Published in final edited form as:

Crit Rev Oncog. 2011 ; 16(1-2): 129–140.

Gadd45 Stress Sensors in Malignancy and Leukemia

Dan A. Liebermann^{1,2}, Jennifer S. Tront¹, Xiogen Sha¹, Ksushiki Mukherjee², Alisha-Mohamed-Hadley¹, and Barbara Hoffman^{1,2}

¹Fels Institute for Cancer Research and Molecular Biology, Temple University, Philadelphia, PA, USA

²Department of Biochemistry, Temple University, Philadelphia, PA, USA

Abstract

Gadd45 proteins, including Gadd45a, Gadd45b and Gadd45g, have been implicated in stress signaling in response to physiological and environmental stress, including oncogenic stress, which can result in cell cycle arrest, DNA repair, cell survival, senescence, and apoptosis. The function of Gadd45 as a stress sensor is mediated via a complex interplay of physical interactions with other cellular proteins implicated in cell cycle regulation and the response of cells to stress, notably PCNA, p21, cdc2/cyclinB1, and the p38 and JNK stress response kinases. Altered expression of Gadd45 has been observed in multiple types of solid tumors as well as in hematopoietic malignancies. Using genetically engineered mouse models and bone-marrow transplantation, evidence has been obtained indicating that Gadd45 proteins can function to either promote or suppress tumor development and leukemia; this is dependent on the molecular nature of the activated oncogene and the cell type, via engagement of different signaling pathways.

Keywords

Gadd45; Cancer; Leukemia

I. Gadd45 in cellular stress responses

The gadd45 family of genes includes three genes, gadd45a, gadd45b, and gadd45g, whose cognate proteins are key players in cellular stress responses. Gadd45 genes encode for small (18 kDa), evolutionarily conserved proteins that are highly homologous to each other (55–57% overall identity at the amino acid level; Figure.1), are highly acidic, and are localized within both the cell cytoplasm and nucleus.^{1–4}

Gadd45a, originally termed gadd45, was cloned as one of many growth arrest and DNA damage-inducible (gadd) genes that are rapidly induced by UV radiation in Chinese hamster ovary (CHO) cells.² Gadd45b, originally termed MyD118, was cloned in this laboratory, as one of many myeloid differentiation primary response (MyD) genes, induced in the absence of protein synthesis following treatment of M1 myeloblastic leukemia cells with differentiation inducers.⁵ Gadd45g, was cloned in this laboratory using an MyD118 (gadd45b) cDNA probe. It was subsequently found to encode for the murine homologue of human CR6⁴, originally cloned as an immediate early response gene in T cells stimulated by interleukin-2.⁶

Each of the gadd45 genes is expressed in multiple murine tissues including heart, brain, spleen, lung, liver, skeletal muscle, kidney and testes, but at different levels,⁴ yet mice null

for each of these genes are viable.⁷⁻⁸ Furthermore, expression of gadd45a, gadd45b, and gadd45g is induced in response to multiple environmental and physiological stresses, including methylmethanesulfonate (MMS), g-irradiation (IR), ultraviolet radiation (UV), VP-16, daunorubicin (DNR), and inflammatory cytokines.^{3,8-9} In all cases the pattern of expression for each gadd45 gene is unique, consistent with each gadd45 family member playing distinct roles in response to each source of stress. During myeloid differentiation, using either normal bone marrow (BM) stimulated with different hematopoietic cytokines or various hematopoietic cell lines induced to undergo terminal myeloid differentiation, each gadd45 gene has a distinctive pattern of expression. Consistent with the distinctive expression patterns, regulation of expression for each gadd45 gene is unique. For instance, gadd45a is a p53 target gene, although its induction can also be p53 independent, whereas gadd45b is a primary response gene to both IL-6 and TGF- β , and gadd45g is induced as a primary response to IL-2 and IL-6.^{1,4,6,10-11}

Gadd45 proteins have been shown to participate in cell cycle arrest, DNA repair, cell survival and apoptosis in response to environmental and physiological stress, as well as having a role in development and carcinogenesis. Gadd45 function by interacting with and modulating the structure/function of partner proteins that are implicated in cell cycle regulation and the response of cells to stress (Figure. 2). The ultimate biological outcome is highly dependent on the magnitude and type of stress stimulus as well as cell type.¹² Proteins that interact with Gadd45 include PCNA and histones, cdk1, p21, MEKK4, MKK7 and p38. There is evidence that interaction of the Gadd45 proteins with PCNA promotes nucleotide excision repair (NER) of DNA.¹³⁻¹⁶ Interaction with PCNA and/or histones may also play a role in epigenetic gene activation by repair-mediated DNA demethylation.¹⁷⁻²¹ Interaction with the cdc2/cyclinB1 complex inhibits the kinase activity of the complex, thereby resulting in G2/M cell cycle arrest.²²⁻²³ It has been suggested that when Gadd45a interacts with p21 it serves to augment its CDKI activity, promoting G1 arrest.^{16,24} Association of Gadd45 proteins with MEKK4 and p38 results in their activation.¹² It was recently reported that the MEKK4 N-terminus binds to its C-terminal segment, thereby inhibiting the C-terminal kinase domain, and that binding of Gadd45 to the MEKK4 N-terminal Gadd45-binding site disrupts this N-C binding, resulting in kinase activation.²⁵ Gadd45 binding can also induce dimerization of MEKK4.²⁵ Gadd45 proteins have also been implicated in activation or repression of JNK activity via interaction with and modulation of the structure/function of upstream kinase modulators^{9,26-32}, thereby promoting apoptosis or cell survival depending on the stimulus and/or cell type. In this context, Gadd45b is a major player in the endogenous NF- κ B-mediated resistance to apoptosis in a variety of cell lines. In fibroblasts this mechanism involves activation of MKK7,²⁹ upstream of JNK, by direct binding with the kinase ATP pocket. Evidence supports the existence of a large complex containing an MKK7-Gadd45b:Gadd45b-MKK7 tetrameric unit whose complexity could be further increased by the dimeric nature of the isolated MKK7.³¹ Using the Technical University of Denmark web site (linked to Entrez), Gadd45 proteins were observed to harbor several threonine and tyrosine phosphorylation sites, as well as a sites which are amenable for acetylation. How may post-translational modifications of Gadd45 proteins alter their function has not been documented, and is currently under investigation. Finally, it is important to note that Gadd45 proteins can form homo- and hetero-oligomers with different family members; however their role in mediating Gadd45 functions has not been established.^{12,33}

If protein-protein interactions govern the many functions of the Gadd45 family of proteins, it can be asked what determines these interactions. It is predicted that the interaction of Gadd45 with its partner protein is regulated by level of expression, cellular localization, and posttranslational modifications of both the Gadd45 proteins and their interacting partners,

which in turn may be determined by the type and magnitude of the stress stimulus and the cell type.

II. Gadd45 as sensors of oncogenic stress that modulate tumor development

The complex role of stress sensors in monitoring oncogenic stress and impacting on tumor development is not fully understood. The best and most studied example of oncogenic stress sensors in tumorigenesis is p53 and its varied cellular functions. Recent observations have implicated Gadd45 proteins as important sensors of oncogenic stress, both *in vitro* and *in vivo*.

It is known that whereas primary mouse cells require introduction of two activated oncogenes for transformation, disruption of certain key growth control genes allows single oncogene transformation.^{34–35} It was shown that for MEFs obtained from Gadd45a^{−/−} mice, H-ras was sufficient for transformation.^{7, 36} The role Gadd45b and/or Gadd45g play in susceptibility of MEFs to single oncogene transformation remains to be assessed.

Evidence was obtained that Gadd45 proteins also play a role in modulation of tumor development *in vivo*. Gadd45a^{−/−} and Gadd45b^{−/−} mice were observed to display increased mutation frequency, and susceptibility to ionizing radiation (IR) and chemical carcinogenesis⁷ (and unpublished). Also, it was documented that NF-κB-mediated repression of Gadd45a and Gadd45g is essential for cancer cell survival.³⁷

The exact frequency of mutations in Gadd45 family members in different types of cancer, remains to be established. Nevertheless, reduced expression of the three Gadd45 family members due to promoter methylation has been frequently observed in several types of human cancer. The Gadd45a promoter is methylated in the majority of breast cancers, resulting in reduced expression when compared with normal breast epithelium.³⁸ In pituitary adenomas, silencing of the Gadd45g gene is seen in 67% of patients. This down-regulation is primarily associated with methylation of the Gadd45g gene and reversal of this epigenetic change results in re-expression of the protein.³⁹ Gadd45g is also down-regulated in anaplastic thyroid cancer and in 65% of hepatocellular carcinomas due to hypermethylation of the Gadd45g promoter.⁴⁰ In another study, all three Gadd45 genes were observed to be methylated and silenced in hepatocellular carcinoma, indicating a strong linkage between Gadd45 gene expression and liver cancer.⁴¹ Furthermore, down-regulation of Gadd45b expression by hepatitis C virus was observed to lead to defective cell cycle arrest. Hypermethylation of the Gadd45b promoter in the presence of HCV was shown to be responsible for this defect, and has been correlated to HCV-associated hepatocellular carcinomas.⁴² Ying and colleagues analyzed the methylation status of two regions in the Gadd45g promoter in a total of 75 cell lines as well as primary tissues and tumors.⁴³ They show that promoter hypermethylation is frequently detected in tumors cell lines, including 85% of non-Hodgkin, 50% of Hodgkin lymphoma, 73% of naso-pharyngeal, 50% of cervical, 29% of esophageal, and 40% of lung carcinoma but not in immortalized normal epithelial cell lines, normal tissues, or peripheral blood mononuclear cells. To gain more insight into the Gadd45g methylation, they also performed high-resolution bisulfite genomic sequencing. They found that densely methylated CpG sites were detected in all silenced cell lines, indicating that epigenetic silencing of Gadd45g could be involved in the pathogenesis of tumors. A methylation-mediated repression of Gadd45a was observed also in prostate cancer.⁴⁴ The role of Gadd45a as a potential therapeutic target has been highlighted by the fact that it is up-regulated on docetaxel treatment and may contribute to docetaxel-mediated cytotoxicity of prostate cancer cells.⁴⁴ Other observations have shown that activated NF-κB leads to repression of GADD45a and GADD45g in various types of cancer.³⁷ Thus,

constitutive activation of NF- κ B in cancers and/or promoter methylation may co-operate to suppress Gadd45 genes in cancer.

Abnormal Gadd45a expression has been documented in pancreatic cancer. One study has shown that gadd45a expression is elevated in several pancreatic ductal adenocarcinoma cell lines, and loss of Gadd45a expression limits growth and survival of one cell line in culture.⁴⁵ In another study, it was observed that ectopic expression of Gadd45a in the PANC1 pancreatic cancer cell line resulted in apoptosis and cell cycle arrest.⁴⁶ These two studies suggest contradictory roles of gadd45a in pancreatic tumor cell growth and survival. Nevertheless, a study in Japan attempted to correlate expression of Gadd45a and p53 inactivation in human pancreatic cancer.⁴⁷ This is important since gadd45a is a p53 target gene, though it has been also shown to be expressed also independently of p53. Interestingly, elevated Gadd45a expression levels were reported in 54% of human pancreatic ductal carcinomas and the frequency of point mutations was found to be almost 14%.⁴⁷ Moreover, over-expression of Gadd45a protein, along with possible p53 loss of function, significantly contributed to poor prognosis, compared with patients with undetectable Gadd45a expression levels.⁴⁷ It was observed that in resectable invasive pancreatic ductal carcinomas, Gadd45a is frequently mutated, and this mutation combined with the p53 status affects the survival of these patients.⁴⁷ We have observed that inhibition of endogenous gadd45a expression in the PANC1 cell line by shRNA limits cell number, due to cell cycle arrest &/or apoptosis.⁴⁸ As such, further investigation is needed to better define a role for Gadd45a and other family members in pancreatic cancer development.

Recent work, conducted in this laboratory, has highlighted the role of Gadd45 proteins, notably Gadd45a, as sensors of oncogenic stress in breast carcinogenesis.^{49–50} Generation and side by side analysis of MMTV-Myc versus MMTV-Ras mice strains, either wild-type or null for gadd45a, have highlighted a unique role for Gadd45a as either a suppressor or promoter of breast cancer development, via employment of distinct signaling pathways in response to distinct oncogene stressors (Figure 3). Our data indicate that the Gadd45a tumor suppressor function, mediated via activation of JNK and p38 stress kinases, contributes to Ras-induced apoptosis and senescence, respectively, and is a unique response to Ras oncogenic stress. In contrast, the tumor promoter function of Gadd45a, mediated through negative regulation of MMP10 expression via the GSK3b / b-catenin signaling cascade, results in increased tumor vascularization, and is a unique response to oncogenic Myc. These novel findings indicate that Gadd45a can function to either promote or suppress breast tumor development through engagement of different signaling pathways, depending on the molecular nature of the activated oncogene.

III. Gadd45 in Leukemia

Expression of gadd45 is rapidly induced by different stressors in hematopoietic cells; this includes differentiation-inducing cytokines and genotoxic agents. Induction of gadd45 genes at the onset of myeloid differentiation suggested that Gadd45 protein(s) play a role in hematopoiesis, yet no apparent abnormalities were observed in either the bone marrow (BM) or peripheral blood compartments of mice deficient for either gadd45a or gadd45b. However, under conditions of hematological stress, including acute stimulation with cytokines, myelo-ablation, inflammation, and genotoxic stress both gadd45a-deficient and gadd45b-deficient mice exhibit abnormal responses.^{8,9,32,51}

The role Gadd45 proteins play as sensors of oncogenic stress and their regulated expression during development of hematopoietic cells and upon response to stress, suggest that altered expression may play a role in leukemogenesis. Recent documented observations, as well as unpublished work conducted in this laboratory, support this notion.

Activating mutations in FLT3 (Fms-like tyrosine kinase) are among the most common genetic lesions in AML, occurring in approximately 30–35% of AML cases.⁵² The most common mutation is an internal tandem duplication (ITD) in the intracellular, juxtamembrane domain present in approximately 20–25% of patients. In a recent study GADD45A mRNA levels were measured in a panel of AML samples to determine the association with the FLT3-ITD mutation.⁵³ It was observed that Gadd45a expression levels were significantly lower in FLT3-ITD⁺ AML than in FLT3-ITD⁻ AML, which is consistent with FLT3-ITD-induced down-regulation of Gadd45a in human AML. Furthermore, Gadd45a expression data for primary AML samples, extracted from a published microarray study,⁵⁴ indicate significant lower expression of Gadd45a relative to normal controls, in a number of AML clusters defined by their gene expression signature, including a cluster consisting only of patients with FLT3-ITD mutations. Down-regulation in a cluster characterized by a t(11q23) rearrangement (predominantly MLL-AF9), may be via a related mechanism as 11q23 events have been associated with increased FLT3 expression in several studies.^{55–56} Lower expression of Gadd45a was observed also in a cluster characterized by the presence of t(8;21) translocations involving the AML-ETO fusion oncoprotein.

Based on observations made with breast cancer mouse models, where Gadd45a was found to behave as a tumor suppressor in response to H-RAS, and as an oncogene in response to Myc^{49–50} (Figure. 2), this laboratory embarked on assessing if and how Gadd45 proteins may modulate leukemogenesis involving constitutive ras signaling, BCR/ABL signaling or deregulated myc expression.

Ras mutations occur at a frequency of 25% in AML, 30% in myeloma and 6–20% in ALL. The highest incidence however is in MDS where the frequency is about 30–40%, with highest frequency (50–70%) in the CMML subset.⁵⁷ The most common mutations are found in N-RAS (~30%), less frequently in K-RAS (~15%), and most rare in H-RAS.^{57–59} Recently, evidence was obtained that oncogenic N-RAS, K-RAS, and H-RAS exhibit different leukemogenic potentials in mice,⁶⁰ with N-RAS inducing either an AML- or CMML-like disease and H-RAS always an AML-like disease, whereas K-RAS invariably induces a CMML-like disease. Using bone marrow transplantation (BMT) in mice, data obtained in this laboratory (unpublished), indicate that loss of Gadd45a impeded N-RAS-driven leukemia. This is in contrast to the tumor suppressor function of Gadd45a in H-RAS driven breast cancer.^{49–50} These observations are in line with the hypothesis that the nature of the Gadd45 oncogenic stress sensor function depends upon the biological setting, including cell type, developmental stage and stress/stimulus. Thus, further understanding the role of Gadd45 proteins as mediators of oncogenic Ras signaling in the context of Ras-driven leukemogenesis, and extending the work to human patients is highly critical.

BCR-ABL is known as the most common translocation in the myeloproliferative (MPD) disorder chronic myelogenous leukemia (CML); it is the first leukemia to be described^{61–62} that is associated with a consistent cytogenetic abnormality, termed the Philadelphia chromosome (Ph1).⁶² The Philadelphia translocation is an acquired somatic mutation in the hematopoietic stem cell,⁶³ and results in fusion of the ABL gene (225 kb) from chromosome 9 to the BCR gene (135 kb) on chromosome 22.^{64–68} The chromosome 9 breakpoint involves a large (200 kb) region within the ABL alternative first exons (1a & 1b) that results in fusion genes that incorporate ABL exon 2.⁶⁴ The breakpoints on chromosome 22 are clustered within three much smaller regions of the BCR gene.⁶⁵ A chimeric mRNA (8.5 kb) is translated to an activated BCR-ABL oncoprotein most commonly 210 kD in size.^{66–67} BCR-ABL is known to localize to the cytoskeleton and to display constitutively active tyrosine kinase activity that leads to the recruitment of downstream effectors of cell proliferation and survival, via several adapter proteins (e.g., GRB2, GAB2, CRKL) and signaling pathways (e.g., RAS, PI3K, JAK-STAT, PDK2-NFkB), all thought to contribute to

the pathogenesis of CML.⁶⁹ Cross-talk signaling between Bcr-Abl/Ras/Gadd45 is depicted in Figure.4. The complex nature by which these signaling pathways contribute to the initiation and progression of CML is only partially understood. Data obtained in this laboratory indicates that loss of either Gadd45a or Gadd4b accelerates the development of BCR-ABL-driven leukemia in mouse BM transplantation studies, and Gadd45 expression was observed to be altered in human CML samples, correlating with disease progression, thereby identifying Gadd45 as a tumor suppressor in the context of BCR-ABL driven-leukemia (unpublished). Clearly elucidating the role Gadd45 plays as a tumor suppressor in the context of BCR-ABL driven leukemia, and what signaling pathways and downstream effectors are modulated by Gadd45 to suppress leukemogenesis is important to better understand the molecular pathology of CML.

Finally, elevated Myc expression is associated with many leukemias, as well as solid tumors including breast (45%), colon (67%), gastric (47%), ovarian (44%), prostate (70%) and medulloblastoma (35%).⁷⁰ Multiple extracellular and intracellular signaling cascades converge to regulate the Myc oncogene.⁷¹ Deregulation of Myc is not restricted to gross genetic abnormalities of the Myc gene, but can also occur as a consequence of direct or indirect mutations of regulatory molecules controlling myc gene expression. This is exemplified by the observations that Myc deregulation is associated with its amplification as well as by activation by other oncogenes associated with myeloid leukemia, including AML1-ETO, PML-RARA, and PLZF-RAR, activating mutations in the FLT3 receptor tyrosine kinase (associated with one-third of AML patients), and BCR-ABL.⁷²⁻⁷⁷ In mouse models, myc was observed to rapidly induce acute myeloid leukemia without evidence of lymphoma-associated antiapoptotic mutations, and McI1 haploinsufficiency was found to protect the mice from myc-induced acute myeloid leukemia.⁷⁸⁻⁷⁹ Data obtained in this laboratory, has shown that loss of gadd45a in myc over-expressing myeloid cells reduced apoptosis, thereby increasing proliferation (data unpublished). This data is consistent with Gadd45a playing a tumor suppressor role in modulating leukemia associated with elevated Myc (unpublished). Thus understanding how Gadd45 proteins modulate myc-driven leukemogenesis, and extending these observations to human AML is important.

CONCLUDING REMARKS

Altered expression of Gadd45 has been observed in multiple types of solid tumors as well as in hematopoietic malignancies. Using genetically engineered mouse models, accumulated evidence indicates that the function of Gadd45 proteins to either promote or suppress tumor development and leukemia depends on the molecular nature of the activated oncogene and the cell type, engaging different signaling pathways. Further research is aimed at better understanding how Gadd45 proteins interface with different signaling cascades to either suppress or promote tumor development and leukemia in response to distinct oncogenic stressors. Thus Gadd45 proteins, and their signaling targets, may represent a novel class of molecules for therapeutic intervention in cancer and leukemia.

Acknowledgments

Supported By NIH grants 1R01HL084114-01 (DAL), 1R01CA122376-01 (DAL), NIH, RO1 CA081168-06 (BH).

Abbreviations

MMS	methylnmethanesulfonate
IR	g-irradiation
UV	ultraviolet radiation

DNR	daunorubicin
BM	bone marrow
HCV	Hepatitis C Virus

References

- Carrier F, Smith ML, Bae I, Kilpatrick KE, Lansing TJ, Chen CY, Engelstein M, Friend SH, Henner WD, Gilmer TM, Kastan MB, Fornace AJ Jr. Characterization of human Gadd45, a p53-regulated protein. *J Biol Chem*. 1994; 269:32672–32677. [PubMed: 7798274]
- Zhan Q, Lord KA, Alamo I Jr, Hollander MC, Carrier F, Ron D, Kohn KW, Hoffman B, Liebermann DA, Fornace AJ Jr. The gadd and MyD genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth. *Mol Cell Biol*. 1994; 14:2361–2371. [PubMed: 8139541]
- Liebermann DA, Hoffman B. MyD genes in negative growth control. *Oncogene*. 1998; 17:3319–3330. [PubMed: 9916994]
- Zhang W, Bae I, Krishnaraju K, Azam N, Fan W, Smith K, Hoffman B, Liebermann DA. CR6: A third member in the MyD118 & Gadd45 gene family which functions in negative growth control. *Oncogene*. 1999; 18:4899–4907. [PubMed: 10490824]
- Abdollahi A, Lord KA, Hoffman-Liebermann B, Liebermann DA. Sequence and expression of a cDNA encoding MyD118: A novel myeloid differentiation primary response gene induced by multiple cytokines. *Oncogene*. 1991; 6:165–167. [PubMed: 1899477]
- Beadling C, Johnson KW, Smith KA. Isolation of interleukin 2-induced immediate-early genes. *Proc Natl Acad Sci USA*. 1993; 90:2719–2723. [PubMed: 7681987]
- 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, Fernandez-Salguero PM, Morgan WF, Deng CX, Fornace AJ Jr. Genomic instability in Gadd45a-deficient mice. *Nat Genet*. 1999; 23:176–184. [PubMed: 10508513]
- Gupta M, Gupta S, Balliet AG, Hollander MC, Fornace AJ, Hoffman B, Liebermann DA. Hematopoietic cells from Gadd45a and Gadd45b deficient mice are sensitized to genotoxic-stress induced apoptosis. *Oncogene*. 2005; 24:7170–7179. [PubMed: 16170381]
- Gupta S, Gupta M, Hoffman B, Liebermann DA. Hematopoietic cells from gadd45a deficient and gadd45b deficient mice exhibit impaired stress responses to acute stimulation with cytokines, myeloablation, and inflammation. *Oncogene*. 2006; 25:5537–5546. [PubMed: 16732331]
- Selvakumaran M, Lin HK, Tjin Tham Sjin R, Reed J, Liebermann DA, Hoffman B. The novel primary response gene MyD118 and the proto-oncogenes myb, myc and bcl-2 modulate transforming growth factor b1-induced apoptosis of myeloid leukemia cells. *Mol Cell Biol*. 1994; 14:2352–2360.
- Yoo J, Ghiassi M, Jirmanova L, Balliet AG, Hoffman B, Fornace AJ Jr, Liebermann DA, Bottinger EP, Roberts AB. TGF-beta-induced apoptosis is mediated by Smad-dependent expression of GADD45B through p38 activation. *J Biol Chem*. 2003; 278:43001–43007. [PubMed: 12933797]
- Liebermann DA, Hoffman B. Gadd45 in stress signaling. *J Mol Signal*. 2008; 3:15. [PubMed: 18789159]
- Smith ML, Kontny HU, Zhan Q, Sreenath A, O'Connor PM, Fornace AJ Jr. Antisense GADD45 expression results in decreased DNA repair and sensitizes cells to u.v.-irradiation or cisplatin. *Oncogene*. 1996; 13:2255–2263. [PubMed: 8950993]
- Vairapandi M, Azam N, Balliet AG, Hoffman B, Liebermann DA. Characterization of MyD118, Gadd45, and PCNA interacting domains: PCNA impedes MyD/Gadd mediated negative growth control. *J Biol Chem*. 2000; 275:16810–16819. [PubMed: 10828065]
- Azam N, Vairapandi M, Zhang W, Hoffman B, Liebermann DA, Azam N, Vairapandi M, Zhang W, Hoffman B, Liebermann DA. Interaction of CR6 (GADD45gamma) with proliferating cell nuclear antigen impedes negative growth control. *J Biol Chem*. 2001; 276:2766–2774. [PubMed: 11022036]

16. Liebermann DA, Hoffman B. Gadd45 in the response of hematopoietic cells to genotoxic stress. *Blood Cells Mol Dis.* 2007; 39:329–335. [PubMed: 17659913]
17. Barreto G, Schäfer A, Marhold J, Stach D, Swaminathan SK, Handa V, Döderlein G, Maltry N, Wu W, Lyko F, Niehrs C. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature.* 2007; 445:671–675. [PubMed: 17268471]
18. Schmitz KM, Schmitt N, Hoffmann-Rohrer U, Schäfer A, Grummt I, Mayer C. TAF12 recruits Gadd45a and the nucleotide excision repair complex to the promoter of rRNA genes leading to active DNA demethylation. *Mol Cell.* 2009; 33:344–353. [PubMed: 19217408]
19. Engel N, Tront JS, Erinle T, Nguyen N, Latham KE, Sapienza C, Hoffman B, Liebermann DA. Conserved DNA methylation in Gadd45a(–/–) mice. *Epigenetics.* 2009; 4:98–9. [PubMed: 19229137]
20. Rai K, Huggins IJ, James SR, Karpf AR, Jones DA, Cairns BR. DNA demethylation in zebrafish involves the coupling of a deaminase, a glycosylase, Gadd45. *Cell.* 2008; 135:1201–1212. [PubMed: 19109892]
21. Ma DK, Jang MH, Guo JU, Kitabatake Y, Chang ML, Pow-Anpongkul N, Flavell RA, Lu B, Ming GL, Song H. Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science.* 2009; 323:1074–1077. [PubMed: 19119186]
22. Wang XW, Zhan Q, Coursen JD, Khan MA, Kontny HU, Yu L, Hollander MC, O'Connor PM, Fornace AJ Jr, Harris CC. GADD45 induction of a G2/M cell cycle checkpoint. *Proc Natl Acad Sci USA.* 1999; 96:3706–3711. [PubMed: 10097101]
23. Vairapandi M, Balliet AG, Hoffman B, Liebermann DA. 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.* 2002; 192:327–338. [PubMed: 12124778]
24. Fan W, Richter G, Cereseto A, Beadling C, Smith KA. Cytokine response gene 6 induces p21 and regulates both cell growth and arrest. *Oncogene.* 1999; 18:6573–6582. [PubMed: 10597261]
25. Miyake Z, Takekawa M, Ge Q, Saito H. Activation of MTK1/MEKK4 by GADD45 through induced N-C dissociation and dimerization-mediated trans autophosphorylation of the MTK1 kinase domain. *Mol Cell Biol.* 2007; 27:2765–2776. [PubMed: 17242196]
26. Papa S, Zazzeroni F, Bubici C, Jayawardena S, Alvarez K, Matsuda S, Nguyen DU, Pham CG, Nelsbach AH, Melis T, De Smaele E, Tang WJ, D'Adamio L, Franzoso G. Gadd45 beta mediates the NF-kappa B suppression of JNK signalling by targeting MKK7/JNKK2. *Nat Cell Biol.* 2004; 6:146–153. [PubMed: 14743220]
27. Takekawa M, Saito H. A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4/MAPKKK. *Cell.* 1998; 95:521–30. [PubMed: 9827804]
28. Hildesheim J, Bulavin DV, Anver MR, Alvord WG, Hollander MC, Vardanian L, Fornace AJ Jr. Gadd45a protects against UV irradiation-induced skin tumors, and promotes apoptosis and stress signaling via MAPK and p53. *Cancer Res.* 2002; 62:7305–7315. [PubMed: 12499274]
29. Zhu N, Shao Y, Xu L, Yu L, Sun L. Gadd45-alpha and Gadd45-gamma utilize p38 and JNK signaling pathways to induce cell cycle G2/M arrest in Hep-G2 hepatoma cells. *Mol Biol Rep.* 2009; 36:2075–2085. [PubMed: 19048389]
30. Amanullah A, Azam N, Balliet A, Hollander C, Hoffman B, Fornace A, Liebermann D. Cell signalling: Cell survival and a Gadd45-factor deficiency. *Nature.* 2003; 424:741–742. [PubMed: 12917673]
31. Tornatore L, Marasco D, Dathan N, Vitale RM, Benedetti E, Papa S, Franzoso G, Ruvo M, Monti SM. Gadd45 beta forms a homodimeric complex that binds tightly to MKK7. *J Mol Biol.* 2008; 378:97–111. [PubMed: 18343408]
32. Gupta M, Gupta S, Hoffman B, Liebermann DA. Gadd45a and gadd45b protect hematopoietic cells from ultraviolet radiation induced apoptosis via distinct signaling pathways. *J Biol Chem.* 2006; 281:17552–17558. [PubMed: 16636063]
33. Kovalsky O, Lung FD, Roller PP, Fornace AJ Jr. Oligomerization of human Gadd45a protein. *J Biol Chem.* 2001; 276:39330–39339. [PubMed: 11498536]
34. Lundberg AS, Hahn WC, Gupta P, Weinberg RA. Genes involved in senescence and immortalization. *Curr Opin Cell Biol.* 2000; 12:705–709. [PubMed: 11063935]

35. Drayton S, Peters G. Immortalisation and transformation revisited. *Curr Opin Genet Dev.* 2002; 12:98–104. [PubMed: 11790562]
36. Bulavin DV, Kovalsky O, Hollander MC, Fornace AJ Jr. Loss of oncogenic H-ras-induced cell cycle arrest and p38 mitogen-activated protein kinase activation by disruption of Gadd45a. *Mol Cell Biol.* 2003; 23:3859–3871. [PubMed: 12748288]
37. Zerbini LF, Wang Y, Czibere A, Correa RG, Cho JY, Ijiri K, Wei W, Joseph M, Gu X, Grall F, Goldring MB, Zhou JR, Liebermann TA. 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 USA.* 2004; 101:13618–13623. [PubMed: 15353598]
38. Wang W, Huper G, Guo Y, Murphy SK, Olson JA Jr, Marks JR. Analysis of methylation-sensitive transcriptome identifies GADD45a as a frequently methylated gene in breast cancer. *Oncogene.* 2005; 24:2705–14. [PubMed: 15735726]
39. Bahar A, Bicknell JE, Simpson DJ, Clayton RN, Farrell WE. Loss of expression of the growth inhibitory gene GADD45gamma, in human pituitary adenomas, is associated with CpG island methylation. *Oncogene.* 2004; 23:93–44.
40. Sun L, Gong R, Wan B, Huang X, Wu C, Zhang X, Zhao S, Yu L. GADD45g, down-regulated in 65 % hepatocellular carcinoma (HCC) from 23 Chinese patients, inhibits cell growth and induces cell cycle G2/M arrest for hepatoma Hep-G2 cell lines. *Mol Biol Rep.* 2003; 30:249–53. [PubMed: 14672412]
41. Na YK, Lee SM, Hong HS, Kim JB, Park JY, Kim DS. Hypermethylation of growth arrest DNA-damage-inducible gene 45 in non-small cell lung cancer and its relationship with clinicopathologic features. *Mol Cells.* 2010; 30:89–92. [PubMed: 20652500]
42. Higgs MR, Lerat H, Pawlowsky JM. Downregulation of Gadd45beta expression by hepatitis C virus leads to defective cell cycle arrest. *Cancer Res.* 2010; 70:4901–11. [PubMed: 20530689]
43. Ying J, Srivastava G, Hsieh WS, Gao Z, Murray P, Liao SK, Ambinder R, Tao Q. The stress-responsive gene GADD45G is a functional tumor suppressor, with its response to environmental stresses frequently disrupted epigenetically in multiple tumors. *Clin Cancer Res.* 2005; 11:6442–9. [PubMed: 16166418]
44. Ramachandran K, Gopisetty G, Gordian E, Navarro L, Hader C, Reis IM, Schulz WA, Singal R. Methylation-mediated repression of GADD45a in prostate cancer and its role as a potential therapeutic target. *Cancer Res.* 2009; 69:1527–35. [PubMed: 19190346]
45. Schneider G, Weber A, Zechner U, Oswald F, Friess HM, Schmid RM, Liptay S. Gadd45a is highly expressed in pancreatic ductal adenocarcinoma cells and required for tumor cell viability. *Int J Cancer.* 2006; 11:2405–2411. [PubMed: 16353139]
46. Li Y, Qian H, Li X, Wang H, Yu J, Liu Y, Zhang X, Liang X, Fu M, Zhan Q, Lin C. Adenoviral-mediated gene transfer of Gadd45a results in suppression by inducing apoptosis and cell cycle arrest in pancreatic cancer cell. *J Gene Med.* 2009; 11:3–13. [PubMed: 19003803]
47. Yamasawa K, Nio Y, Dong M, Yamaguchi K, Itakura M. Clinicopathological significance of abnormalities in Gadd45 expression and its relationship to p53 in human pancreatic cancer. *Clin Cancer Res.* 2002; 8:2563–2569. [PubMed: 12171884]
48. Cretu A, Sha X, Tront J, Hoffman B, Liebermann DA. Stress Sensor Gadd45 genes as Therapeutic Targets in Cancer. *Cancer Therapy.* 2009; 7(A):268–276. [PubMed: 19652693]
49. Tront JS, Hoffman B, Liebermann DA. Gadd45a Suppresses Ras-driven Mammary Tumorigenesis by Activation of JNK and p38 Stress Signaling Resulting in Apoptosis and Senescence. *Cancer Res.* 2006; 66:8448–8454.
50. Tront JS, Huang Y, Fornace A, Hoffman B, Liebermann DA. Gadd45a Promotes or Suppresses Breast Cancer Dependent on the Oncogenic Stress. *Cancer Research.* 2010; 70:9671–9681. [PubMed: 21098706]
51. Hoffman B, Liebermann DA. Gadd45 modulation of intrinsic and extrinsic stress responses in myeloid cells. *J Cell Physiol.* 2009; 218:26–31. [PubMed: 18780287]
52. Parcells BW, Ikeda AK, Simms-Waldrup T, Moore TB, Sakamoto KM. FMS-like tyrosine kinase 3 in normal hematopoiesis and acute myeloid leukemia. *Stem Cells.* 2006; 24:1174–1184. [PubMed: 16410383]

53. Perugini M, Kok CH, Brown AL, Wilkinson CR, Salerno DG, Young SM, Diakiw SM, Lewis ID, Gonda TJ, D'Andrea RJ. Repression of Gadd45a by activated FLT3 and GM-CSF receptor mutants contributes to growth, survival and blocked differentiation. *Leukemia*. 2009; 23:729–38. [PubMed: 19151789]
54. Valk PJ, Verhaak RG, Beijen MA, Erpelinck CA, Barjesteh van Waalwijk van Doorn-Khosrovani S, Boer JM, Beverloo HB, Moorhouse MJ, van der Spek PJ, Löwenberg B, Delwel R. Prognostically useful gene-expression profiles in acutemyeloid leukemia. *N Engl J Med*. 2004; 350:1617–1628. [PubMed: 15084694]
55. Kuchenbauer F, Kern W, Schoch C, Kohlmann A, Hiddemann W, Haferlach T, Schnittger S. Detailed analysis of FLT3 expression levels in acute myeloid leukemia. *Haematologica*. 2005; 90:1617–1625. [PubMed: 16330434]
56. Ono R, Nakajima H, Ozaki K, Kumagai H, Kawashima T, Taki T, Kitamura T, Hayashi Y, Nosaka T. Dimerization of MLL fusion proteins and FLT3 activations synergize to induce multiple-lineage leukemogenesis. *J Clin Invest*. 2005; 115:919–929. [PubMed: 15761502]
57. Ikeda A, Shankar DB, Watanabe M, Tamanoi F, Moore TB, Sakamoto KM. Molecular targets and the treatment of myeloid leukemia. *Molecular Genetics and Metabolism*. 2006; 88:216–224. [PubMed: 16678459]
58. Reimann C, Arola M, Bierings M, Karow A, van den Heuvel-Eibrink MM, Hasle H, Niemeyer CM, Kratz CP. A novel somatic K-Ras mutation in juvenile myelomonocytic leukemia. *Leukemia*. 2006; 20:1637–8. [PubMed: 16826224]
59. Miyauchi J, Asada M, Sasaki M, Tsunematsu Y, Kojima S, Mizutani S. Mutations of the N-ras gene in juvenile chronic myelogenous leukemia. *Blood*. 1994; 83:2248–2254. [PubMed: 8161790]
60. Parikh C, Subrahmanyam R, Ren R. Oncogenic NRAS, KRAS, and HRAS Exhibit Different Leukemogenic Potentials in Mice. *Cancer Res*. 2007; 67:71397–146.
61. Virchow R. Weisses Blut. Froiep's Notzien. 1845; 36:151.
62. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. *Journal of the National Cancer Institute*. 1960; 25:85–109. [PubMed: 14427847]
63. Fialkow PJ, Gartler SM, Yoshida A. Clonal origin of chronic myelocytic leukemia in man. *Proc Natl Acad Sci USA*. 1967; 58:1468–1471. [PubMed: 5237880]
64. Bernards A, Rubin CM, Westbrook CA, Paskind M, Baltimore D. The first intron in the human c-abl gene is at least 200 kilobases long and is a target for translocations in chronic myelogenous leukemia. *Mol Cell Biol*. 1987; 7:3231–3236. [PubMed: 3313010]
65. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell*. 1984; 36:93–99. [PubMed: 6319012]
66. de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, Spurr NK, Heisterkamp N, Groffen J, Stephenson JR. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. *Nature*. 1982; 300:765–767. [PubMed: 6960256]
67. Heisterkamp N, Stam K, Groffen J, de Klein A, Grosveld G. Structural organization of the bcr gene and its role in the Ph' translocation. *Nature*. 1985; 315:758–761. [PubMed: 2989703]
68. Melo JV. BCR-ABL gene variants. *Baillieres Clin Haematol*. 1997; 10:203–222. [PubMed: 9376660]
69. Tefferi AD, Gilliland DG. Oncogenes in Myeloproliferative Disorders. *Cell Cycle*. 2007; 6:550–566. [PubMed: 17351342]
70. Vita M, Henriksson M. The Myc oncoprotein as a therapeutic target for human cancer. *Seminars in Cancer Biology*. 2006; 16:318–330. [PubMed: 16934487]
71. Ponzielli R, Katz S, Barsyte-Lovejoy D, Penn LZ. Cancer therapeutics: Targeting the dark side of Myc. *Eur J of Cancer*. 2005; 41:2485–2501. [PubMed: 16243519]
72. Afar DE, Goga A, McLaughlin J, Witte ON, Sawyers CL. Differential complementation of Bcr-Abl point mutants with c-Myc. *Science*. 1994; 264:424–426. [PubMed: 8153630]
73. Alitalo K, Saksela K, Winqvist R, Alitalo R, Keski-Oja J, Laiho M, Ilvonen M, Knuutila S, de la Chapelle A. Acute myelogenous leukaemia with c-myc amplification and double minute chromosomes. *Lancet*. 1985; 2:1035–1039. [PubMed: 2865517]

74. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. *Blood*. 2002; 100:1532–1542. [PubMed: 12176867]
75. Sawyers CL, Callahan W, Witte ON. Dominant negative MYC blocks transformation by ABL oncogenes. *Cell*. 1992; 70:901–910. [PubMed: 1525828]
76. Müller-Tidow C, Steffen B, Cauvet T, Tickenbrock L, Ji P, Diederichs S, Sargin B, Köhler G, Stelljes M, Puccetti E, Ruthardt M, deVos S, Hiebert SW, Koeffler HP, Berdel WE, Serve H. Translocation products in acute myeloid leukemia activate the Wnt signaling pathway in hematopoietic cells. *Mol Cell Biol*. 2004; 24:2890–2904.
77. Slovak ML, Ho JP, Pettenati MJ, Khan A, Douer D, Lal S, Traweek ST. Localization of amplified MYC gene sequences to double minute chromosomes in acute myelogenous leukemia. *Genes Chromosomes Cancer*. 1994; 9:62–67. [PubMed: 7507702]
78. Luo H, Li Q, O'Neal J, Kreisel F, Le Beau MM, Tomasson MH. c-Myc rapidly induces acute myeloid leukemia in mice without evidence of lymphoma-associated antiapoptotic mutations. *Blood*. 2005; 106:2452–2461. [PubMed: 15972450]
79. Xiang Z, Luo H, Payton JE, Cain J, Ley TJ, Opferman JT, Tomasson MH. Mcl1 haploinsufficiency protects mice from myc-induced acute myeloid leukemia. *J Clin Invest*. 2010; 120:2109–2118. [PubMed: 20484815]

Figure 1. Comparison of Gadd45 protein sequences

Figure 2. Gadd45 function in stress signaling

Summary of the various protein-protein interactions of Gadd45 proteins that affect cellular processes such as cell cycle arrest, DNA repair, survival, apoptosis, senescence as well as epigenetic gene activation.

Figure 3. Schematic diagram demonstrating how Gadd45a modulates mammary tumor development in RAS-driven compared to Myc-driven tumors

Gadd45a suppresses RAS-driven tumorigenesis via Jnk-mediated apoptosis and p38-mediated senescence. In contrast Gadd45a promotes Myc-driven tumorigenesis via GSK3B/B-catenin signaling which suppresses MMP10 expression, resulting in increased tumor vascularization, decreased apoptosis and senescence, ultimately accelerating tumor growth.

Figure 4. Schematic Diagram of Bcr-Abl/Ras/Gadd45 signaling