metalloproteinases by suppressing DeltaNp63alpha and beta-catenin via p38 MAP kinase and APC complex activation. Oncogene 23:1829–1837. https://doi.org/10.1038/sj.onc.1207301

Hildesheim J, Salvador JM, Hollander MC, Fornace AJ (2005) Casein kinase 2- and protein kinase A-regulated adenomatous polyposis coli and beta-catenin cellular localization is dependent on p38 MAPK. J Biol Chem 280:17221–17226. https://doi.org/10.1074/jbc.M410440200

Hollander MC, Fornace AJ (2002) Genomic instability, centrosome amplification, cell cycle checkpoints and Gadd45a. Oncogene 21:6228–6233. https://doi.org/10.1038/sj.onc.1205774

Hollander MC, Sheikh MS, Bulavin DV, Lundgren K, Augeri-Henmueller L, Shehee R, Molinaro TA, Kim KE, Tolosa E, Ashwell JD, Rosenberg MP, Zhan Q, Fernández-Salguero PM, Morgan WF, Deng CX, Fornace AJ (1999) Genomic instability in Gadd45a-deficient mice. Nat Genet 23:176–184. https://doi.org/10.1038/13802

Hollander MC, Kovalsky O, Salvador JM, Kim KE, Patterson AD, Hairnes DC, Fornace AJ Jr (2001) DMBA carcinogenesis in Gadd45a-null mice is associated with decreased DNA repair and increased mutation frequency. Cancer Res 61:2487–2491

Hollander MC, Philburn RT, Patterson AD, Wyatt MA, Fornace AJ (2005a) Genomic instability in Gadd45a-/- cells is coupled with S-phase checkpoint defects. Cell Cycle 4:704–709. https://doi.org/10.4161/cc.4.5.1675

Hollander MC, Philburn RT, Patterson AD, Velasco-Miguel S, Friedberg EC, Linnoila RI, Fornace AJ (2005b) Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. Proc Natl Acad Sci U S A 102:13200–13205. https://doi.org/10.1073/pnas.0503133102

Husain A, He G, Venkatraman ES, Spriggs DR (1998) BRCA1 up-regulation is associated with repair-mediated resistance to cis-diamminedichloroplatinum(II). Cancer Res 58:1120–1123

Ijiri K, Zerbini LF, Peng H, Correa RG, Lu B, Walsh N, Zhao Y, Taniguchi N, Huang XL, Otu H, Wang H, Wang JF, Komiya S, Ducy P, Rahman MU, Flavell RA, Gravallese EM, Oettgen P, Libermann TA, Goldring MB (2005) A novel role for GADD45beta as a mediator of MMP-13 gene expression during chondrocyte terminal differentiation. J Biol Chem 280:38544–38555. https://doi.org/10.1074/jbc.M504202200

Ishiguro H, Kimura M, Takahashi H, Tanaka T, Mizoguchi K, Takeyama H (2016) GADD45A expression is correlated with patient prognosis in esophageal cancer. Oncol Lett 11:277–282. https://doi.org/10.3892/ol.2015.3882

Jackman J, Alamo I, Fornace AJ (1994) Genotoxic stress confers preferential and coordinate messenger RNA stability on the five gadd genes. Cancer Res 54:5656–5662

Jean S, Bideau C, Bellon L, Halimi G, De Méo M, Orsière T, Dumenil G, Bergé-Lefranc JL, Botta A (2001) The expression of genes induced in melanocytes by exposure to 365-nm UVA: study by cDNA arrays and real-time quantitative RT-PCR. Biochim Biophys Acta 1522:89–96. https://doi.org/10.1016/s0167-4781(01)00326-8

Jinawath N, Vasoontara C, Yap KL, Thiaville MM, Nakayama K, Wang TL, Shih IM (2009) NAC-1, a potential stem cell pluripotency factor, contributes to paclitaxel resistance in ovarian cancer through inactivating Gadd45 pathway. Oncogene 28:1941–1948. https://doi.org/10.1038/onc.2009.37

Johnen H, González-Silva L, Carramolino L, Flores JM, Torres M, Salvador JM (2013) Gadd45g is essential for primary sex determination, male fertility and testis development. PLoS One 8:e58751. https://doi.org/10.1371/journal.pone.0058751

Johnson D, Hastwell PW, Walmsley RM (2013) The involvement of WT1 in the regulation of GADD45a in response to genotoxic stress. Mutagenesis 28:393–399. https://doi.org/10.1093/mutage/get015

Ju S, Zhu Y, Liu L, Dai S, Li C, Chen E, He Y, Zhang X, Lu B (2009) Gadd45b and Gadd45g are important for anti-tumor immune responses. Eur J Immunol 39:3010–3018. https://doi.org/10.1002/eji.200839154

Jung N, Yi YW, Kim D, Shong M, Hong SS, Lee HS, Bae I (2000) Regulation of Gadd45gamma expression by C/EBP. Eur J Biochem 267:6180–6187

Jung HJ, Kim EH, Mun JY, Park S, Smith ML, Han SS, Seo YR (2007) Base excision DNA repair defect in Gadd45a-deficient cells. Oncogene 26:7517–7525. https://doi.org/10.1038/sj.onc.1210557

Karger S, Weidinger C, Krause K, Sheu SY, Aigner T, Gimm O, Schmid KW, Dralle H, Fuhrer D (2009) FOXO3a: a novel player in thyroid carcinogenesis. Endocr Relat Cancer 16:189–199. https://doi.org/10.1677/ERC-07-0283

Karin M (2014) Whipping NF-κB to submission via GADD45 and MKK7. Cancer Cell 26:447–449. https://doi.org/10.1016/j.ccell.2014.09.012

Kastan MB, Zhan Q, el-Deiry WS, Carrier F, Jacks T, Walsh WV, Plunkett BS, Vogelstein B, Fornace AJ (1992) A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in

ataxia-telangiectasia. Cell 71:587–597. https://doi.org/10.1016/0092-8674(92)90593-2

Kaufmann LT, Gierl MS, Niehrs C (2011) Gadd45a, Gadd45b and Gadd45g expression during mouse embryonic development. Gene Expr Patterns 11:465–470. https://doi.org/10.1016/j.gep.2011.07.005

Kearsey JM, Coates PJ, Prescott AR, Warbrick E, Hall PA (1995) Gadd45 is a nuclear cell cycle regulated protein which interacts with $p21^{Cip1}$. Oncogene 11:1675–1683

Kirkland D, Aardema M, Henderson L, Müller L (2005) Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens I. Sensitivity, specificity and relative predictivity. Mutat Res 584:1–256. https://doi.org/10.1016/j.mrgentox.2005.02.004

Kodama S, Negishi M (2011) Pregnane X receptor PXR activates the GADD45beta gene, eliciting the p38 MAPK signal and cell migration. J Biol Chem 286:3570–3578. https://doi.org/10.1074/jbc.M110.179812

Krewski D, Andersen ME, Tyshenko MG, Krishnan K, Hartung T, Boekelheide K, Wambaugh JF, Jones D, Whelan M, Thomas R, Yauk C, Barton-Maclaren T, Cote I (2020) Toxicity testing in the 21st century: progress in the past decade and future perspectives. Arch Toxicol 94:1–58. https://doi.org/10.1007/s00204-019-02613-4

Lal A, Gorospe M (2006) Egad, more forms of gene regulation: the gadd45a story. Cell Cycle 5:1422–1425. https://doi.org/10.4161/cc.5.13.2902

Lee HY, Youn SW, Kim JY, Park KW, Hwang CI, Park WY, Oh BH, Park YB, Walsh K, Seo JS, Kim HS (2008) FOXO3a turns the tumor necrosis factor receptor signaling towards apoptosis through reciprocal regulation of c-Jun N-terminal kinase and NF-kappaB. Arterioscler Thromb Vasc Biol 28:112–120. https://doi.org/10.1161/ATVBAHA.107.153304

Lee D, Hokinson D, Park S, Elvira R, Kusuma F, Lee JM, Yun M, Lee SG, Han J (2019) ER stress induces cell cycle arrest at the G2/M phase through eIF2α phosphorylation and GADD45α. Int J Mol Sci 20(24):6309. https://doi.org/10.3390/ijms20246309

Li S, Ting NS, Zheng L, Chen PL, Ziv Y, Shiloh Y, Lee EY, Lee WH (2000) Functional link of BRCA1 and ataxia telangiectasia gene product in DNA damage response. Nature 406:210–215. https://doi.org/10.1038/35018134

Li HH, Aubrecht J, Fornace AJ (2007) Toxicogenomics: overview and potential applications for the study of non-covalent DNA interacting chemicals. Mutat Res 623:98–108. https://doi.org/10.1016/j.mrfmmm.2007.03.013

Li HH, Hyduke DR, Chen R, Heard P, Yauk CL, Aubrecht J, Fornace AJ (2015) Development of a toxicogenomics signature for genotoxicity using a dose-optimization and informatics strategy in human cells. Environ Mol Mutagen 56:505–519. https://doi.org/10.1002/em.21941

Li HH, Chen R, Hyduke DR, Williams A, Frötschl R, Ellinger-Ziegelbauer H, O'Lone R, Yauk CL, Aubrecht J, Fornace AJ (2017) Development and validation of a high-throughput transcriptomic biomarker to address 21st century genetic toxicology needs. Proc Natl Acad Sci U S A 114:E10881–E10889. https://doi.org/10.1073/pnas.1714109114

Li T, Xu L, Teng J, Ma Y, Liu W, Wang Y, Chi X, Shao S, Dong Y, Zhan Q, Liu X (2020) GADD45G interacts with E-cadherin to suppress the migration and invasion of esophageal squamous cell carcinoma. Dig Dis Sci 65:1032–1041. https://doi.org/10.1007/s10620-019-05836-8

Lin CR, Yang CH, Huang CE, Wu CH, Chen YS, Sheen-Chen SM, Huang HW, Chen KH (2011) GADD45A protects against cell death in dorsal root ganglion neurons following peripheral nerve injury. J Neurosci Res 89:689–699. https://doi.org/10.1002/jnr.22589

Liu B, Suyeoka G, Papa S, Franzoso G, Neufeld AH (2009) Growth arrest and DNA damage protein 45b (Gadd45b) protects retinal ganglion cells from injuries. Neurobiol Dis 33:104–110. https://doi.org/10.1016/j.nbd.2008.09.020

Liu Z, Huang R, Roberts R, Tong W (2019) Toxicogenomics: a 2020 vision. Trends Pharmacol Sci 40:92–103. https://doi.org/10.1016/j.tips.2018.12.001

Lu B (2006) The molecular mechanisms that control function and death of effector CD4+ T cells. Immunol Res 36:275–282. https://doi.org/10.1385/IR:36:1:275

Lucas A, Mialet-Perez J, Daviaud D, Parini A, Marber MS, Sicard P (2015) Gadd45γ regulates cardiomyocyte death and post-myocardial infarction left ventricular remodelling. Cardiovasc Res 108:254–267. https://doi.org/10.1093/cvr/cvv219

Luzy AP, Orsini N, Linget JM, Bouvier G (2013) Evaluation of the GADD45α-GFP GreenScreen HC assay for rapid and reliable in vitro early genotoxicity screening. J Appl Toxicol 33:1303–1315. https://doi.org/10.1002/jat.2793

Ma DK, Guo JU, Ming GL, Song H (2009) DNA excision repair proteins and Gadd45 as molecular players for active DNA demethylation. Cell Cycle 8:1526–1531. https://doi.org/10.4161/cc.8.10.8500

Ma P, Pan Y, Yang F, Fang Y, Liu W, Zhao C, Yu T, Xie M, Jing X, Wu X, Sun C, Li W, Xu T, Shu Y (2020) KLF5-modulated lncRNA NEAT1 contributes to tumorigenesis by acting as a scaffold for BRG1 to silence GADD45A in gastric cancer. Mol Ther Nucleic Acids 22:382–395. https://doi.org/10.1016/j.omtn.2020.09.003

Maekawa T, Sano Y, Shinagawa T, Rahman Z, Sakuma T, Nomura S, Licht JD, Ishii S (2008) ATF-2 controls transcription of Maspin and GADD45 alpha genes independently from p53 to suppress mammary tumors. Oncogene 27:1045–1054. https://doi.org/10.1038/sj.onc.1210727

Magimaidas A, Madireddi P, Maifrede S, Mukherjee K, Hoffman B, Liebermann DA (2016) Gadd45b deficiency promotes premature senescence and skin aging. Oncotarget 7:26935–26948. https://doi.org/10.18632/oncotarget.8854

Mita H, Tsutsui J, Takekawa M, Witten EA, Saito H (2002) Regulation of MTK1/MEKK4 kinase activity by its N-terminal autoinhibitory domain and GADD45 binding. Mol Cell Biol 22:4544–4555

Moffat I, Chepelev N, Labib S, Bourdon-Lacombe J, Kuo B, Buick JK, Lemieux F, Williams A, Halappanavar S, Malik A, Luijten M, Aubrecht J, Hyduke DR, Fornace AJ, Swartz CD, Recio L, Yauk CL (2015) Comparison of toxicogenomics and traditional approaches to inform mode of action and points of departure in human health risk assessment of benzo[a]pyrene in drinking water. Crit Rev Toxicol 45:1–43. https://doi.org/10.3109/10408444.2014.973934

Mukherjee K, Sha X, Magimaidas A, Maifrede S, Skorski T, Bhatia R, Hoffman B, Liebermann DA (2017) Gadd45a deficiency accelerates BCR-ABL driven chronic myelogenous leukemia. Oncotarget 8:10809–10821. https://doi.org/10.18632/oncotarget.14580

Na YK, Lee SM, Hong HS, Kim JB, Park JY, Kim DS (2010) Hypermethylation of growth arrest DNA-damage-inducible gene 45 in non-small cell lung cancer and its relationship with clinicopathologic features. Mol Cells 30:89–92. https://doi.org/10.1007/s10059-010-0092-1

Notas G, Alexaki VI, Kampa M, Pelekanou V, Charalampopoulos I, Sabour-Alaoui S, Pediaditakis I, Dessirier V, Gravanis A, Stathopoulos EN, Tsapis A, Castanas E (2012) APRIL binding to BCMA activates a JNK2-FOXO3-GADD45 pathway and induces a G2/M cell growth arrest in liver cells. J Immunol 189:4748–4758. https://doi.org/10.4049/jimmunol.1102891

Papa S, Zazzeroni F, Fu YX, Bubici C, Alvarez K, Dean K, Christiansen PA, Anders RA, Franzoso G (2008) Gadd45beta promotes hepatocyte survival during liver regeneration in mice by modulating JNK signaling. J Clin Invest 118:1911–1923. https://doi.org/10.1172/JCI33913

Papathanasiou MA, Kerr NC, Robbins JH, McBride OW, Alamo I, Barrett SF, Hickson ID, Fornace AJ (1991) Induction by ionizing radiation of the gadd45 gene in cultured human cells: lack of mediation by protein kinase C. Mol Cell Biol 11:1009–1016. https://doi.org/10.1128/mcb.11.2.1009

Park MA, Seok YJ, Jeong G, Lee JS (2008) SUMO1 negatively regulates BRCA1-mediated transcription, via modulation of promoter occupancy. Nucleic Acids Res 36:263–283. https://doi.org/10.1093/nar/gkm969

Paruthiyil S, Cvoro A, Tagliaferri M, Cohen I, Shtivelman E, Leitman DC (2011) Estrogen receptor β causes a G2 cell cycle arrest by inhibiting CDK1 activity through the regulation of cyclin B1, GADD45A, and BTG2. Breast Cancer Res Treat 129:777–784. https://doi.org/10.1007/s10549-010-1273-5

Passos JF, Nelson G, Wang C, Richter T, Simillion C, Proctor CJ, Miwa S, Olijslagers S, Hallinan J, Wipat A, Saretzki G, Rudolph KL, Kirkwood TB, von Zglinicki T (2010) Feedback between p21 and reactive oxygen production is necessary for cell senescence. Mol Syst Biol 6:347. https://doi.org/10.1038/msb.2010.5

Perugini M, Iarossi DG, Kok CH, Cummings N, Diakiw SM, Brown AL, Danner S, Bardy P, Bik To L, Wei AH, Lewis ID, D'Andrea RJ (2013) GADD45A methylation predicts poor overall survival in acute myeloid leukemia and is associated with IDH1/2 and DNMT3A mutations. Leukemia 27:1588–1592. https://doi.org/10.1038/leu.2012.346

Pietrasik S, Zajac G, Morawiec J, Soszynski M, Fila M, Blasiak J (2020) Interplay between BRCA1 and GADD45A and its potential for nucleotide excision repair in breast cancer pathogenesis. Int J Mol Sci 21(3):870. https://doi.org/10.3390/ijms21030870

Reinhardt HC, Hasskamp P, Schmedding I, Morandell S, van Vugt MA, Wang X, Linding R, Ong SE, Weaver D, Carr SA, Yaffe MB (2010) DNA damage activates a spatially distinct late cytoplasmic cell-cycle checkpoint network controlled by MK2-mediated RNA stabilization. Mol Cell 40:34–49. https://doi.org/10.1016/j.molcel.2010.09.018

Röckner G, Wahlberg V, Olund A (1989) Episiotomy and perineal trauma during childbirth. J Adv Nurs 14:264–268. https://doi.org/10.1111/j.1365-2648.1989.tb03412.x

Salvador JM, Mittelstadt PR, Guszczynski T, Copeland TD, Yamaguchi H, Appella E, Fornace AJ, Ashwell JD (2005a) Alternative p38 activation pathway mediated by T cell receptor-proximal tyrosine kinases. Nat Immunol 6:390–395. https://doi.org/10.1038/ni1177

Salvador JM, Mittelstadt PR, Belova GI, Fornace AJ, Ashwell JD (2005b) The autoimmune suppressor Gadd45alpha inhibits the T cell alternative p38 activation pathway. Nat Immunol 6:396–402. https://doi.org/10.1038/ni1176

Schmitz KM, Schmitt N, Hoffmann-Rohrer U, Schäfer A, Grummt I, Mayer C (2009) TAF12 recruits Gadd45a and the nucleotide excision repair complex to the promoter of rRNA genes leading to active DNA demethylation. Mol Cell 33:344–353. https://doi.org/10.1016/j.molcel.2009.01.015

Selvakumaran M, Lin HK, Sjin RT, Reed JC, Liebermann DA, Hoffman B (1994) The novel primary response gene MyD118 and the proto-oncogenes myb, myc, and bcl-2 modulate transforming growth factor beta 1-induced apoptosis of myeloid leukemia cells. Mol Cell Biol 14:2352–2360. https://doi.org/10.1128/mcb.14.4.2352

Sen GL, Reuter JA, Webster DE, Zhu L, Khavari PA (2010) DNMT1 maintains progenitor function in self-renewing somatic tissue. Nature 463:563–567. https://doi.org/10.1038/nature08683

Sengupta A, Molkentin JD, Paik JH, DePinho RA, Yutzey KE (2011) FoxO transcription factors promote cardiomyocyte survival upon induction of oxidative stress. J Biol Chem 286:7468–7478. https://doi.org/10.1074/jbc.M110.179242

Sha X, Hoffman B, Liebermann DA (2018) Loss of Gadd45b accelerates BCR-ABL-driven CML. Oncotarget 9:33360–33367. https://doi.org/10.18632/oncotarget.26076

Shao S, Wang Y, Jin S, Song Y, Wang X, Fan W, Zhao Z, Fu M, Tong T, Dong L, Fan F, Xu N, Zhan Q (2006) Gadd45a interacts with aurora-A and inhibits its kinase activity. J Biol Chem 281:28943–28950. https://doi.org/10.1074/jbc.M600235200

Simpson K, Bevan N, Hastwell P, Eidam P, Shah P, Gogo E, Rees S, Brown A (2013) The BlueScreen-384 assay as an indicator of genotoxic hazard potential in early-stage drug discovery. J Biomol Screen 18:441–452. https://doi.org/10.1177/1087057112470858

Smith ML, Chen IT, Zhan Q, Bae I, Chen CY, Gilmer TM, Kastan MB, O'Connor PM, Fornace AJ (1994) Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. Science 266:1376–1380. https://doi.org/10.1126/science.7973727

Smith ML, Ford JM, Hollander MC, Bortnick RA, Amundson SA, Seo YR, Deng CX, Hanawalt PC, Fornace AJ (2000) p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking p53, p21, and/or gadd45 genes. Mol Cell Biol 20:3705–3714. https://doi.org/10.1128/mcb.20.10.3705-3714.2000

Snyder RD, Green JW (2001) A review of the genotoxicity of marketed pharmaceuticals. Mutat Res 488:151–169. https://doi.org/10.1016/s1383-5742(01)00055-2

Snyder AR, Morgan WF (2004) Gene expression profiling after irradiation: clues to understanding acute and persistent responses. Cancer Metastasis Rev 23:259–268. https://doi.org/10.1023/B:CANC.0000031765.17886.fa

Song L, Li J, Zhang D, Liu ZG, Ye J, Zhan Q, Shen HM, Whiteman M, Huang C (2006) IKKbeta programs to turn on the GADD45alpha-MKK4-JNK apoptotic cascade specifically via p50 NF-kappaB in arsenite response. J Cell Biol 175:607–617. https://doi.org/10.1083/jcb.200602149

Takahashi S, Saito S, Ohtani N, Sakai T (2001) Involvement of the Oct-1 regulatory element of the gadd45 promoter in the p53-independent response to ultraviolet irradiation. Cancer Res 61:1187–1195

Takekawa M, Saito H (1998) A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. Cell 95:521–530. https://doi.org/10.1016/s0092-8674(00)81619-0

Todd MD, Lee MJ, Williams JL, Nalezny JM, Gee P, Benjamin MB, Farr SB (1995) The CAT-Tox (L) assay: a sensitive and specific measure of stress-induced transcription in transformed human liver cells. Fundam Appl Toxicol 28:118–128. https://doi.org/10.1006/faat.1995.1153

Tong T, Ji J, Jin S, Li X, Fan W, Song Y, Wang M, Liu Z, Wu M, Zhan Q (2005) Gadd45a expression induces Bim dissociation from the cytoskeleton and translocation to mitochondria. Mol Cell Biol 25:4488–4500. https://doi.org/10.1128/MCB.25.11.4488-4500.2005

Tornatore L, Sandomenico A, Raimondo D, Low C, Rocci A, Tralau-Stewart C, Capece D, D'Andrea D, Bua M, Boyle E, van Duin M, Zoppoli P, Jaxa-Chamiec A, Thotakura AK, Dyson J, Walker BA, Leonardi A, Chambery A, Driessen C, Sonneveld P, Morgan G, Palumbo A, Tramontano A, Rahemtulla A, Ruvo M, Franzoso G (2014a) Cancer-selective targeting of the NF-κB survival pathway with GADD45β/MKK7 inhibitors. Cancer Cell 26:495–508. https://doi.org/10.1016/j.ccr.2014.07.027

Tornatore L, Sandomenico A, Raimondo D, Low C, Rocci A, Tralau-Stewart C, Capece D, D'Andrea D, Bua M, Boyle E, van Duin M, Zoppoli P, Jaxa-Chamiec A, Thotakura AK, Dyson J, Walker BA, Leonardi A, Chambery A, Driessen C, Sonneveld P, Morgan G, Palumbo A, Tramontano A, Rahemtulla A, Ruvo M, Franzoso G (2014b) Cancer-selective targeting of the NF-κB survival pathway with GADD45β/MKK7 inhibitors. Cancer Cell 26:938. https://doi.org/10.1016/j.ccell.2014.11.021

Tran H, Brunet A, Grenier JM, Datta SR, Fornace AJ, DiStefano PS, Chiang LW, Greenberg ME (2002) DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. Science 296:530–534. https://doi.org/10.1126/science.1068712

Tront JS, Willis A, Huang Y, Hoffman B, Liebermann DA (2013) Gadd45a levels in human breast cancer are hormone receptor dependent. J Transl Med 11:131. https://doi.org/10.1186/1479-5876-11-131

Vairapandi M, Balliet AG, Hoffman B, Liebermann DA (2002) GADD45b and GADD45g are cdc2/cyclinB1 kinase inhibitors with a role in S and G2/M cell cycle checkpoints induced by genotoxic stress. J Cell Physiol 192:327–338. https://doi.org/10.1002/jcp.10140

Vinayagam A, Stelzl U, Foulle R, Plassmann S, Zenkner M, Timm J, Assmus HE, Andrade-Navarro MA, Wanker EE (2011) A directed protein interaction network for investigating intracellular signal transduction. Sci Signal 4:rs8. https://doi.org/10.1126/scisignal.2001699

Walmsley RM, Tate M (2012) The GADD45a-GFP GreenScreen HC assay. Methods Mol Biol 817:231–250. https://doi.org/10.1007/978-1-61779-421-6_12

Wan X, Yao B, Ma Y, Liu Y, Tang Y, Hu J, Li M, Fu S, Zheng X, Yin D (2020) MicroRNA-128-1-5p attenuates myocardial ischemia/reperfusion injury by suppressing Gadd45g-mediated apoptotic signaling. Biochem Biophys Res Commun 530:314–321. https://doi.org/10.1016/j.bbrc.2020.07.009

Wang XW, Zhan Q, Coursen JD, Khan MA, Kontny HU, Yu L, Hollander MC, O'Connor PM, Fornace AJ, Harris CC (1999) GADD45 induction of a G2/M cell cycle checkpoint. Proc Natl Acad Sci U S A 96:3706–3711. https://doi.org/10.1073/pnas.96.7.3706

Wang X, Wang RH, Li W, Xu X, Hollander MC, Fornace AJ, Deng CX (2004) Genetic interactions between Brca1 and Gadd45a in centrosome duplication, genetic stability, and neural tube closure. J Biol Chem 279:29606–29614. https://doi.org/10.1074/jbc.M312279200

Wang L, Xiao X, Li D, Chi Y, Wei P, Wang Y, Ni S, Tan C, Zhou X, Du X (2012) Abnormal expression of GADD45B in human colorectal carcinoma. J Transl Med 10:215. https://doi.org/10.1186/1479-5876-10-215

Wang Y, Liu W, Yang Q, Yu M, Zhang Z (2015) Di (2-ethylhexyl) phthalate exposure during pregnancy disturbs temporal sex determination regulation in mice offspring. Toxicology 336:10–16. https://doi.org/10.1016/j.tox.2015.07.009

Wang H, Huang Z, Zhao X, Guo B, Ji Z (2020) miR-4270 regulates cell proliferation and apoptosis in patients with Sertoli cell-only syndrome by targeting GADD45A and inactivating the NOTCH signaling pathway. Am J Transl Res 12:5730–5740

Warr N, Carre GA, Siggers P, Faleato JV, Brixey R, Pope M, Bogani D, Childers M, Wells S, Scudamore CL, Tedesco M, del Barco Barrantes I, Nebreda AR, Trainor PA, Greenfield A (2012) Gadd45γ and Map3k4 interactions regulate mouse testis determination via p38 MAPK-mediated control of Sry expression. Dev Cell 23:1020–1031. https://doi.org/10.1016/j.devcel.2012.09.016

Weinstein JN, Myers TG, O'Connor PM, Friend SH, Fornace AJ, Kohn KW, Fojo T, Bates SE, Rubinstein LV, Anderson NL, Buolamwini JK, van Osdol WW, Monks AP, Scudiero DA, Sausville EA, Zaharevitz DW, Bunow B, Viswanadhan VN, Johnson GS, Wittes RE, Paull KD (1997) An information-intensive approach to the molecular pharmacology of cancer. Science 275:343–349. https://doi.org/10.1126/science.275.5298.343

Wingert S, Rieger MA (2016) Terminal differentiation induction as DNA damage response in hematopoietic stem cells by GADD45A. Exp Hematol 44:561–566. https://doi.org/10.1016/j.exphem.2016.04.006

Wingert S, Thalheimer FB, Haetscher N, Rehage M, Schroeder T, Rieger MA (2016) DNA-damage response gene GADD45A induces differentiation in hematopoietic stem cells without inhibiting cell cycle or survival. Stem Cells 34:699–710. https://doi.org/10.1002/stem.2282

Wu J, Lu LY, Yu X (2010) The role of BRCA1 in DNA damage response. Protein Cell 1:117–123. https://doi.org/10.1007/s13238-010-0010-5

Xin L, Wang J, Wu Y, Guo S (2015) The development of GADD45α luciferase reporter assays in human cells for assessing the genotoxicity of environmental pollutants. Toxicol Mech Methods 25:136–142. https://doi.org/10.3109/15376516.2014.1003357

Yamamoto Y, Moore R, Flavell RA, Lu B, Negishi M (2010) Nuclear receptor CAR represses TNFalpha-induced cell death by interacting with the anti-apoptotic GADD45B. PLoS One 5:e10121. https://doi.org/10.1371/journal.pone.0010121

Yamasawa K, Nio Y, Dong M, Yamaguchi K, Itakura M (2002) Clinicopathological significance of abnormalities in Gadd45 expression and its relationship to p53 in human pancreatic cancer. Clin Cancer Res 8:2563–2569

Yang Z, Song L, Huang C (2009) Gadd45 proteins as critical signal transducers linking NF-kappaB to MAPK cascades. Curr Cancer Drug Targets 9:915–930. https://doi.org/10.2174/156800909790192383

Yang F, Zhang W, Li D, Zhan Q (2013) Gadd45a suppresses tumor angiogenesis via inhibition of the mTOR/STAT3 protein pathway. J Biol Chem 288:6552–6560. https://doi.org/10.1074/jbc.M112.418335

Yang P, Ni F, Deng RQ, Qiang G, Zhao H, Yang MZ, Wang XY, Xu YZ, Chen L, Chen DL, Chen ZJ, Kan LX, Wang SY (2015) MiR-362-5p promotes the malignancy of chronic myelocytic leukaemia via down-regulation of GADD45α. Mol Cancer 14:190. https://doi.org/10.1186/s12943-015-0465-3

Yoo J, Ghiassi M, Jirmanova L, Balliet AG, Hoffman B, Fornace AJ, Liebermann DA, Bottinger EP, Roberts AB (2003) Transforming growth factor-beta-induced apoptosis is mediated by Smad-dependent expression of GADD45b through p38 activation. J Biol Chem 278:43001–43007. https://doi.org/10.1074/jbc.M307869200

You W, Xu Z, Shan T (2019) Regulatory roles of GADD45α in skeletal muscle and adipocyte. Curr Protein Pept Sci 20:918–925. https://doi.org/10.2174/1389203720666190624143503

Yuan C, Liu X, Liu X, Yang N, Liu Z, Yan S, Shen K, Kong B (2015) The GADD45A (1506T>C) polymorphism is associated with ovarian cancer susceptibility and prognosis. PLoS One 10:e0138692. https://doi.org/10.1371/journal.pone.0138692

Zerbini LF, Wang Y, Czibere A, Correa RG, Cho JY, Ijiri K, Wei W, Joseph M, Gu X, Grall F, Goldring MB, Zhou JR, Libermann TA, Zhou JR (2004) NF-kappa B-mediated repression of growth arrest- and DNA-damage-inducible proteins 45alpha and gamma is essential for cancer cell survival. Proc Natl Acad Sci U S A 101:13618–13623. https://doi.org/10.1073/pnas.0402069101

Zhan Q, Lord KA, Alamo I, Hollander MC, Carrier F, Ron D, Kohn KW, Hoffman B, Liebermann DA, Fornace AJ (1994) The gadd and MyD genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth. Mol Cell Biol 14:2361–2371. https://doi.org/10.1128/mcb.14.4.2361

Zhan Q, Chen IT, Antinore MJ, Fornace AJ (1998) Tumor suppressor p53 can participate in transcriptional induction of the GADD45 promoter in the absence of direct DNA binding. Mol Cell Biol 18:2768–2778. https://doi.org/10.1128/mcb.18.5.2768

Zhan Q, Antinore MJ, Wang XW, Carrier F, Smith ML, Harris CC, Fornace AJ (1999) Association with Cdc2 and inhibition of Cdc2/Cyclin B1 kinase activity by the p53-regulated protein Gadd45. Oncogene 18:2892–2900. https://doi.org/10.1038/sj.onc.1202667

Zhang W, Bae I, Krishnaraju K, Azam N, Fan W, Smith K, Hoffman B, Liebermann DA (1999) CR6: a third member in the MyD118 and Gadd45 gene family which functions in negative growth control. Oncogene 18:4899–4907. https://doi.org/10.1038/sj.onc.1202885

Zhang W, Li T, Shao Y, Zhang C, Wu Q, Yang H, Zhang J, Guan M, Yu B, Wan J (2010) Semi-quantitative detection of GADD45-gamma methylation levels in gastric, colorectal and pancreatic cancers using

methylation-sensitive high-resolution melting analysis. J Cancer Res Clin Oncol 136:1267–1273. https://doi.org/10.1007/s00432-010-0777-z

Zhang L, Yang Z, Liu Y (2014) GADD45 proteins: roles in cellular senescence and tumor development. Exp Biol Med (Maywood) 239:773–778. https://doi.org/10.1177/1535370214531879

Zhang D, Zhang W, Li D, Fu M, Chen R, Zhan Q (2015) GADD45A inhibits autophagy by regulating the interaction between BECN1 and PIK3C3. Autophagy 11:2247–2258. https://doi.org/10.1080/15548627.2015.1112484

Zhao Z, Gao Y, Guan X, Liu Z, Jiang Z, Liu X, Lin H, Yang M, Li C, Yang R, Zou S, Wang X (2018) GADD45B as a prognostic and predictive biomarker in stage II colorectal cancer. Genes (Basel) 9(7):361. https://doi.org/10.3390/genes9070361