

GADD45A: With or without you

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Abstract

The growth arrest and DNA damage inducible (GADD)45 family includes three small and ubiquitously distributed proteins (GADD45A, GADD45B, and GADD45G) that regulate numerous cellular processes associated with stress signaling and injury response. Here, we provide a comprehensive review of the current literature investigating GADD45A, the first discovered member of the family. We first depict how its levels are regulated by a myriad of genotoxic and non-genotoxic stressors, and through the combined action of intricate transcriptional, posttranscriptional, and even, post-translational mechanisms. GADD45A is a recognized tumor suppressor and, for this reason, we next summarize its role in cancer, as well as the different mechanisms by which it regulates cell cycle, DNA repair, and apoptosis. Beyond these

Abbreviations: AD, Alzheimer disease; AILI, acetaminophen-induced liver injury; AP-1, activator protein-1; APEX, DNA-(apurinic or apyrimidinic site) endonuclease; ATF4, activating transcription factor 4; AUF1, AU-rich element RNA-binding factor 1; BAX, BCL2 associated X protein; BRCA1, breast cancer type 1 susceptibility protein; C/EBP β , CCAAT/enhancer-binding protein β ; CDK1 (CDC2), cyclin-dependent kinase 1; CDKN1A (p21), cyclin dependent kinase inhibitor 1; CHOP, C/EBP homologous protein; circRNA, circular RNA; EF1, elongation factor 1; EGR1, early growth response protein 1; eIF2 α , eukaryotic translation initiation factor 2 α ; ER, endoplasmic reticulum; ERCC5, DNA excision repair protein (or xeroderma pigmentosum group G-complementing protein, XPG); ERK1/2, extracellular signal-regulated kinase 1/2; ER α and ER β , estrogen receptor α and β ; FOXO3a, forkhead box protein O3a; GADD45, growth arrest and DNA damage inducible 45; GSK3 β , glycogen synthase kinase 3 β ; IKK, I κ B (inhibitor of NF- κ B) kinase; IL, interleukin; JNK, JUN (c-Jun) N-terminal kinase; lncRNA, long noncoding RNA; MAPK, mitogen-activated protein kinase; MEK4, MEK Kinase 4 or MAPK kinase kinase 4; miRNA, microRNA; MKK7, MAPK kinase kinase MAP2K7; MMP, matrix metalloproteinase; NFAT, nuclear factor of activated T-cells; NFYA, nuclear transcription factor Y subunit α ; NF- κ B, nuclear factor- κ B; PCNA, proliferating cell nuclear antigen; PKB, Protein kinase B; POU2F1, POU domain, class 2, transcription factor 1; PPAR, peroxisome proliferator-activated receptor; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; STAT, signal transducer and activator of transcription; TAF12, transcription initiation factor TFIID subunit 12; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor α ; UCHL1, ubiquitin carboxyl terminal esterase L1; UPR, unfolded protein response; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells; WT1, Wilms tumor protein 1.

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most well-known actions, GADD45A may also influence catabolic and anabolic pathways in the liver, adipose tissue and skeletal muscle, among others. Not surprisingly, GADD45A may trigger AMP-activated protein kinase activity, a master regulator of metabolism, and is known to act as a transcriptional coregulator of numerous nuclear receptors. GADD45A has also been reported to display a cytoprotective role by regulating inflammation, fibrosis and oxidative stress in several organs and tissues, and is regarded an important contributor for the development of heart failure. Overall data point to that GADD45A may play an important role in metabolic, neurodegenerative and cardiovascular diseases, and also autoimmune-related disorders. Thus, the potential mechanisms by which dysregulation of GADD45A activity may contribute to the progression of these diseases are also reviewed below.

KEYWORDS

cancer, cell cycle control, cell metabolism, GADD45A, inflammation

1 | INTRODUCTION: THE GADD45 FAMILY, A BAND WITH THREE COMPONENTS

The growth arrest and DNA damage inducible (GADD)45 family includes three small and highly acidic proteins named GADD45A (also known as GADD45, GADD45 α or DNA damage-inducible transcript [DDIT]1 protein), GADD45B (GADD45 β or myeloid differentiation primary response 118, MYD118), and GADD45G (GADD45 γ , DDIT2, GADD-related protein 17 kDa, GRP17, or cytokine-responsive protein 6, CR6). These proteins are evolutionarily conserved and highly homologous to each other, sharing a 55-57% similarity at the amino acid level.¹ In humans, the *Gadd45* genes are located on three different chromosomes: 1p31.3 (*Gadd45a*), 19p13.3 (*Gadd45b*), and 9q22.2 (*Gadd45g*).² The first member described, *Gadd45a*, was initially identified as one of several genes whose mRNA levels were rapidly induced in Chinese hamster ovary cells by agents that cause DNA damage, such as UV radiation and X-ray irradiation.³ Later, *Gadd45b* was found to be a primary response gene transiently expressed in myeloid precursor cells following induction of growth arrest by interleukin (IL)-6 or lipopolysaccharide (LPS).⁴ Finally, *Gadd45g* was identified as an immediate early response gene in T cells stimulated by IL-2.⁵

The GADD45 family members are ubiquitously distributed in adult mammalian tissues, although they have distinctive expression patterns.¹ At the subcellular level, they are primarily localized within the cell nucleus, but may also be found in the cytoplasm. These proteins display low abundance in normal quiescent cells, peaking in the G1 phase of the cycle and decreasing during the S phase.⁶ In fact, their levels are regulated by numerous factors at the transcriptional, posttranscriptional, and posttranslational levels. Different transcriptional binding sites are located within the promoter region of the *Gadd45* genes, including early growth response protein 1 (EGR1), Wilms tumor protein 1 (WT1), POU domain, class 2, transcription factor 1 (POU2F1 or octamer-binding protein 1, OCT-1),

nuclear transcription factor Y subunit α (NFYA), and nuclear factor- κ B (NF- κ B).² *Gadd45a* also contains binding motifs for the p53 protein, activator protein-1 (AP-1), and nuclear factor of activated T-cells (NFAT).²

GADD45 proteins are involved in a variety of cellular processes frequently associated with stress signaling and response to cell injury.^{7–9} Indeed, they are rapidly induced in response to oncogenic stress, as well as by terminal differentiation and apoptotic cytokines, being able to modulate tumor formation. For example, GADD45B is the only member induced by the transforming growth factor (TGF)- β , whereas GADD45A is a selective p53 target.⁸ To operate as stress sensors, they must physical interact with other cellular proteins. For instance, all three members interact with the DNA replication and repair protein PCNA (proliferating cell nuclear antigen) and the cyclin dependent kinase inhibitor 1 (CDKN1A or p21).¹⁰ They also directly associate with, and inhibit, the cyclin-dependent kinase 1 (CDK1, also known as cell division control protein 2 homolog or CDC2), which plays a pivotal role, together with cyclin B1, in regulating the progression from G2 to M phase during the cell cycle.^{9–11} This interaction accounts for their role in nucleotide excision repair and in G1 and G2 arrest in response to DNA damage.¹⁰ GADD45 proteins also bind to nucleosome histones, thereby altering accessibility to DNA, particularly where chromatin is damaged, and enabling their role in DNA repair.⁹ In fact, they interact with other partners involved in DNA repair, including DNA-(apurinic or apyrimidinic site) endonuclease (APEX1), DNA excision repair protein ERCC5 (also known as xeroderma pigmentosum group G-complementing protein, XPG), and p53 itself.⁹

Mitogen-activated protein kinases (MAPK) are important regulators of cell proliferation, survival, and differentiation; the extracellular signal-regulated kinase (ERK)1/2 pathway is often associated with cell survival and proliferation, whereas JUN N-terminal kinase (JNK) and p38 MAPK are mostly activated by stimuli that lead to apoptosis or inhibit cell growth. Interestingly, GADD45 proteins associate with the MAPK kinase kinase 4 (MEKK4 or MAP3K4), which in turn activates both p38 and JNK to drive apoptosis and increase cytokine production in response to environmental stressors.⁷ In contrast, NF- κ B-mediated induction of GADD45B blunts JNK activation through its association with the MAPK kinase kinase MAP2K7 (or MKK7).⁷ Thus, GADD45 proteins may carry out antiapoptotic effects via opposing but complementary mechanisms: the activation of the antiapoptotic p38/NF- κ B pathway by GADD45A and the inhibition of the proapoptotic MKK7/JNK pathway by GADD45B.¹² Of note, both p38 and JNK are also able to regulate the transcription of *Gadd45* genes.⁹

GADD45 proteins perform similar but not identical roles, depending on the cell type and the particular stress response pathway that is specifically activated. They participate in cell cycle arrest and growth, DNA repair, and cell apoptosis, survival, and senescence, hence playing a critical role in the carcinogenic process. However, there are specific dissimilarities among them. Thus, GADD45A and GADD45G are known to induce cell growth arrest and apoptosis,¹³ but the role of GADD45B is still controversial and considered cell- and stimulus-specific. Although most data demonstrate a tumor suppressor role,^{14–17} other studies point to an antiapoptotic effect of GADD45B in hematopoietic cells,¹⁴ hepatocytes,¹⁸ or neurons.¹⁹ Further, and similar to GADD45A, GADD45B and GADD45G play pivotal roles in innate immune responses and in a variety of inflammatory diseases.^{20–23} Besides this, GADD45B can regulate angiogenesis, cell differentiation and migration, and oxidative stress, and it has recently been demonstrated that it modulates gluconeogenesis and lipid metabolism in the liver,^{24–27} and neuroplasticity and neuroprotection in the nervous system.²⁸ In contrast, GADD45G has been reported to participate in primary sex determination and male fertility,²⁹ and the development of heart failure.¹³ Despite their many functional similarities, this review will be focused on the first discovered and most well-characterized member of the family, GADD45A.

2 | GADD45A: THE MOST FAMOUS MEMBER OF THE BAND

2.1 | Regulation of GADD45A levels

Gadd45a is expressed in brain, heart, kidney, liver, skeletal muscle, spleen, and lung. A myriad of genotoxic and non-genotoxic stressors may induce its gene expression, including UV and γ radiation, X-rays, hypoxia, peroxynitrite free

radicals, low pH, xenobiotics (arsenic, hexavalent chromium compounds, cisplatin, ethanol, cigarette smoke), oncogenic stress, nutrient depletion, and many soil, air, and water pollutants.² *Gadd45a* expression varies significantly throughout the cell cycle; it is usually low at rest, but it increases significantly during the G1 phase and decreases during the S phase, displaying a pivotal role in both S-phase and G2/M arrest.³⁰ Following exposure to stress, *Gadd45a* expression is upregulated through the combined action of transcriptional and posttranscriptional derepression, transcription factors activation, and the recruitment of posttranscriptional inducers.³¹ To further complicate it, GADD45A is a short-lived protein that can also be induced at the posttranslational level, through a process that involves a reduction in its ubiquitination.³² In addition, the activity of GADD45A may be modulated by its nuclear translocation through the carrier protein nucleophosmin 1.³³

The signaling pathways responsible for regulating *Gadd45a* expression are intricate and involve several transcription factors that are differentially activated depending on the causative stress (Figure 1). Historically, increased *Gadd45a* gene expression has been mostly recognized as a downstream event of p53 activation, a tumor suppressor protein that plays a crucial role in protecting cells from malignant transformation and contributes to the maintenance of genomic stability.³⁴ Specifically, in response to many agents associated with DNA damage, apoptosis, cell cycle control, and cell injury, p53 stimulates *Gadd45a* transcription through the binding to a conserved site located within the third intron of its gene.^{9,35} Not surprisingly, *Gadd45a*-null mice exhibit a phenotype resembling that of p53-deficient mice, including genomic instability as a consequence of perturbations in G2/M cell cycle progression, and impaired DNA repair.³⁴ For this reason, GADD45A is regarded as the link between the p53-dependent DNA repair and cell cycle checkpoints, which are vital responses to ensure the maintenance of genomic fidelity in the event of DNA damage.³⁶ However, p53 can also contribute to increase *Gadd45a* levels without binding to its promoter, suggesting the occurrence of other mechanisms involving protein-protein interactions.³⁷ Additionally, several p53-independent pathways are also involved in the transcriptional regulation of *Gadd45a*. As with other family members, EGR1, forkhead box protein O3a (FOXO3a), NFYA, POU2F1, and the tumor suppressor WT1 are involved in its induction by directly binding to specific motifs within its promoter.^{33,35,38} WT1, in particular, positively regulates *Gadd45a* expression through both p53-dependent and p53-independent mechanisms in response to DNA damage or chromosome missegregation.³⁵ FOXO3a, a transcription factor activated in response to oxidative stress, directly induces *Gadd45a* at the same time it suppresses the expression of *Gadd45b*.⁹ FOXO3a is known for its ability to block the transition between G2/M cell cycle phases and to trigger the repair of damaged DNA, and this is probably due to a mechanism involving, at least in part, the FOXO3a-mediated increase in *Gadd45a*.^{2,39} Other growth-arrest-associated regulatory factors that may transcriptionally regulate *Gadd45a*, either directly or indirectly, include breast cancer type 1 susceptibility protein (BRCA1), CCAAT/enhancer-binding protein (C/EBP) β , and activating transcription factor 4 (ATF4).⁹ BRCA1 directly upregulates its transcription in a JUN- and FOS-independent manner, thus ruling out the participation of other immediate-responsive genes.^{2,40} ATF4, which also has a pivotal role in cellular stress response, enhances *Gadd45a* transcription following arsenite exposure, leucine starvation, or endoplasmic reticulum (ER) stress, by directly interacting with an ATF/CREB (cAMP response element binding protein)-related binding element within its promoter.⁹ Another activating transcription factor, ATF2, which is a p38 and JNK target involved in stress-induced apoptosis, may also indirectly stimulate *Gadd45a* transcription through its binding to POU2F1 and BRCA1.⁴¹

GADD45A, as well as the other two members of the family, are downstream targets of the NF- κ B transcriptional activity involved in regulating cell survival and apoptosis.¹⁴ However, the role of this transcription factor in regulating *Gadd45a* is still controversial. It was first demonstrated that NF- κ B inhibition following IKK β (inhibitor of NF- κ B [I κ B] kinase β) suppression caused an augment of *Gadd45a* mRNA expression.⁴² Since no changes were observed in the activity of several regulators of *Gadd45a* transcription (AKT/PKB or protein kinase B, FOXO3a, and p53), this study pointed to that the IKK β /NF- κ B signaling pathway regulates GADD45A through a posttranscriptional mechanism involving an increase in its mRNA stability.⁴² Later studies have demonstrated that NF- κ B acts as a pro-survival factor that favors apoptosis escape of cancer cells by indirectly reducing the expression of *Gadd45a*, in a process that involves an increase in MYC repressor, and the downregulating of nucleolin.⁹

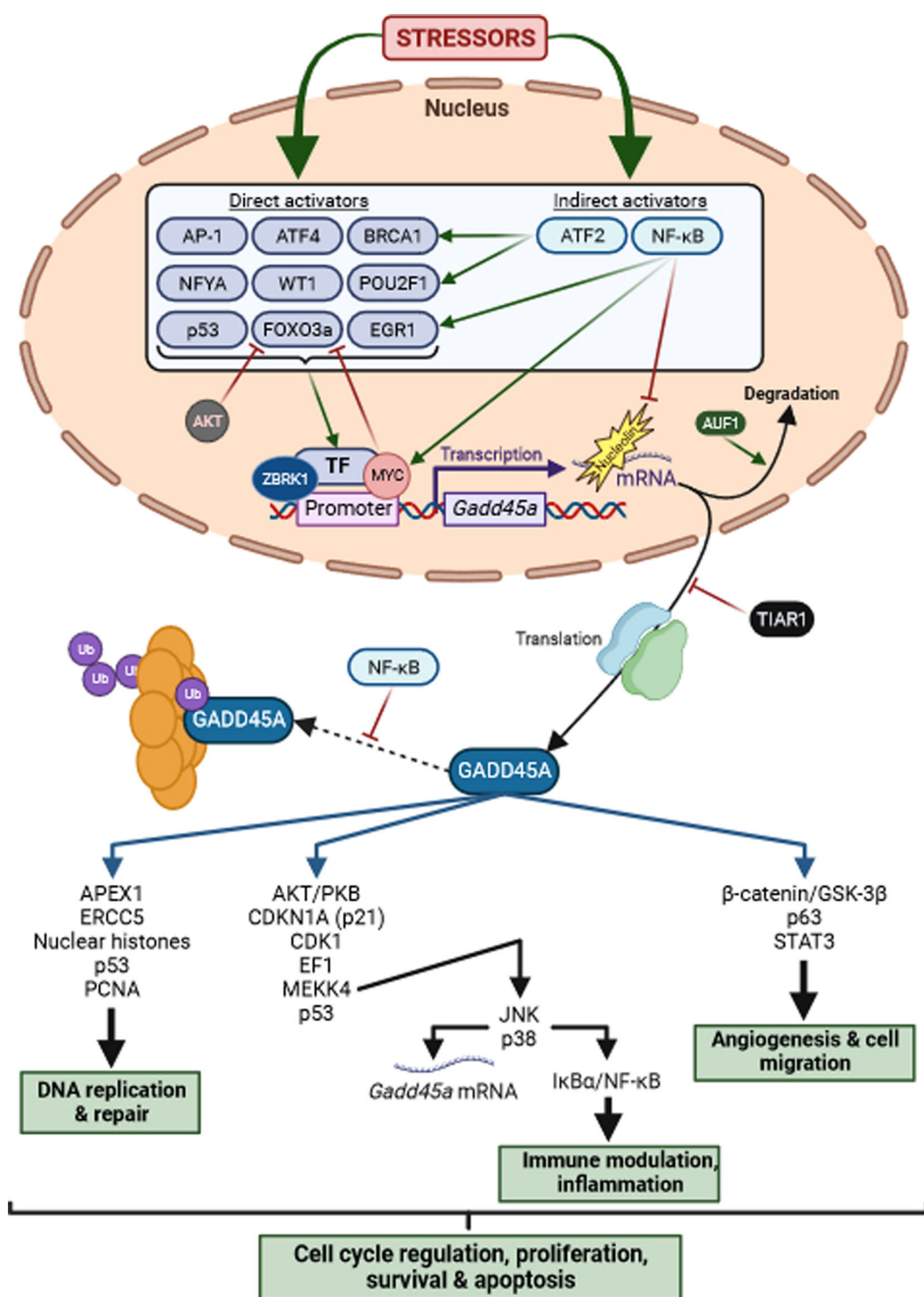


FIGURE 1 (See caption on next page).

Nucleolin is a nucleolar protein that increases *Gadd45a* mRNA stability and acts as a transcriptional coactivator of POU2F1. Supporting the importance of nucleolin, another report demonstrated that the increase in *Gadd45a* mRNA expression observed in IKK β -deficient fibroblasts was due to an increase in nucleolin levels.⁴² On the contrary, NF- κ B has been reported to induce cell death in UVB-exposed keratinocytes by activating *Gadd45a* transcription, in a process mediated by EGR1.⁴³ In fact, it is known that the p65 (RELA) subunit of NF- κ B activates the transcription of *Gadd45b* by directly binding to κ B elements in the promoter, but *Gadd45a* does not contain these response elements in its promoter region.⁹ Anyway, this differential regulation of GADD45A protein levels by NF- κ B might contribute to both pro- and antiapoptotic properties observed for this transcription factor. Another related transcription factor that directly increases *Gadd45a* expression is AP-1, which in a similar way to NF- κ B controls a number of cellular processes including inflammation, differentiation, proliferation, and apoptosis, in response to a variety of stimuli (cytokines, growth factors, stress, and bacterial or viral infection).³³ More specifically, p38 and JNK activate the AP-1 subunit JUN to enhance *Gadd45a* transcription.⁹

The mRNA levels of *Gadd45a* are finely tuned by several microRNAs (miRNA; e.g. miR-19a-3p, miR-130b, miR-138, miR-148a, miR-1283, miR-374a-5p, miR-376c, miR-454-3p, miR-499, miR-4270), long noncoding RNAs (lncRNA; lncRNA-DiGeorge syndrome critical region gene 5 or DGCR5), and even circular RNAs (circRNAs; circ_0004904).^{44–53} Downregulation of *Gadd45a* gene expression caused by these noncoding RNAs has been related to changes in cell proliferation and apoptosis, invasion, migration and epithelial-mesenchymal transition in

FIGURE 1 Regulation of GADD45A levels and its major interacting proteins. GADD45A levels are regulated through the combined action of transcriptional, posttranscriptional and posttranslational mechanisms. At the transcriptional level, a myriad of genotoxic and non-genotoxic stressors may induce *Gadd45a* gene expression through both direct and indirect mechanisms. Among the first stand out p53, although several p53-independent pathways are also involved in its transcriptional initiation, including AP-1, ATF4, BRCA1, EGR1, FOXO3a, NFYA, POU2F1, and WT1. ATF2 may also indirectly stimulate *Gadd45a* transcription through its binding to POU2F1 and BRCA1. NF- κ B may induce *Gadd45a* mRNA levels through the activation of EGR1, but may also reduce its expression by increasing the MYC repressor and downregulating the levels of nucleolin, a nucleolar protein that increases mRNA stability and acts as a transcriptional coactivator of POU2F1. To maintain constitutively low mRNA levels in non-stressed cells, *Gadd45a* is negatively regulated by transcriptional (MYC, ZBRK1) and posttranscriptional repressors (AUF1, TIAR1). AUF1 renders instability to *Gadd45a* mRNA and favors its degradation, while TIAR1 blocks its translation. MYC and AKT are able to indirectly downregulate *Gadd45a* expression by inhibiting FOXO3a. Finally, the p50 subunit of NF- κ B is able to reduce the ubiquitination and subsequent proteasome-dependent degradation of GADD45A. To carry out its numerous functions, GADD45A must regulate several proteins. Thus, it interacts with partners involved in DNA replication and repair (APEX1, ERCC5, p53, PCNA and nuclear histones), while it associates with CDK1, CDKN1A (p21), EF1, and p53 itself to control cell cycle, proliferation and survival. GADD45A induces MEKK4, which in turn activates both p38 and JNK MAPKs to drive apoptosis and regulate immune functions. Of note, both p38 and JNK are able to regulate the transcription of *Gadd45a* and the activity of NF- κ B. GADD45A has also a role in reducing cell survival through the modulation of the AKT, p38 MAPK and STAT5 signaling pathways. Finally, other less-known activities for GADD45A have also been reported. It negatively regulates p63 oncogene and the GSK3 β / β -catenin cascade, which are involved in extracellular matrix remodeling, angiogenesis and the invasion and metastasis of cancer cells. By suppressing STAT3 transcriptional activity, GADD45A also lessens the formation of tumor blood vessels. Created with BioRender.com. AP-1, activator protein-1; ATF4, activating transcription factor 4; AUF1, AU-rich element RNA-binding factor 1; BRCA1, breast cancer type 1 susceptibility protein; CDK1, cyclin-dependent kinase 1; CDKN1A (p21), cyclin dependent kinase inhibitor 1; EGR1, early growth response protein 1; ERCC5, DNA excision repair protein (or xeroderma pigmentosum group G-complementing protein, XPG); FOXO3a, forkhead box protein O3a; GADD45, growth arrest and DNA damage inducible 45; GSK3 β , glycogen synthase kinase 3 β ; I κ B, inhibitor of NF- κ B; JNK, JUN (c-Jun) N-terminal kinase; MAPK, mitogen-activated protein kinase; MEKK4, MEK Kinase 4 or MAPK kinase kinase 4; NF- κ B, nuclear factor- κ B; NFYA, nuclear transcription factor Y subunit α ; PCNA, proliferating cell nuclear antigen; PKB, Protein kinase B; POU2F1, POU domain, class 2, transcription factor 1; STAT, signal transducer and activator of transcription; WT1, Wilms tumor protein 1. [Color figure can be viewed at wileyonlinelibrary.com]

various types of cancer (hepatocellular carcinoma, high-grade glioma, non-small-cell lung cancer), and even other diseases or conditions (acute myocardial infarction, autoimmunity, diabetic cardiomyopathy, male infertility, pre-eclampsia).

Once the *Gadd45a* gene is transcribed, the pre-mRNA is spliced and the resulting mRNA exported out of the nucleus to serve as a template for protein synthesis.³¹ To maintain constitutively low mRNA levels in non-stressed cells, *Gadd45a* is negatively influenced by transcriptional and posttranscriptional repressors (Figure 1). This is, for instance, the case of MYC and AKT, which are frequently overexpressed in a variety of cancers and are able to indirectly downregulate *Gadd45a* expression by inhibiting FOXO3a.⁹ FOXO proteins are tumor suppressors frequently inactivated in cancer cells through its AKT-mediated phosphorylation and subsequent nuclear exclusion. Following cell growth stimulation, MYC is rapidly recruited to the chromatin and selectively contributes to repress the FOXO3a-mediated expression of *Gadd45a*.⁵⁴ MYC is also able to inhibit both basal and induced transcription of *Gadd45a* by directly binding to its promoter.³³ On the contrary, in follicular thyroid carcinoma, AKT activation and cytoplasmatic accumulation of FOXO3a correlate with increased expression of *Gadd45a*, suggesting that other transcription factors are regulating *Gadd45a* in these cells.⁵⁵ More recently, the zinc finger protein 350 (ZBRK1) has been demonstrated to function as a transcriptional repressor of *Gadd45a* in resting cells, and genomic methylation of specific cytosine residues of CpG dinucleotides within its promoter also contributes to negatively regulate its transcription.³³ Among the posttranscriptional repressors, stand out RNA-binding proteins that may render instability to *Gadd45a* mRNA and favor its degradation (AUF1; AU-rich element RNA-binding factor 1), or block their translation (TIAR; T-cell-restricted intracellular antigen-1 (TIA-1)-related protein), both preventing the translation of those transcripts that escape the transcriptional repression in non-stressed cells.³³ In the presence of stress, AUF1 dissociates from mRNA and this results in the increase of translation.

2.2 | GADD45A: The conductor of the cell cycle and apoptotic choral orchestra

In response to certain genotoxic stresses, GADD45A interacts with CDK1 and provokes the dissociation of the CDK1-cyclin B1 complex, that, in turn, results in the inhibition of CDK1 kinase activity and the subsequent cell cycle G2/M arrest and cell growth suppression (Figure 1).^{8,56} In support of this, the tumor suppressor protein CR6-interacting factor 1 (CRIF1) boosts the activity of GADD45 family proteins and, by this means, acts as a negative regulator of the cell cycle through the subsequent inhibition of CDK1.⁵⁷ In human hepatoma HepG2 cells, GADD45A also induces G2/M arrest through the activation of p38 and JNK signaling pathways.⁵⁸ GADD45A binds to p21 as well, another p53 target gene that inhibits cyclin dependent kinases implicated in G1/S and G2/M cell cycle arrest, overall suggesting that it is involved in the regulation of cell cycle at different stages.⁵⁹ A more recent study also depicts a role for the prolonged activation of the GADD45A/p21 pathway in cellular senescence and mitochondrial dysfunction induced by oxidative and other stresses, in a process in which the p38 MAPK is involved.¹ On the other hand, ER stress and subsequent induction of the unfolded protein response (UPR) induces cell cycle arrest at the G2/M phase by reducing the amount of cyclin B1.⁶⁰ Interestingly, cell death caused by ER stress in mouse embryonic fibroblasts is diminished after knocking down *Gadd45a* expression due to cell cycle arrest at the G2/M phase, and the UPR branch involved in such effect was demonstrated to be the eukaryotic translation initiation factor 2 (eIF2) α signaling pathway.⁶⁰ Another branch of the UPR, which is driven by the endonuclease activity of the inositol-requiring enzyme 1 (IRE1), may induce pre-miR-301a degradation and, thus, elevate *Gadd45a* mRNA expression to switch the ER stressed cells to apoptosis.⁶¹

Apoptotic cells display a remarkable increase in GADD45A protein levels, thus pointing to a pivotal role for this protein in the signal transduction of the apoptotic cascade.³³ GADD45A may prevent oncogene-induced cell proliferation through direct interaction with p38 MAPK, in a process mediated, at least in part, by p53 activity (Figure 1).⁶² On the other hand, suppression of JNK activity by NF- κ B favors tumorigenesis and metastasis in several cancer types, since it enhances cell survival and allow them to escape from apoptosis^{63,64} and, interestingly,

GADD45A binds to and activates MEKK4 to inhibit cell growth and induce apoptosis through the activation of the p38 and JNK pathways.⁶⁵ As a consequence, defective activation of MEKK4 and both JNK and p38 MAPK in *Gadd45a* knockout mice could account for the reduced apoptosis in keratinocytes that display these mice after UV radiation exposure.⁶⁶ And the opposite is also true; GADD45A activation, through the stimulation of p38 and JNK pathways, induces apoptosis and regulates nuclear import of CDK1 kinase to promote sustained G2/M arrest.^{67,68} Further, JNK regulates both the intrinsic and extrinsic apoptotic pathways and, consistent with this, GADD45A is also capable of inducing mitochondrial dysfunction and the JUN-dependent AP-1 transcriptional activity during cell death response.³³ Strikingly, both JNK and p38 kinases are able to induce *Gadd45a* transcription in response to certain genotoxic stressors, thus leading to a positive feedback loop in their apoptotic effect.³³ Also, despite p53 is widely recognized as an upstream transcriptional activator of *Gadd45a*, the latter could contribute to withstand p53 activity through the activation of the JNK and p38 MAPK pathways, sustaining another proapoptotic positive feedback regulation.³³

2.3 | GADD45A: A first chorus voice in DNA repair and (de)methylation

GADD45A is regarded as an important regulator in nucleotide excision repair, base excision, and post replication repair, and also contributes to avoid abnormal centrosome duplication and amplification, which are essential steps for an effective mitosis, thus preventing uncontrolled cell transformation and tumorigenesis.^{69,70} However, GADD45A lacks the catalytic activity necessary to directly carry out such effects, and the mechanisms involved have not been fully elucidated. It is known that its binding to PCNA has an important role in nucleotide excision DNA repair in response to UV radiation,^{2,71} in the inhibition of DNA replication,³⁰ as well as in growth suppression by inhibiting the entry of cells into S phase (Figure 1).³⁶ In particular, GADD45A recruits PCNA to the damaged chromatin sites, and also acts as a downstream mediator of FOXO3a to induce G2/M phase arrest and repair damaged DNA under low level of stress condition.³³ On the other hand, GADD45A can form homo-oligomers and hetero-oligomers with the other two members of the family and, although the physiological relevance of this phenomenon is still not completely understood, some studies point to that it could be involved in the modulation of the accessibility to damaged chromatin and subsequent DNA repair process.^{72,73} In fact, the two distinct regions of GADD45A involved in self-association partially overlap with PCNA binding domains and, thus, might lead to functional consequences.⁷³ Besides PCNA, GADD45A has been reported to interact with several other DNA repair-related proteins, including histones, single-stranded DNA cytosine deaminase (AID or AICDA), methyl-CpG-binding domain protein 4 (MBD4), G/T mismatch-specific thymine DNA glycosylase (TDG), ERCC5, and the transcription initiation factor TFIID subunit 12 (TAF12) (Figure 1).⁷⁰ It has been demonstrated that GADD45A binds to damaged chromatin and enables the accessibility of other proteins involved in DNA repair, such as the endonuclease ERCC5,⁷⁰ and also participates in base excision repair by hampering the interaction of APEX1 with PCNA, a fact which protects from carcinogenic base damage and contributes to preserve genomic stability.⁷¹

DNA methylation at specific cytosine residues within the regulatory promoter regions of the genes causes chromatin condensation and, as a result, it results in the inhibition of the binding of the transcription machinery. GADD45A-mediated DNA demethylation allows specific epigenetic activation of genes related to cellular responses to stress, nuclear hormones, or differentiation, but also might account for hypermethylation and subsequent inactivation of tumor suppressor genes in the presence of low GADD45A activity.^{70,74} In support of a role for GADD45A in active demethylation, its overexpression by using genetic tools or its DNA-damage-induced increase promoted DNA demethylation at specific CpG residues within the regulatory regions of genes encoding for ribosomal proteins (rDNA), whereas *Gadd45a* downregulation led to hypermethylation and rDNA transcription silencing.⁷⁵ The latter study further demonstrated that GADD45A-driven demethylation is initiated by promoting association of TAF12 to the rDNA gene, followed by recruitment of the nucleotide excision repair machinery that removes methylated cytosines. Likewise, the tumor suppressor inhibitor of growth protein 1 (ING1)⁷⁶ and

ten-eleven-translocation (TET) proteins⁷⁷ cooperate with GADD45A for targeting active DNA demethylation to specific regions. GADD45A also promotes DNA demethylation by bringing on the interaction between thymine DNA glycosylase and activation-induced deaminase in a multimeric complex, and by promoting the DNA repair machinery, which replaces methylated cytosines by unmethylated nucleotides.^{74,78,79} Some data also suggest that GADD45A might promote demethylation of damaged double-strand DNA, but also it could hamper remethylation of hemi-methylated sites through the inhibition of the DNA (cytosine-5)-methyltransferase 1 (DNMT1).⁸⁰ Besides relaxing chromatin through DNA demethylation, GADD45A may also locally modify chromatin accessibility by relaxing topoisomerase and increasing its cleavage activity, an effect that can destabilize histone-DNA interactions.^{81,82} In any case, and due to unknown reasons, other studies have been unable to quantify any change in DNA demethylation after *Gadd45a* overexpression or suppression⁸³ and, thus, whether GADD45A promotes DNA demethylation remains still unsolved.

3 | GADD45A: A MULTI-INSTRUMENTALIST ARTIST WITH ANTICANCER ACTIVITY

GADD45A is a tumor suppressor that is capable of delaying, or even preventing, tumorigenesis at multiple levels (Figure 2 and Table 1). As stated above, it stands out by its important role in regulating apoptosis and senescence, but it participates in DNA repair and inhibits cell migration as well, by this means avoiding the conversion of damaged cells into malignant cells.⁸ In agreement with these beneficial effects, GADD45A protein levels are reduced in many human tumors and cancer cell lines, and several tumor suppressors induce its transcription.⁹ Further, *Gadd45a* expression levels positively correlate

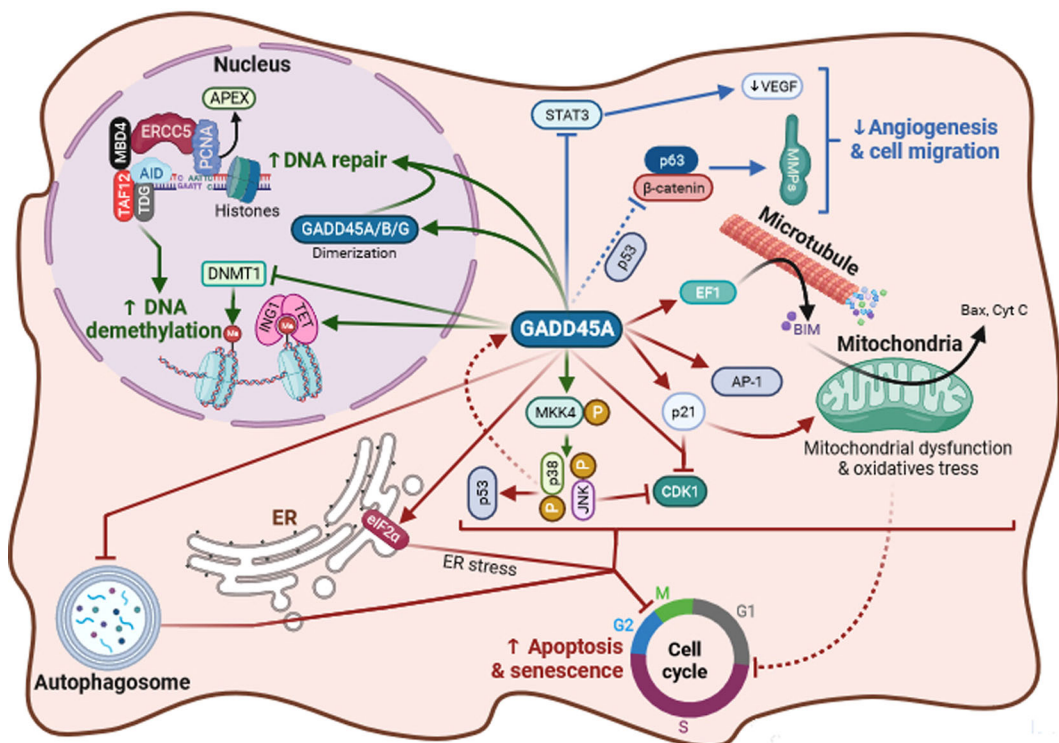


FIGURE 2 (See caption on next page).

with a higher survival rate and a better prognosis in patients with esophageal squamous cell carcinoma,⁹⁴ and epigenetic modulation of GADD45a is related to its reduced gene expression in cancer, since its promoter region is hypermethylated in several CpG residues of some breast cancers, gastric cardia adenocarcinoma or prostate cancer.^{95,96,114} It has even been suggested that serum levels or methylation degree of GADD45A could be used as a biomarkers to predict the response and survival rate after neoadjuvant chemotherapy in gastric cancer patients,⁹⁷ or to distinguish patients with benign prostate cancer from malignant.⁹⁸ Even though genetic mutation and inactivation of this protein is a very infrequent cause of cancer,^{14,17,99,100,115} there are some studies that report an association between GADD45A mutations or polymorphisms and the development of cancer. For example, an investigation examining the genotype distribution between patients and age-matched healthy women found out a GADD45A polymorphism (+1506 T > C) that was associated with ovarian cancer susceptibility and prognosis, and for this reason, the authors pointed to that it could be successfully used as a biomarker.¹¹⁶ This same genotype and others (+3204 G > C) have been associated with reduced susceptibility to sporadic and

FIGURE 2 GADD45A: A tumor suppressor that acts at multiple levels. GADD45A is an important regulator of nucleotide excision repair, base excision, and post replication repair, thus preventing tumorigenesis. To carry out such effects, it binds to PCNA and hampers its interaction with APEX1, thus enabling the accessibility of other proteins involved in nucleotide excision DNA repair (such as ERCC5). Besides PCNA, GADD45A may interact with several other DNA repair-related proteins, including histones, AID, MBD4, TAF12, and TDG. Further, GADD45A can homodimerize and even heterodimerize with the other two members of the family (GADD45B and G) to modulate the accessibility to damaged chromatin and subsequent DNA repair process. On the other hand, GADD45A-mediated DNA demethylation allows specific epigenetic activation of genes related to cellular responses to stress or differentiation. The tumor suppressor ING1 and TET proteins cooperate with GADD45A for targeting active DNA demethylation to specific regions. GADD45A is also able to promote DNA demethylation at specific CpG residues within the regulatory regions of genes encoding for ribosomal proteins (rDNA), in a process which requires TAF12 and subsequent recruitment of the nucleotide excision repair machinery. In a similar way, GADD45A also promotes DNA demethylation by bringing on the interaction between TDG and AID in a multimeric complex. GADD45A can also hamper remethylation through the inhibition of DNMT1. Another antitumoral activity of GADD45A encompasses induction of apoptosis and cellular senescence. In response to certain stresses, GADD45A interacts with and inhibits CDK1, leading to subsequent cell cycle G2/M arrest and cell growth suppression. The binding to MEKK4 and subsequent activation of p38 and JNK signaling pathways seems to be also involved in the induction of the G2/M arrest of the cell cycle by GADD45A. Thus, the stimulation of p38 and JNK pathways regulates nuclear import of CDK1 kinase and activates p53 to promote sustained G2/M arrest and apoptosis. Interestingly, both JNK and p38 kinases are able to induce *Gadd45a* transcription in response to certain genotoxic stressors, thus leading to a positive feedback loop in their apoptotic effect. GADD45A binds to p21 as well, a CDK inhibitor involved in G1/S and G2/M cell cycle arrest, suggesting that it is involved in the regulation of cell cycle at different stages. In fact, prolonged activation of the GADD45A/p21 pathway induces cellular senescence due to mitochondrial dysfunction and oxidative stress. GADD45A may also induce cell death by activating the eIF2 α branch of the UPR, which results in a reduction in cyclin B1 levels, by inhibiting autophagy, or by inducing the JUN-dependent AP-1 transcriptional activity. Finally, GADD45A can initiate apoptosis by physically interacting with the elongation factor EF1. This results in the release of the proapoptotic protein BIM from microtubule protein complexes, which boosts the BAX/cytochrome c-dependent apoptosis. Additional mechanisms may account for the anticancer activities of GADD45A. For instance, it negatively regulates p63 and β -catenin oncogenes, resulting in reduced expression of genes involved in extracellular matrix remodeling such as endopeptidases and MMP. MMP expression inhibition by GADD45A probably involves both p38 MAPK and p53 activation. Finally, GADD45A, by suppressing STAT3 transcriptional activity, reduces the secretion of VEGF and lessens angiogenesis. Created with BioRender.com. AID (AICDA), single-stranded DNA cytosine deaminase; AP-1, activator protein-1; APEX, DNA-(apurinic or apyrimidinic site) endonuclease; BAX, BCL2 associated X protein; CDK1 (CDC2), cyclin-dependent kinase 1; DNMT1, DNA methyltransferase 1; EF1, elongation factor 1; eIF2 α , eukaryotic translation initiation factor 2 α ; ER, endoplasmic reticulum; ERCC5, DNA excision repair protein (or xeroderma pigmentosum group G-complementing protein, XPG); GADD45, growth arrest and DNA damage inducible 45; ING1, inhibitor of growth protein 1; JNK, JUN (c-Jun) N-terminal kinase; MAPK, mitogen-activated protein kinase; MBD4, methyl-CpG-binding domain protein 4; MEKK4, MEK Kinase 4 or MAPK kinase kinase 4; MMP, matrix metalloproteinase; PCNA, proliferating cell nuclear antigen; STAT, signal transducer and activator of transcription; TAF12, transcription initiation factor TFIID subunit 12; TDG, G/T mismatch-specific thymine DNA glycosylase; TET, ten-eleven-translocation; UPR, unfolded protein response; VEGF, vascular endothelial growth factor. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 The multifaceted role of GADD45A in human diseases.

Organ/tissue and disease or condition	GADD45A levels	GADD45A-associated functions	References
Cancer			
Breast cancer (triple-negative)		Acts as a tumor suppressor that:	[40–42, 57, 59–65, 72, 75, 76, 78]
Chronic myelogenous leukemia		– Prevents cell transformation and proliferation.	
Esophageal squamous cell carcinoma		– Induces cell apoptosis and senescence.	
Gastric cardia adenocarcinoma		– Promotes DNA demethylation and repair.	
Hepatocellular, ovarian & vascular tumors	↓	– Reduces extracellular matrix remodeling.	[1, 7, 62, 72, 80]
Lung tumor		– Negatively regulates migration-associated proteins, thus reducing cell invasion and metastasis.	
Medulloblastoma		– Lessens tumor angiogenesis.	
Pancreatic cancer			
Prostate cancer			[37, 74]
Melanoma (UV-induced)			
MYC-driven breast cancer	↑	Enhances tumor vascularization and growth. Prevents apoptosis.	
Pancreatic ductal adenocarcinoma			
Radiotherapy and some therapeutic drugs	↑	Induces cancer cell apoptosis and inhibits autophagy. Reduces tumor recurrence.	
Adipose tissue			
Obesity	↑	Promotes white adipocyte differentiation through the upregulation of the expression of cell cycle related genes and the activation of PPARγ. Regulates energy metabolism by reducing lipolysis, mitochondrial biogenesis, and cell proliferation. Enhances lipogenesis.	[84–86]
Cardiovascular system			
Atherosclerosis		Promotes endothelial cell apoptosis and activation, and cardiomyocyte apoptosis.	[87–93]

(Continues)

TABLE 1 (Continued)

Organ/tissue and disease or condition	GADD45A levels	GADD45A-associated functions	References
Cardiac hypertrophy	↑		
Heart failure			
Pre-eclampsia			
CNS and neuronal-related diseases			
Alzheimer disease		Plays a cytoprotective role by preventing neuron cell death.	[82, 83, 94–100]
Epileptic seizures		Prevents oxidative stress and genomic DNA damage.	
Kainate- or glutamate-induced oxidative neurotoxicity	↑	Regulates DNA excision and repair.	
Peripheral nerve injury			
Transient ischemia			
Immunological system and autoimmunity			
Systemic lupus erythematosus	↓	Induces p38 MAPK in dendritic cells, thus favoring Th1 polarization. Acts as a negative regulator of T cells. May contribute to autoantibody formation.	[101–104]
Psoriasis	↑	Promotes keratinocyte proliferation and the production of pro-inflammatory mediators.	[102]
Rheumatoid arthritis	↔↑	Reduces susceptibility.	[105, 106]
Liver			
Acetaminophen-induced liver injury	↑	Hepatoprotective role since it:	[107–112]
Liver steatosis		Triggers AMPK activity, thus inducing energy-producing pathways (glucose uptake, glycolysis, glycogenolysis, FA β-oxidation, and lipolysis) and inhibiting anabolic processes (gluconeogenesis, and biosynthesis of FA, cholesterol, and proteins).	
Nonalcoholic steatohepatitis		Induces apoptosis to protect hepatocytes from excess ER stress.	
Chronic liver injury and cirrhosis	↓	- Prevents inflammation and the activation of the TGF-β signaling pathway, thus reducing the subsequent accumulation of extracellular matrix proteins and fibrosis. - Promotes scavenging of reactive oxygen species and upregulation of antioxidant enzymes, thus preventing oxidative stress.	

TABLE 1 (Continued)

Organ/tissue and disease or condition	GADD45A levels	GADD45A-associated functions	References
Skeletal muscle			
Muscle atrophy induced by fasting, aging or disuse	↑	Represses genes involved in anabolic signaling, protein synthesis, glucose uptake, glycolysis, oxygen delivery, mitochondrial biogenesis, citric acid cycle, and oxidative phosphorylation. Induces the expression of genes involved in autophagy and caspase-mediated proteolysis. Antiapoptotic effect on myofibers, probably by blocking NF-κB signaling.	[51, 113]
Skin			
Diabetic foot ulcers	↑	Hinders wound healing.	[77]

Abbreviations: AMPK, AMP-activated protein kinase; CNS, central nervous system; ER, endoplasmic reticulum; FA, fatty acid; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PPAR, peroxisome proliferator-activated receptor; TGFβ, transforming growth factor β.

non-BRCA1/2 familial breast cancers in Chinese populations.¹¹⁵ Finally, point mutations in exon 4 of GADD45A gene have been related to increased frequency of some human pancreatic carcinomas.¹¹⁷

Studies on genetically modified mouse models thoroughly support the anticancer role of GADD45A. *Gadd45a* deletion in mice increases mutation rate and enhances susceptibility to carcinogenesis in the presence of genotoxic stressors. In particular, *Gadd45a* knockout mice showed an increased prevalence of ovarian, hepatocellular, and vascular tumors.^{34,84,85} Other reports demonstrate that suppression of *Gadd45a* alone hastens lung tumor progression,⁸⁶ and render mice more prone to UV-induced skin tumors.⁶⁸ Further supporting this, GADD45A overexpression in UV-exposed keratinocytes mediates a p38 and JNK MAPK-dependent proapoptotic response that avoids the accumulation of damaged and potentially carcinogenic cells.³³ GADD45A has also a tumor suppressor role in chronic myelogenous leukemia, since its downregulation in mice resulted in a more aggressive phenotype and a reduced survival time, through the modulation of the AKT, p38 MAPK and signal transducer and activator of transcription (STAT)5 signaling pathways.¹⁰⁷

In breast cancer, the GADD45A levels depend on the hormone receptor status; triple-negative breast cancer tumors display low GADD45A protein levels, whereas luminal A and luminal B subtypes have high levels.¹¹⁸ In this regard, the estrogen receptor (ER)β has a protective role in breast cancer development through the inhibition of cell proliferation, and some findings demonstrate that ERβ causes a G2 cell cycle arrest by inactivating CDK1 through the repression of cyclin B1 and stimulation of *Gadd45a* expression.¹¹⁹ Other studies demonstrate that GADD45A inhibits mammary tumor onset and growth by inducing the JNK-activated apoptosis and p38-mediated cellular senescence, whereas its suppression has an opposite effect.⁸⁵ In support of this, the anti-tumorigenic effects of the hypoglycemic peroxisome proliferator-activated receptor (PPAR)γ agonist troglitazone in breast cancer models positively correlated with the increase in *Gadd45a* mRNA expression and protein levels, the activation of the JNK pathway, and apoptosis.¹⁰⁸ On the other hand, breast cancer cases are often associated with mutations in BRCA1 protein, which is implicated in the cellular response to DNA damage that triggers apoptosis through activation of JNK, and also may induce GADD45A through the interaction with other transcription factors to upregulate apoptosis.^{109,110} In the presence of genotoxic stress, p53 protein induces *Gadd45a* transcription, but in the absence of DNA damage, BRCA1 represses it.¹¹⁰ Further, GADD45A acts synergistically with BRCA1 to regulate centrosome duplication and increase the genomic stability.^{120,121}

An important player in cancer development is NF- κ B. NF- κ B is constitutively triggered due to the activation of IKK in human pancreatic ductal adenocarcinoma and in several pancreatic cancer cell lines, where it exerts an antiapoptotic role.¹¹¹ Interestingly, GADD45A levels are reduced by NF- κ B in pancreatic cancer cells,¹¹¹ and constitutive activation of NF- κ B in several types of cancer leads to the repression of GADD45A, probably through MYC induction, an effect that is essential for cancer cell survival and tumor formation.^{8,63} Accordingly, inhibition of NF- κ B boosts GADD45A-dependent induction of apoptosis in cancer cells, hence reducing tumor growth, in a process mediated by JNK activation.^{63,111} However, it has also been demonstrated that, in response to some cancer therapeutic drugs (e.g. arsenite), the IKK β /NF- κ B pathway may activate the JNK signaling cascade through the induction of GADD45A.⁶⁴ This effect would be dependent on the p50 subunit of NF- κ B, but independent of p65, and would involve a reduction in the ubiquitination and subsequent proteasome-dependent degradation of GADD45A. In addition, GADD45A may stimulate I κ B degradation and NF- κ B translocation into the nucleus.² Thus, NF- κ B might regulate the expression of *Gadd45a* at the same time it acts as its regulatory target, in a sort of positive feedback loop. Overall data suggest that the IKK β /NF- κ B axis may mediate both pro- or antiapoptotic effects of the GADD45A/JNK pathway by inducing or inhibiting, respectively, its activity, depending on the triggering stimulus.

Additional mechanisms may account for the proapoptotic effects of GADD45A in cancer cells. For instance, GADD45A may induce apoptosis by physically interacting with the elongation factor (EF)1, a microtubule-severing protein, thus releasing the proapoptotic protein BIM from microtubule protein complexes, which will induce BAX/cytochrome c-dependent apoptosis.¹¹² Not surprisingly, suppression of endogenous BIM greatly inhibits GADD45A-induced apoptosis. A more recent study also demonstrated that GADD45A may induce cancer cell death and reduce tumor recurrence in several murine models and cancer cell lines through a combined action involving stimulation of apoptosis and inhibition of autophagy, in particular after radiotherapy and chemotherapy treatments, when it is strongly induced.¹²² Other less-known anticancer activities for GADD45A have also been reported. GADD45A negatively regulates p63 and β -catenin oncogenes.⁶⁶ β -catenin is a transcription factor that induces the expression of genes involved in extracellular matrix remodeling such as endopeptidases and the matrix metalloproteinases (MMP)3 and MMP9, thus enabling cell proliferation, and the invasion and metastasis of cancer cells. For instance, in medulloblastomas, GADD45A negatively regulates cancer cell invasion and migration-associated proteins, such as MMP9 and β -catenin, thus reducing epithelial-mesenchymal transition and its metastatic potential.¹²³ Another study demonstrated that GADD45A hinders epidermal tumor cell migration and invasion under UV exposure by inhibiting MMP expression, in a process involving p38 MAPK and p53 activation, and association with the adenomatous polyposis coli (APC) complex.¹¹³ On the contrary, and for an unknown reason, GADD45A positively regulates the expression of the MMP9 in non-tumoral keratinocytes.⁸⁷ In fact, the protein levels of GADD45A and MMP9, an enzyme that also hinders wound healing, are upregulated in skin tissues from patients with diabetic foot ulcers and in diabetic rodents, and in cultured human keratinocytes exposed to advanced glycation end products.⁸⁷ The same study demonstrated that *Gadd45a* suppression prevented the increase in *Mmp9* transcription by inhibiting demethylation in its promoter, without affecting cell proliferation or apoptosis, while *Gadd45a* overexpression resulted in the opposite. Finally, angiogenesis is another important factor contributing to tumorigenesis, and GADD45A, by suppressing STAT3 transcriptional activity, reduces the secretion of vascular endothelial growth factor (VEGF) and lessens the formation of tumor blood vessels.⁸⁸

Notwithstanding its well-established tumor suppressor properties, an antiapoptotic and pro-survival role has also been described for GADD45A in some malignancies, and evidence exist of a positive correlation between its gene expression and certain tumors development. For instance, it exerts a pro-survival activity in hematopoietic cells exposed to UV radiation and certain anticancer drugs, since its suppression results in defective G2/M arrest and increased apoptosis.¹²⁴ Additional studies demonstrated that GADD45A boosts the NF- κ B survival pathway through the activation of p38 MAPK and subsequent phosphorylation-mediated ubiquitination of its inhibitory protein I κ B.¹² Likewise, despite GADD45A suppresses the RAS-driven breast tumorigenesis, it is capable of promoting the MYC-driven breast cancer.^{85,89} In this latter case, GADD45A activation results in a strong decrease in the angiogenic enzyme MMP10 levels via the glycogen synthase kinase (GSK3) β / β -catenin cascade, which enhances tumor vascularization and growth.⁸⁹ Further supporting this, *Gadd45a*

downregulation in MYC-driven breast cancer enhances apoptosis or cellular senescence.^{85,89} GADD45A overexpression also prevents melanoma cell death by inhibiting the mitochondrial apoptotic pathway.³³ Finally, *Gadd45a* gene expression and protein levels are elevated in a vast majority of pancreatic ductal adenocarcinomas, all contributing to worsen their prognosis compared to those patients with low GADD45A levels.⁸ Studies in vitro have demonstrated that knockdown of *Gadd45a* impairs proliferation and induces apoptosis of pancreatic cancer cells, suggesting that this protein carries out a pro-survival role in these malignant cells.^{1,111} On the other hand, cisplatin resistance in some melanomas has been related to increased expression of *Gadd45a* due to the activation of the ERK1/2 MAPK pathway, while suppression of *Gadd45a* sensitized melanoma cells to this drug by enhancing chemotherapy-induced DNA damage.⁹⁰

4 | GADD45A EFFECTS BEYOND CANCER

4.1 | Central nervous system: Rethinking the role of GADD45A

Alzheimer's disease (AD) is characterized by the extracellular deposition of neurotoxic amyloid β -peptide (A β) in the brain, which correlates with the activation of AP-1 and NF- κ B, and relates to DNA damage due to double-strand breaks, a characteristic of apoptosis. DNA rupture is regarded as an early sign of neuron degeneration that may be frequently detected in the cortex of AD patients. Interestingly, brain tissue samples obtained from AD patients display increased GADD45A protein levels in the hippocampus and the occipital lobe, which colocalizes with neurons upregulating the antiapoptotic protein BCL-2 (B-cell lymphoma protein 2) and positively correlates with DNA damage (Figure 3 and Table 1).¹²⁵ Previous studies have demonstrated that exposure of human preneural NT2 cells to A β provoke a strong and rapid increase in the expression of *Gadd45a*, an indicator of DNA damage and

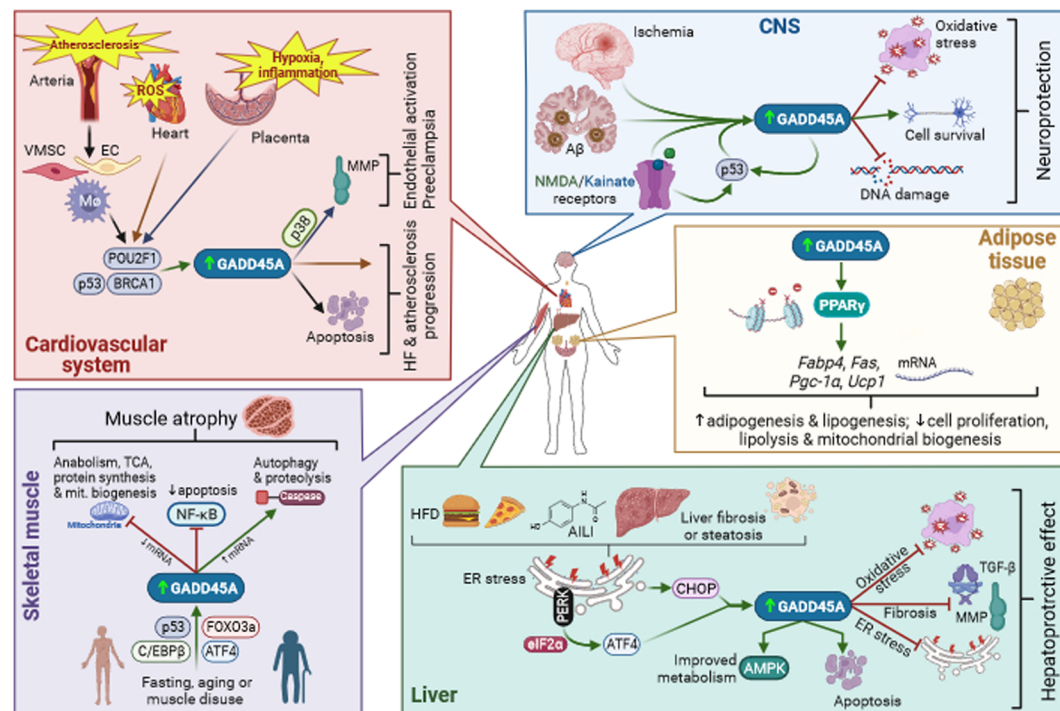


FIGURE 3 (See caption on next page).

FIGURE 3 The multiple roles of GADD45A beyond cancer. Brain tissue from AD patients displays increased GADD45A protein levels, which colocalizes with neurons upregulating the antiapoptotic protein BCL-2 and positively correlates with DNA damage. Further, GADD45A has a crucial function in the response to DNA strand breakage induced by A β in neurons. On the other hand, ischemia or stimulus that enhance oxidative stress and cell death through the activation of the N-methyl-D-aspartate or kainate receptors are associated with a rise of GADD45A mRNA and protein levels. This increase may be both dependent or independent of p53 transcriptional control, and is aimed to counteract oxidative stress and DNA damage induced by this stimulus, thus improving neuron survival and conferring neuroprotection to the injured brain. In both white and brown adipose tissue, GADD45A induces adipogenesis and lipid accumulation by promoting PPAR γ demethylation in the promoter region. In brown adipose tissue, this results in enhanced PPAR γ transcription and activity, and the subsequent modulation of its target genes, such as *Fabp4*, *Fas*, *Pgc-1 α* , and *Ucp1*. As a result, increased GADD45A activity reduces mitochondrial biogenesis, cell proliferation, and triglyceride lipolysis, while increases lipid accumulation, overall resulting in reduced glucose uptake and insulin sensitivity. Acetaminophen-induced liver injury (AILI) induces a rapid increase in GADD45A levels in the liver, where it exerts an hepatoprotective effect through the activation of AMPK, a master regulator of metabolism that induces the expression of genes related to energy-producing pathways, such as glucose uptake, glycolysis, glycogenolysis, fatty acid β -oxidation, and lipolysis, while inhibiting anabolic processes, such as the gluconeogenesis and the biosynthesis of fatty acids, cholesterol, and proteins. Liver steatosis induced by a high-fat diet feeding is linked to ER stress, which if not corrected may result in hepatocyte apoptosis and excessive lipids liberation, thus contributing to fat accumulation. This is accompanied by increased expression of *Atf4*, *Chop* and *Gadd45a* in the liver, which is coupled with increased steatosis, fibrosis and inflammation. In this context, the PERK branch of the unfolded protein response activated during ER stress boosts hepatocyte apoptosis via ATF4 by regulating *Gadd45a* expression, while CHOP itself elicits *Gadd45a* gene expression to attenuate oxidative stress and ensuing inflammatory signaling, and to induce apoptosis to protect cells from excess ER stress. Finally, during hepatic fibrosis, GADD45A reduces the expression of extracellular matrix proteins and counteracts fibrosis by inhibiting the TGF- β signaling pathway, while it also reduces local inflammation (TNF- α , IL-6 rise) and oxidative stress. Skeletal muscle atrophy may be caused by diverse stresses, such as aging, malnutrition, starvation, muscle disuse, any serious injury. In such process, upregulation of GADD45A levels by several upstream regulators has been recognized, including ATF4, C/EBP β , FOXO and p53. GADD45A transcriptionally repress genes involved in anabolic signaling, protein synthesis, glucose uptake, glycolysis, oxygen delivery, mitochondrial biogenesis, citric acid cycle, and oxidative phosphorylation, but induces genes involved in autophagy and caspase-mediated proteolysis, overall resulting in muscle atrophy. In addition, GADD45A probably cooperates with other transcriptional regulators (e.g., NF- κ B) to drive skeletal muscle atrophy. During atherosclerosis, VSMC growth factors induce a robust increase in *Gadd45a* during the S phase, and a huge increase after cell division, in a process transcriptionally regulated by p53 and BRCA1. In a similar way, PPAR γ activation with the antidiabetic thiazolidinediones induces apoptosis by enhancing POU2F1-mediated transcription of *Gadd45a* in endothelial cells, monocyte-derived macrophages, and also VSMC, thus reducing neointima formation during the atherosclerotic process. GADD45A might also have an important role during heart disease. Oxidative stress-induced apoptosis in cardiomyocytes is accompanied by a remarkable increased expression of several p53 upstream regulators, and several downstream effectors such as GADD45A. GADD45A is regarded an important contributor toward myocardial apoptosis and ensuing development of heart failure. Finally, GADD45A also acts as an upstream modulator of pathophysiological changes occurring during pre-eclampsia. Placental stresses during pregnancy, including hypoxia, inflammatory cytokine secretion or hypertonic stress, induce GADD45A and its downstream effector p38 MAPK, both contributing to endothelial activation and the pathogenesis of pre-eclampsia through a mechanism involving the regulation of *Mmp2* and *Mmp9* expression and the inhibition of oxidative stress, which could promote the invasion and migration of trophoblast cells during early pregnancy. Created with BioRender.com. A β , amyloid β peptide; AD, Alzheimer disease; AILI, acetaminophen-induced liver injury; AMPK, AMP-activated protein kinase; ATF4, activating transcription factor 4; BCL-2, B-cell lymphoma protein 2; BRCA1, breast cancer type 1 susceptibility protein; C/EBP β , CCAAT/enhancer-binding protein β ; CHOP, C/EBP homologous protein; ER, endoplasmic reticulum; FABP4, fatty acid binding protein 4; FAS, fatty acid synthase; FOXO3a, forkhead box protein O3a; GADD45, growth arrest and DNA damage inducible 45; IL, interleukin; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NF- κ B, nuclear factor- κ B; PERK, protein kinase R-like ER kinase; PGC-1 α , PPAR γ coactivator-1 α ; POU2F1, POU domain, class 2, transcription factor 1; PPAR, peroxisome proliferator-activated receptor; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor α ; UCP1, uncoupling protein 1; VSMC, vascular smooth muscle cells. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

DNA excision–repair processes.^{91,126} The authors of these studies concluded that GADD45A has a crucial function in the response to DNA strand breakage induced by A β in neurons.

Oxidative stress induced by glutamate plays a key role in cell death during the development of several neuronal-related diseases, and is often associated with an increase of GADD45A mRNA and protein levels (Figure 3).⁹² In mouse hippocampal neuronal cells, p53 genetic ablation suppressed glutamate-induced increase in GADD45A and the subsequent cell death.⁹² The latter was also prevented by *Gadd45a* knockdown. Likewise, excitotoxic injury induced with the N-methyl-D-aspartate receptor agonist quinolinic acid enhanced p53 mRNA and protein expression in brain, together with GADD45A and the proapoptotic BAX, all involved in the response to damage.⁹³ However, GADD45A increase preceded that of p53, suggesting that the latter was not involved in its transcriptional upregulation. GADD45A levels are also induced in neurons of rats with either epileptic seizures or hippocampal oxidative neurotoxicity induced by kainate, thus supporting the hypothesis that GADD45A has a cytoprotective role in the injured brain.^{92,101,127} Actually, GADD45A is regarded as a specific marker of peripheral nerve injury in adult primary sensory and motor neurons, where it plays a role in protecting neurons against cell death.¹⁰² In a similar way, GADD45A protein is induced in the brain following transient ischemia in rodents, probably as a protective response to prevent oxidative stress and genomic DNA damage.^{103,127} In fact, brain injury that occurs as a consequence of an ischemic event is characterized by the presence of both single-stranded and double-stranded DNA breakage, which is followed by an increase of the GADD45A levels in pyramidal neurons of the affected hemisphere.¹⁰⁴ Thus, GADD45A is probably upregulated in response to DNA damage as a mechanism to enhance cell survival after the ischemic insult.^{104,128}

Activity regulated cytoskeletal-associated protein (Arc) is among some of the genes that are transcriptionally demethylated and turned on upon neuronal activation in several neurological and psychiatric disorders. Its expression is associated with synaptic plasticity in learning and memory and in pathological conditions such as depression or AD.¹⁰⁵ Interestingly, studies performed in vitro and in vivo models have reported reduced mRNA expression for all three members of the *Gadd45* family in hippocampal neurons, and have pointed to that GADD45 proteins, by demethylating the immediate early activity gene *Arc*, might become suitable therapeutic targets for pathological conditions such as depression or AD.¹⁰⁵ On the other hand, it has been reported that valproic acid, which is utilized in the treatment of bipolar disorder and works by improving the efficiency of neurotransmission with several neurotrophic effects, induces neurite outgrowth through the JNK-paxillin signaling cascade in a process mediated by GADD45A.¹⁰⁶

4.2 | Regulatory roles of GADD45A in adipose tissue and the liver

Apart from its most well-known actions as stress sensor, GADD45A may also influence metabolism (Figure 3 and Table 1). Transcriptomic analyzes have found a positive correlation *Gadd45a* mRNA expression in adipose tissue and fat deposition and obesity.¹²⁹ GADD45A promotes white adipocyte differentiation and has also a role in regulating energy metabolism of adipose tissue.¹³⁰ In brown adipose tissue, *Gadd45a* suppression resulted in enhanced lipolysis, mitochondrial biogenesis, and cell proliferation, but reduced lipogenesis, while its overexpression displayed an opposite effect.¹²⁹ As a result, *Gadd45a* knockdown in vivo improved insulin sensitivity, glucose uptake, and energy expenditure.¹²⁹ The adipogenic effect of GADD45A is due to the upregulation of the expression of cell cycle related genes, but also to the stimulation of PPAR γ transcriptional activity over genes related to lipid metabolism (*Ppara*, fatty acid binding protein 4, fatty acid synthase, and *Ppar γ* itself, among others).¹²⁹ A very recent study corroborates this adipogenic role for GADD45A, but involving a different mechanism. Thus GADD45A expression positively correlated with subcutaneous fat deposition and obesity in humans and animals, whereas its overexpression in mature adipocytes obtained from porcine and mouse subcutaneous adipose tissue promoted their differentiation and lipid accumulation.¹³¹ In consonance with this, GADD45A deletion in mice rendered them resistant to high-fat diet-induced obesity, and boosted the expression of

mitochondria-related genes. Importantly, these effects required the interaction of GADD45A with STAT1 and subsequent inhibition of the latter.¹³¹

Acetaminophen-induced liver injury (AILI), a disorder characterized by hepatic metabolism dysregulation, induces a rapid increase in GADD45A levels in livers of patients and in vivo and in vitro models, where it exerts an hepatoprotective effect.¹³² For this reason, it has been suggested that *Gadd45a* gene expression in the liver might be used as a biomarker to identify those patients with drug-induced liver injury.¹³³ In particular, and within the context of AILI, GADD45A triggers AMP-activated protein kinase (AMPK) activity, a master regulator of metabolism that induces the expression of genes related to energy-producing pathways, such as glucose uptake, glycolysis, glycogenolysis, fatty acid β -oxidation, and lipolysis, while inhibiting anabolic processes, such as the gluconeogenesis and the biosynthesis of fatty acids, cholesterol, and proteins.¹³² In support of a role for GADD45A, caffeic acid, an organic compound that is capable of attenuating AILI, abrogates the EGR1-mediated increase in GADD45A in the liver.¹³⁴

All three GADD45 family proteins contain at least one LXXLL signature motif, which is known to be involved in protein-protein interactions.¹⁰ Actually, GADD45 proteins are known to interact and activate several nuclear receptors, including constitutive androstane receptor (CAR), ER α , retinoic acid receptor (RAR) α , retinoid X receptor (RXR) α , and the three PPAR subtypes,^{10,135} suggesting that they may act as transcriptional coregulators. GADD45 proteins could be important, for instance, in DNA demethylation processes at specific gene promoter locus, since nuclear receptors, once bound, may recruit the demethylation machinery.⁷⁰

Liver steatosis induced by a high-fat diet feeding in mice is linked to ER stress, which if not corrected may result in hepatocyte apoptosis and excessive lipids liberation, thus contributing to fat accumulation. This is accompanied by increased mRNA expression of *Atf4*, *Chop* (C/EBP homologous protein), and *Gadd45a* in the liver, which is coupled with increased steatosis and inflammation.^{136–138} The increased expression of these genes is aimed to induce apoptosis, to protect cells from excess ER stress.¹³⁷ A recent study has demonstrated that the PERK (protein kinase R-like ER kinase) branch of the unfolded protein response, which is activated during ER stress, boosts hepatocyte apoptosis via ATF4 by regulating *Gadd45a* expression,¹³⁶ while CHOP itself elicits *Gadd45a* gene expression to stimulate apoptosis (Figure 3).¹³⁸ All these effects were prevented by the treatment with the PPAR β/δ agonist GW0742 or the antidiabetic drugs linagliptin, a dipeptidyl peptidase-4 inhibitor, and empagliflozin, an inhibitor of the sodium glucose co-transporter-2.^{137–140} In consonance with this, the GLP-1 receptor agonist semaglutide might be useful in controlling liver steatosis by reducing inflammation, insulin resistance, and ER stress through a mechanism involving GADD45A, and independently of weight loss or any effect on lipogenesis.¹³⁶ In a rodent model of nonalcoholic steatohepatitis induced by methionine- and choline-deficient diet, *Gadd45a* suppression boosted hepatic inflammation, fibrosis, and apoptosis, which were accompanied by disrupted fatty acid metabolism and greater oxidative and ER stress.¹⁴¹ The same study demonstrated that ER stress alone was able to induce *Gadd45a* mRNA expression in hepatocytes, in an attempt to attenuate cellular stress and ensuing inflammatory signaling.

Hepatic fibrosis, which may lead to cirrhosis and hepatocellular carcinoma, is characterized by the accumulation of extracellular matrix proteins, including collagens, proteoglycans, fibronectin, and several glycoproteins. This fibrogenic response is accompanied by increased cell proliferation, hepatic stellate cells activation, hepatocyte apoptosis, and local inflammation (tumor necrosis factor α , TNF- α , and IL-6 rise), overall leading to liver injury and dysfunction. Of note, most of these changes are driven by TGF- β -mediated pathways in a GADD45A-dependent manner (Figure 3). Thus, a significant downregulation of GADD45A, but not GADD45B or GADD45G, together with the activation of the TGF- β signaling pathway was observed in fibrotic liver tissues of mice and isolated hepatic stellate cells with chronic liver injury, while forced overexpression of GADD45A reduced the expression of extracellular matrix proteins and counteracted fibrosis by inhibiting the TGF- β signaling pathway.¹⁴² Scavenging of reactive oxygen species and upregulation of antioxidant enzymes contributed to the beneficial effects of GADD45A on the liver.

4.3 | A critical mediator of muscle atrophy

Muscle atrophy is a complex and debilitating disorder that may be caused by diverse stresses, such as aging, malnutrition, starvation, muscle disuse, muscle denervation, any serious illness or injury. It is often characterized by a reduction in AKT signaling and an increase in Atf4 expression, GSK-3 β signaling, caspase-3 activity, and autophagy. It is worth mentioning that *Gadd45a* expression, which is widely recognized as a major inducer of muscle atrophy, is induced by fasting, aging or disuse in skeletal muscle, in a process at least partially regulated by ATF4 (Figure 3).^{78,143} Ensuing increased nuclear GADD45A levels transcriptionally repress genes involved in anabolic signaling, protein synthesis, glucose uptake, glycolysis, oxygen delivery, mitochondrial biogenesis, citric acid cycle, and oxidative phosphorylation, but induces the mRNA levels of genes involved in autophagy and caspase-mediated proteolysis, overall resulting in muscle atrophy derepression (Table 1).⁷⁸ In fact, GADD45A overexpression in muscle or cultured myotubes also induces atrophy in the absence of any upstream stress.⁷⁸ Interestingly, diabetic patients often develop a specific skeletal muscle pathology characterized by mitochondrial function impairment, oxidative stress and type II fiber atrophy, which are all linked to exercise intolerance.¹⁴⁴ In support of a role for GADD45A in such effects, overexpression of this protein in mice promoted atrophy of type II fibers, while *Gadd45a* expression downregulation by using RNA interference technology provoked the opposite effect.⁷⁸ In fact, type II fibers are more prone to atrophy also during fasting and denervation.⁷⁸

ATF4 is not the only pathway regulating GADD45A in muscle atrophy, but also other potential upstream regulators might be involved, including C/EBP β , FOXO and p53.⁷⁸ C/EBP β may work alone or in cooperation with ATF4 to induce *Gadd45a* expression and muscle atrophy through the binding to specific sites within the promoter of its gene.¹⁴³ In addition, GADD45A probably cooperates with other transcriptional regulators (e.g. FOXO itself, NF- κ B, and myogenin) to drive skeletal muscle gene expression leading to atrophy.⁷⁸ In this regard, muscle atrophy due to accelerated catabolism of proteins is rapidly observed after peripheral nerve transection or spinal cord injury, and GADD45A, together with insulin-like growth factor 1 (IGF-1) and myogenin, are strongly regulated during the initial stages of this process.¹⁴⁵ In any case, beyond this specific role in myofiber atrophy, GADD45A might also have an antiapoptotic effect on these cells, probably by blocking NF- κ B signaling, which is itself activated during atrophy due to denervation.¹⁴⁵

4.4 | GADD45A in cardiovascular diseases: Good or evil?

The pathogenesis of atherosclerosis involves the proliferation of vascular smooth muscle cells (VSMC), a process transcriptionally regulated by the immediately early genes EGR1, FOS/AP-1, and MYC.¹⁴⁶ In early stages, apoptosis of VSMC reduces neointimal thickening and hastens atherosclerosis, but in later stages it may confer greater instability to atherosclerotic plaque. It has been hypothesized that, during atherosclerosis, VSMC growth factors would induce an initial and transient downregulation of the basal *Gadd45a* expression before the beginning of the S phase, which would be followed of a robust increase during the S phase and, lastly, a huge increase after cell division.¹⁴⁶ Regulation of the transcriptional activity of p53 and BRCA1 would account for these changes in *Gadd45a* gene expression (Figure 3). In agreement with this, TNF- α -induced apoptosis of human umbilical vein endothelial cells correlates with increased expression of *Gadd45a* (Table 1).¹⁴⁷ Likewise, PPAR γ activation with the antidiabetic thiazolidinediones troglitazone and rosiglitazone induced apoptosis in endothelial cells, monocyte-derived macrophages, and VSMC, thus reducing neointima formation during the atherosclerotic process.^{11,148} Using genetic approaches or drug treatments, the authors demonstrated that this caspase-mediated apoptosis occurred through a PPAR γ -dependent pathway, and required the activation of p53 and subsequent increase in *Gadd45a* expression, but was independent of BAX and BCL-2 expression.¹¹ In particular, PPAR γ activation by thiazolidinediones led to VSMC apoptosis and growth arrest by inducing POU2F1-mediated transcription of

Gadd45a. In contrast, other authors have reported that these thiazolidinedione-induced proapoptotic effects are independent of PPAR γ activation, although the reasons for this discrepancy are currently unknown.¹⁴⁸

On the other hand, GADD45A acts as an upstream modulator of the pathophysiological changes occurring during pre-eclampsia (Figure 3).¹⁴⁹ Placental stresses during pregnancy, including hypoxia, inflammatory cytokine secretion or hypertonic stress, induce GADD45A and its downstream effector p38 MAPK, both contributing to endothelial activation and the pathogenesis of pre-eclampsia.^{149,150} It has been suggested that silencing *Gadd45a* could be useful in the prevention of pre-eclampsia through a mechanism involving the regulation of *Mmp2* and *Mmp9* expression and the inhibition of oxidative stress, which could promote the invasion and migration of trophoblast cells during early pregnancy.¹⁵¹

GADD45A might also have an important role during heart disease (Figure 3 and Table 1). Adult mammalian cardiomyocytes are refractory to proliferation and, as a consequence, any change in heart size due to stress, increased/decreased workload or disease must occur through hypertrophy or atrophy of the individual cells. Of note, some of the effector pathways regulating cardiomyocyte size are also involved in the transcriptional regulation of *Gadd45a*, including those controlled by NFAT, AKT, FOXO and MAPK.¹⁵² All these pathways regulate protein turnover and, in general, cell hypertrophy takes place when protein synthesis outweighs degradation, whereas the opposite occurs in atrophy. In consonance with this, a recent work aimed to identify differentially expressed genes between hypertrophic cardiomyopathy patients and healthy controls has demonstrated that GADD45A is a hub protein underlying this disease.¹⁵³

The PERK-eIF2 α -ATF4 branch of the ER also intervenes in regulating cardiomyocyte size, since it induces autophagy to resolve accumulation of misfolded proteins, and, as stated above, ATF4 is also relevant in controlling *Gadd45a* transcription.¹⁵² On the other hand, oxidative stress-induced apoptosis in neonatal rat cardiomyocytes and the heart of mice is accompanied by a remarkable increased expression of several p53 upstream regulators, and several downstream effectors such as GADD45A and PUMA, a p53-regulated modulator of apoptosis.^{154,155} Thus, GADD45A, together with p53, is regarded an important contributor toward myocardial apoptosis and ensuing development of heart failure.

GADD45A might also have a role in the cardiac disturbances occurring in people with diabetes. This so-called diabetic cardiomyopathy, which is defined as the myocardial dysfunction that develops in diabetic patients and is not directly attributable to other cardiovascular diseases, is characterized by metabolic disturbances that are often accompanied by local inflammation, oxidative stress, mitochondrial dysfunction, ER stress, cardiomyocyte apoptosis, and fibrosis, which affect cardiac output and eventually lead to heart failure. The diabetic heart exhibits an increase in *Gadd45a* expression, but reduced protein levels, suggesting a strong effect of posttranscriptional mechanisms in regulating GADD45A protein and mRNA levels.⁵⁰ The same study demonstrated that PPAR α , whose activity is often dysregulated in the diabetic heart, interacted with GADD45A, although the functional consequences of these interactions are still unknown.

4.5 | Immunomodulatory effects of GADD45A

Activation of naive T lymphocytes into Th1 cells is a complex process crucial in the adaptive immune system, and in which p38 MAPK plays a crucial role, but that is also dependent on dendritic cells through the secretion of diverse cytokines.¹⁵⁶ Interestingly, GADD45A regulates the activation of both cell types: it induces p38 MAPK in dendritic cells, which favors Th1 polarization, while in T cells it inhibits the alternative activation pathway of this kinase (Table 1).^{156,157} As a result, *Gadd45a* knockout mice display impaired Th1 cell development and production of Th1-related cytokines, since deficient p38 MAPK activation in dendritic cells overcomes increased p38 activity in T cells.¹⁵⁶ Chronic activation of alternatively activated p38 MAPK in T cells in the absence of GADD45A might be involved, for instance, in autoimmune disease development in mice.^{158,159} In a similar way, *Gadd45a* knockout mice are prone to develop an autoimmune disease that resembles human systemic lupus erythematosus (SLE), which

suggests that this protein acts as a negative regulator of T cells.¹⁵⁸ However, another study performed in T cells from patients with SLE demonstrated that GADD45A might contribute to autoantibody formation and autoimmunity by promoting DNA demethylation of CD11a and CD70 regulatory regions in CD4⁺ T cells.¹⁶⁰

Li et al.²⁰ have examined the association of polymorphisms in the promoter region of the *Gadd45a* gene in patients with rheumatoid arthritis (RA) or SLE, since these polymorphisms have been related with an increase in the susceptibility to these and other inflammatory diseases. They found out a specific genotype (−589 CC) that is less susceptible to RA in DR4-negative individuals, and that is also related to the disease state in SLE patients. Although *Gadd45a* gene expression was not modified in peripheral blood mononuclear cells of these RA patients,²⁰ another study has reported a higher expression of *Gadd45a* in the adventitia of the ascending aorta of patients with RA compared to non-RA patients (Table 1).¹⁶¹

In patients with psoriasis, *Gadd45a* is expressed at higher levels in peripheral and in dermal infiltrating T cells, although no differences are found regarding p38 MAPK activity.¹⁵⁷ The same authors reported that the GADD45A target *Uchl1* (ubiquitin carboxyl terminal esterase L1) was hypermethylated at its promoter in psoriatic skin, and that *Uchl1* lower expression fairly correlated with reduced GADD45A levels, overall data pointing to that GADD45A promotes keratinocyte hyper-proliferation and the production of pro-inflammatory mediators in psoriasis patients.¹⁵⁷ Likewise, silencing of *Gadd45a* expression correlates with reduced *Uchl1* levels in endothelial cells due to methylation changes in its promoter.¹⁶² Of note, UCHL1 is capable of inhibiting NF-κB and inducible nitric oxide synthase activity and, thus, its reduction might contribute to the inflammatory process in this pathology.¹⁵⁷

5 | CONCLUDING REMARKS

The stress-responsive GADD45 family consists of three proteins (GADD45A, GADD45B and GADD45G) that are ubiquitously distributed in adult mammalian tissues. However, they display distinctive expression patterns and partially overlapping functions, depending on the causative stress. Similar to the other family members, GADD45A is often associated with stress signaling and cellular injury response by means of its ability to control cell cycle arrest and growth, cell migration, and cell apoptosis, survival and senescence. Through these multiple actions, GADD45A has reportedly been recognized as a tumor suppressor capable of delaying, or even preventing, tumor development. Besides these, the regulation that GADD45A exerts on DNA repair, and even its ability to modulate gene expression through epigenetic mechanisms, might also partly account for its tumor suppressor properties. Anyhow, there are also few studies indicating that GADD45A might operate as a tumor promoter due to some antiapoptotic and pro-survival properties in certain malignancies. Likewise, some authors have reported a positive correlation between the mRNA expression of *Gadd45a* and the development of specific tumors. Thus, overall data indicate that GADD45A may function as either tumor promoter or suppressor, depending on the oncogenic stimuli and the distinct signaling pathways activated. Further studies are warranted to elucidate the effects and potential benefits of GADD45A modulation during tumorigenesis.

Strikingly, more recent studies point to additional roles for GADD45A, although the involved mechanisms are much less studied. For instance, it regulates catabolic and anabolic pathways in many tissues, thus controlling body's overall metabolism. In adipose tissue, GADD45A regulates adipogenesis, lipid accumulation, and thermogenic metabolism, whereas its overexpression in animal models prevents hepatic inflammation, fibrosis, apoptosis, and oxidative and ER stress, while it improves fatty acid metabolism. In this regard, and due to the impact it has on glucose uptake and insulin sensitivity, some authors have pointed to GADD45A might be therapeutically targeted to counteract some of the effects contributing to obesity and diabetes. The transcriptional regulation of genes involved in metabolism, mitochondrial biogenesis and function, autophagy and proteolysis also account for the pivotal role that GADD45A plays during muscle atrophy. Finally, GADD45A has also been reported to display a cytoprotective effect against oxidative stress and inflammation. In this regard, GADD45A dysregulation in the cardiovascular system has been linked to atherosclerosis, or heart failure development due to diverse causes.

However, the opposite is also true, and GADD45A inhibition has been pointed out as a potential strategy to prevent hypertension during early pregnancy.

To summarize, GADD45A is a multifaceted protein whose effects extend beyond controlling cell cycle. Thus, it is not surprising that its mRNA and protein levels are tightly regulated by the combined action of transcriptional, posttranscriptional, and posttranslational mechanisms. In fact, besides the renowned tumor suppressor p53, several other transcription factors have been reported to regulate *Gadd45a* mRNA expression, either directly or indirectly, including ATF4, BRCA1, C/EBP β , EGR1, FOXO3a, NFYA, POU2F1, WT1, and even the pro-inflammatory and profibrotic regulators NF- κ B, AP-1 and NFAT. In fact, NF- κ B and AP-1 are also both involved in the regulation of cell differentiation, proliferation, and apoptosis in response to diverse stimuli. Overall, data presented in this review highlight an important role for GADD45A dysregulation not only in cancer, but also in autoimmune, cardiovascular, metabolic and neurodegenerative disorders. Therefore, on this basis it may be tempting to speculate that the modulation of GADD45A activity might become a potential target for the development of new drugs for the treatment of these diseases. In support of this, GADD45A is considered as an essential step for mediating the anticancer activity of some chemotherapeutic drugs. Just as an example, induction of *Gadd45a* after treatment of prostate cancer cells with a DNA methyltransferase inhibitor increases tumoral cell sensitivity to chemotherapy.⁹⁶ In any case, much more studies are still needed to be able to transfer all this knowledge to clinical practice.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Although not applicable for this review, all original data used for this study will be made available upon reasonable request to the corresponding author.

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