Minireview

GADD45 proteins: roles in cellular senescence and tumor development

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Abstract

The growth arrest and DNA damage 45 (GADD45) family genes regulate DNA repair, cell cycle, cell survival, apoptosis, senescence, and DNA demethylation in the cells under various stress stimuli, such as oxidative stress, UV radiation, and oncogenic stress. Recent studies have provided important insights regarding how different oncogenic stresses activate GADD45 signaling pathway and lead to disparate influences on tumor initiation. In this review, we discuss the deregulation and cellular function of GADD45 proteins in the context of cancer development. We also highlight recent advances in exploring the tumor suppressive function of GADD45 proteins-triggered cellular senescence.

Keywords: GADD45, cell senescence, cancer

***Experimental Biology and Medicine* 2014; 239: 773–778. DOI: 10.1177/1535370214531879**

Introduction

Stress response is an essential physiological mechanism for cells in response to environmental stress. Depending on the strength and nature of stresses, the response may integrate different pathways and trigger multiple cellular processes, including cell arrest, apoptosis, senescence, and metabolic changes.[1,2] Among the stress-related genes, GADD45 family members are the sensors that can mediate rapid responses to a variety of stressors in mammals.[3,4] GADD45 gene family encodes three proteins: GADD45A, GADD45B, and GADD45G. The GADD45 genes are located on different chromosomes (chr 1 for GADD45A, chr 19 for GADD45B and chr 9 for GADD45G), and these cognate proteins are small (about 18 kDa), highly homologous (55–57% similarity), and localized to both the cell nucleus and cytoplasm.[5,6]

The GADD45A was identified as a rapidly induced gene after ultraviolet (UV) exposure of the Chinese hamster ovary (CHO) cells,[7] and then defined as one of the p53 target genes.[8] GADD45B was first cloned from myeloid leukemia cell lines in the presence of interleukin-6 (IL-6) treatment, and known as the myeloid differentiation primary response (MyD) 118.[9] Because its structure was similar to GADD45A, it was named GADD45B. GADD45G, murine homolog of the human CR6 gene, was originally found as an IL-2 immediate-early response gene in T-cell.[10,11] All GADD45 proteins are able to interact with each other and form homo- or hetero-dimers, which are crucial for GADD45 functions.[6,12]

GADD45 family proteins are ubiquitously distributed in mammalian tissues. Each member of GADD45 genes has a distinctive expression pattern in mouse tissues. For example, GADD45A is expressed in skeletal muscle, kidney, spleen, heart, lung, brain, and liver, but with low levels in testis; GADD45B is mostly detected in skeletal muscle, lungs, and liver, but lowly expressed in kidney, spleen, brain, heart, and testis; GADD45G is highly expressed in kidney and liver, whereas relative low expression levels are observed in heart, brain, spleen, lung, and testis. However, the specific function of these individual members in physiological and pathological processes is not well defined.[11] In mice, genetic ablation of GADD45A causes abnormal mitosis, cytokinesis, and growth control, resulting in genomic instability, increased radiation carcinogenesis, and low frequency of exencephaly.[13] In mice with the administration of a single dose of TCPOBOP (1,4-bis[2-(3,5)-dichoropyridyloxy] benzene), GADD45B deficiency impairs the transcriptional activity of the nuclear receptor constitutive androstane receptor (CAR), and leads to a marked delay in the liver growth.[14,15] Knockout of GADD45G does not yield discernible phenotypic change. There is no evidence that shows that ablation of one of the members has effect on the expression or activity of other GADD45 proteins.

The notion that expression of GADD45 proteins usually causes cell cycle arrest in most types of cells and downregulation of these proteins is often observed in human cancer

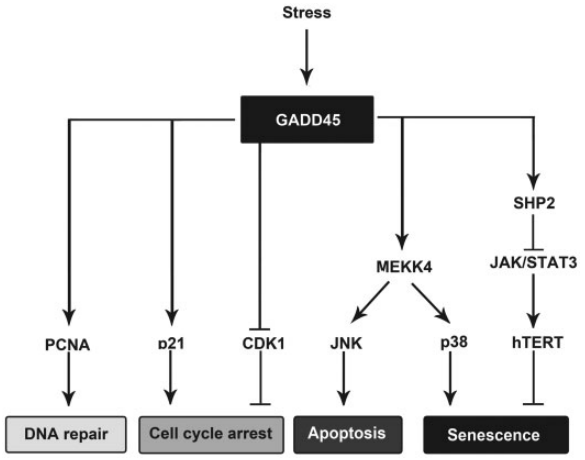

Figure 1 The GADD45 signaling pathway. GADD45 functions in stress signaling and the modulation of cell proliferation and survival. The interaction of GADD45 with PCNA regulates DNA replication and repair by stimulating nucleotide excision repair. GADD45 can induce cell cycle arrest through directly interacting with cell cycle-related protein CDK1 and p21. Direct phosphorylation of MEKK4 by GADD45 leads to activation of JNK and p38 kinase, resulting in cellular apoptosis and senescence, respectively. GADD45g-mediated cellular senescence may involve the downregulation of JAK/STAT3 pathway and hTERT inhibition

supports that GADD45 proteins have tumor suppressive function. The mechanisms underlying GADD45-mediated inhibition of tumor development involve their ability to interact with MEK kinase 4(MEKK4), CDK1, $p21^{waf}$, and PCNA (Figure 1).[16–19] The cross-talk among these proteins ensures a tightly coordinated cellular response. For example, GADD45-mediated auto-phosphorylation of MEKK4 is required for efficient p38/JNK activation and subsequent $p21^{waf}$ expression,[20] which is actually also a key target of GADD45. Thus, conceivably, the interactions of GADD45 proteins with these critical molecules are the determinants of cell fate when cells confront with certain stress signals.

GADD45 proteins and cancer

GADD45 expression in cancer

The expression of GADD45 family genes is frequently downregulated in several types of cancers. Analysis of clinical hepatocellular carcinoma samples indicates that GADD45B expression is downreguated in 83.35% cases.[21] Consistent with this result, aberrant expression of GADD45A and GADD45G are also observed in breast cancer.[22,23] In addition, GADD45A mRNA level is approximately 10 times lower in non-small cell lung carcinoma (NSCLC) compared to normal lung tissues, and this low intratumoral GADD45A expression is significantly associated with a poorer histological grading.[24]

In recent years, numerous researchers have shown that the loss of GADD45 expression in cancerous tissues is a consequence of their promotor hypermethylation. One of the most detailed studies was conducted on lung cancer, showing that the methylation frequencies are 1.4%, 7.2%, and 31.6% for GADD45A, GADD45B, and GADD45G in NSCLC, respectively.[24] In addition, by methylation-specific PCR (MSP) analysis, GADD45A is hypermethylated in breast cancer cell lines.[22] Compared to GADD45A and GADD45B, hypermrthylation occurs more frequently in GADD45G promoter in a variety of cancers. For example, GADD45G is heavily methylated in multiple cancer cell lines, including 85% of non-Hodgkin, 50% of Hodgkin lymphoma, 73% of nasopharyngeal, 50% of cervical, 29% of esophageal, and 40% of lung carcinoma.[23] Consistently, the frequency of GADD45G methylation is also much higher in hepatocellular carcinoma, gastric, colorectal, and pancreatic cancers compared to normal cells.[25] In GADD45 family, there is no evidence showing that GADD45B and GADD45G contain mutations in clinical samples. Based on DNA sequencing, in resectable invasive ductal carcinomas patients, 13.6% of them harbor point mutations in exon 4 of GADD45A. However, there is no correlation between the GADD45A mutations and clinicopathological factors including age, gender, histological grade, clinical stage, pT, pN, M, and vascular invasion.[26] All of the evidences indicate that epigenetic silencing of GADD45 may be critical in the development of certain types of cancer, through enhancing the fitness of cancer cells or transformed cells to stress signals.

Besides the promotor methylation, the silence of GADD45 family members can be specifically controlled through A variety of regulators in cancer cells. p53 and NF-kB are two main regulators that can modify the activation of GADD45 pathways. In fact, GADD45A gene contains highly conserved sequences in its promoter region, where p53 can bind in the cells with the treatment of UV or IR. Consistent with this observation, co-expression of p53 and WT1 in breast carcinoma cell lines strongly augments GADD45A promoter activity.[27] p53 mutations, which occur in 50% of human cancers,[28] may be partially responsible for the downregulation of GADD45 expression. The clinical evidence from 72 pancreatic IDC patient samples, however, suggested that the expression of GADD45A was not necessarily correlated with the levels of p53 protein.[26] NF-kB can suppress the transcription of GADD45A and GADD45G via upregulating c-MYC expression.[29,30] Functionally, the activation of GADD45A and GADD45G was shown responsible for the NF-kB attenuation-induced apoptosis in prostate cancer cell line DU145.[30] In addition to p53, BRCA1, a potential target of cancer therapy, is also a positive modulator of GADD45a expression.[31] All together, GADD45 functions are reduced in most of the cancers and deregulation of these regulators of GADD45 expression may result in increased cell proliferation and tumorigenic potential.

GADD45 proteins act as tumor suppressor

The properties of cancer cells are characterized by resistance to apoptosis, unlimited replicative potential, uncontrolled invasion and metastases, avoidance of oncogene-induced senescence, and improved drug resistance.[32] Stress signaling impacts almost all of these processes. GADD45 proteins have generally been considered as tumor suppressors. Indeed, forced expression of GADD45 proteins inhibits proliferation and induces apoptosis in a

number of cancer cell lines. In mice, the knockout of GADD45A alleles enhances tumor initiation.[13,33,34]

The tumor-suppressive activity of GADD45 proteins is largely attributed to the hyperactivation of the MAPK (p38/JNK) signaling that usually yields cytostatic or proapoptotic effects. GADD45A deletion promoted both cancer initiation and progression in Ras-driven mouse mammary carcinogenesis.[33] Similarly, H-Ras overexpression in these GADD45A−/− mouse embryo fibroblast causes malignant transformation.[35] Notably, the treatment with the inhibitors of p38 or JNK pathways reveals that the major inhibitory effect mediated by GADD45A is related to p38 rather than to JNK activation in Ras-driven transformation.[35] Interestingly, the blockage of either the p38 or JNK pathways can effectively potentiate cell cycle progression in HepG2 liver cancer cells with GADD45 expression,[36] suggesting that the role of JNK activation in GADD45 pathway for the suppression of cancer cell proliferation is still controversial.

In consistent with the tumor suppressive function of GADD45 proteins, induction of cell apoptosis by several mechanistically distinct chemotherapeutics is attributable, at least in part, to GADD45 activation, suggesting that pharmacological or genetic activation of GADD45 family members may provide novel strategies in anti-cancer therapeutics. Sorafenib is the first chemotherapeutic drug that shows survival benefit for patients with advanced hepatocellular carcinoma. This multitargeted tyrosine kinase inhibitor suppresses tumor cell growth inhibition by JNK-induced apoptosis.[37] Disruption of GADD45B or JNK kinase can limit the pro-apoptotic effects of sorafenib in Huh7 liver cancer cells.[38] In addition to GADD45B, knockdown of GADD45A abrogates the troglitazone-induced apoptosis in MCF7 human breast cancer cells.[39] Furthermore, GADD45 genes participate in several drugs-induced apoptosis, including CD437, trichostatin A (TSA), (-)-xanthatin, docetaxel, and NSAID.[40–44]

Apart from their intrinsic tumor-suppressive activities in tumor cells, GADD45 proteins may have regulatory function in immune surveillance of tumors. IFN-gamma, a cytokine that involves in various immune responses to intrinsic or extrinsic stimuli, plays a pivotal role in immunosurveillance process of tumor suppression in nude mice.[45] Interestingly, GADD45B overexpression in naive $CD4^+$ T cells selectively increases IFN-gamma production.[46] Further study has demonstrated that GADD45G promotes IFN-gamma expression in MEKK4+/+ T cells, but not in MEKK4−/− cells or in cells treated with a p38 inhibitor.[47] Most importantly, in mice bearing B16 melanoma, GADD45B deficiency largely enhances tumor growth compared to GADD45B+/− littermate controls, and the augment is attributable to the reduced expression of IFN-gamma, granzyme B, and CCR5 in GADD45B−/−CD8+ T cells at the tumor site.[48] All together, in immune system, disruption of GADD45 proteins in hematopoietic cells may impair tumor immunosurveillance in hosts.

GADD45A acts as tumor promoter

Besides the antitumor function of GADD45A protein through the induction of cellular apoptosis or senescence in cancer cells, GADD45A has been shown to have an important role in facilitating tumorigenesis in certain cancers. A report analyzing invasive ductal carcinomas (IDCs) of the pancreas samples demonstrates that in patients with p53(+) IDC, the GADD45A(+) group has a significantly lower survival rate than does the GADD45A(−) group.[26] In line with the observation, GADD45A is overexpressed in pancreatic ductal adenocarcinoma at the mRNA and protein levels, and disruption of GADD45A expression reduces proliferation and induces apoptosis in pancreatic cancer cells.[49] Furthermore, GADD45A is commonly upregulated in Ptch1-associated tumors and Ptch1 null embryos.[50] The most direct evidence for the supportive function of GADD45A in tumorigenesis is provided by a mouse model of Myc-driven breast cancer, in which loss of GADD45A attenuates the onset of mammary tumor formation. Mechanistically, GADD45A activates β-Catenin-dependent MMP10 suppression and leads to increased capillary vascularization in Myc-driven breast tumors.[34] Therefore, these results highlight the complex roles of GADD45A in cancer development and that the nature of oncogenic signals and the differences in cellular context may determine the direction of the function of GADD45 proteins.

GADD45-induced cellular senescence

GADD45 and cell arrest

Decades of the research efforts attempting to illustrate the pathways of anti-tumor therapy have added significant knowledge to our understanding of the mechanisms of cellular senescence. These process involves several steps including promoting the DNA damage, generating DNA repair signal, eliciting permanent cell cycle arrest, and the final entry of senescence.

Several members of the GADD45 family have been shown to inhibit cell cycle. In human cells, cellular G2/M checkpoint is impaired after exposure of UV radiation due to knockdown endogenous expression of GADD45A, GADD45B, or GADD45G. Thus, theoretically, the accumulation of the GADD45 proteins should promote the cellular machinery responsible for DNA repair, resulting in temporal or permanent arrest of cell cycle process. Early evidence shows that microinjecting the expression vector containing GADD45A into human primary fibroblasts arrested the cells in G2/M phase.[51] Consistent with this observation, ectopic expression of GADD45 proteins in ML1 human myeloblstic leukemia and H1299 lung carcinoma induces cell cycle arrest in the G1/S phase.[52] However, it has been revealed that GADD45G overexpression in U2OS and HeLa cancer cells does not trigger apoptosis or cell arrest under normal culture conditions; instead, after serum withdrawal, cell cycle arrest at G2/M phase is more prominent in HeLa cells with GADD45G expression than in the control cells,[3,53,54] suggesting that the ability of GADD45-induced cell cycle arrest depends on cell types and the environment of culture.

Mechanistically, the cell arrest action of GADD45 proteins is based on their interaction with a variety of proteins including CDK1/Cyclin B1 and p21. All three GADD45

proteins can interact directly with CDK1 and inhibit the kinase activity of the CDK1/Cyclin B1 complex, resulting in G2/M cell cycle arrest. GADD45A and GADD45B dissociate the CDK1/Cyclin B1 complex, whereas GADD45G inhibits the complex without disrupting interaction.[17] There may be a considerable cross-talk among these different GADD45-interacting proteins. In response to DNA damage, $p21^{waf}$ interacts with CDK/Cyclin complex and inhibits the cell cycle checkpoint.[55] Compared to CDK/Cyclin B1, GADD45A, GADD45B, and GADD45G interact with $p21^{waf}$ and lead to both G1/S and G2/M phase arrest in mammalian cells.[18]

GADD45 and cellular senescence

Cellular senescence is a state of irreversible growth arrest in response to various types of stresses. It has been reported that somatic cells, even tumor cells, undergo senescence in response to DNA damage agents, oxidative stress, and ionizing radiation. Compared to replicative senescence triggered by a cell-intrinsic mechanism, stress-induced senescence occurs rapidly within a few days. In fact, the overexpression of stress-related protein, such as p38 or its upstream MEKK6, results in rapid premature senescence in breast cancer cell lines.[56,57] Further strengthening the links between stress and senescence is the observation that transformed cells such as Sk-Hep1 and HCT116 undergo senescence in response to chemotherapeutic drugs or low dose of ionizing radiation.[58–61] Therefore, the stress-induced premature senescence, termed as extrinsic mechanism for inhibition of tumor proliferation, plays an important role in cancer therapy.

Since GADD45 proteins are present at the core of the signaling network that governs cell cycle process, manipulation of GADD45 function has become of increasing interest for the induction of cellular senescence. Evidence from the senescence-accelerated mice (SAMP1) model has revealed that GADD45B exhibits a higher expression in the aging articular cartilage of SAMP1 mice compared to that in control mice.[62] GADD45A is also involved in cellular senescence in response to diverse stresses. p53 preferentially occupies the promoters of growth arrest genes p21 and GADD45A in normal human diploid fibroblasts undergoing replicative senescence.[62] Furthermore, an increase in GADD45A expression is found in H_2O_2 stress-induced senescence.[63] In our study, we have demonstrated that ectopic GADD45G expression can directly induce senescence in HCC Sk-Hep1, SMMC-7721, and Hep3B cells. Notably, knockdown of GADD45G in Sk-Hep1 tumor cells by small interfere RNA (siRNA) attenuated MG132-induced senescence.[64] All of these observations suggest that GADD45 proteins play an important role in cellular senescence processes.

The details underlying the pro-senescent activities of GADD45 proteins have not been well defined. p21 accumulation seems to be critically involved in the induction of cellular senescence. GADD45 proteins can regulate p21 expression at transcriptional level and physically interact with p21.[62] At the same time, the oxidative stress-triggered senescence process is associated with long-term activation of p21 through the GADD45-MAPK14 (p38MAPK) -GRB2-TGFβR2-TGFβ signal pathway.[65] Interestingly, our results have demonstrated that GADD45G-induced senescence process does not require the presence of p53, p16, p21, and Rb in HCC cells.[64] Thus, this finding highlights that, in addition to classical initiators of cell senescence, other proteins such as GADD45G may trigger cellular senescence to inhibit tumor development in an independent manner or as a complementary mechanism.

STAT3 downregulation in GADD45G-induced senescence

Compared to the transient activation in somatic cells, aberrant constitutive activation of STAT3 is frequently observed in many human solid and hematological tumors.[66] The abnormality of STAT3 signaling greatly benefits cancer cells in angiogenesis, proliferation, invasion, and metastasis.[67] In both normal and tumor cells, STAT3 regulates the expression of hTERT, MCL1, and BCL2,[68,69] which usually render to cancer cells the capabilities of evading replicative senescence or apoptosis. Thus, the inhibition of aberrant hyperactivated STAT3 activity is believed as a potential therapeutic strategy for cancer treatment.

Recent work conducted in our laboratory has demonstrated that the tumor suppressor function of GADD45G is mainly through negative regulation of JAK/STAT3 signaling and induction a p53/p16/Rb-independent senescence. Restoration of STAT3 activity by either SHP2 inhibition or the expression of constitutive activated form of STAT3 efficiently counteracts the GADD45G-induced senescence (Figure 1).[64] In line with our finding, ablation of STAT3 signaling in breast cancer cells induces cellular senescence and hence promotes antitumor immune response that inhibits the growth and metastasis of breast cancer.[70] Interestingly, another report shows that STAT3 binds to upstream regulatory elements of GADD45G and suppresses its transcriptional activation.[71] Therefore, the interaction between GADD45G and JAK/STAT3 pathway establishes a positive feedback loop that is likely to be important in regulating cell fate. Altogether, these findings provide novel insight into the regulatory complexity of how GADD45G and JAK/STAT3 signaling pathway cooperate to orchestrate cell malignant transformation and tumor initiation.

Conclusion

The GADD45 pathways function as a central signaling node that regulate tumor development. Downregulation of GADD45 proteins, which occurs frequently in clinical tumor tissues, may potentiate tumor cell survival by escaping from cellular senescence and apoptosis. Inactivation of JAK/STAT3 pathway is a critical event in GADD45G-induced senescence. Pharmacological or genetic activation of GADD45 function may be considered as a therapeutic strategy potential for certain types of cancer.

Authors' contributions: LZ and YL wrote the manuscript. ZY helped in checking the manuscript. LZ, ZY, and YL read and approved the final version.

ACKNOWLEDGEMENTS

This study was supported by grants from National Natural Science Foundation of China (No. 81201542), Natural Science Foundation of Shanghai (No. 12ZR1430100) and the State Key Laboratory of Oncogenes and Related Genes (No. 91-12-03).

REFERENCES

1. Haigis MC, Yankner BA. The aging stress response. *Mol Cell* 2010;**40**:333–44
2. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011;**11**:85–95
3. Liebermann DA, Hoffman B. Gadd45 in stress signaling. *J Mol Signal* 2008;**3**:15
4. Salvador JM, Brown-Clay JD, Fornace AJ Jr. Gadd45 in stress signaling, cell cycle control, and apoptosis. *Adv Exp Med Biol* 2013;**793**:1–19
5. Moskalev AA, Smit-McBride Z, Shaposhnikov MV, Plyusnina EN, Zhavoronkov A, Budovsky A, Tacutu R, Fraifeld VE. Gadd45 proteins: relevance to aging, longevity and age-related pathologies. *Ageing Res Rev* 2012;**11**:51–66
6. Tamura RE, de Vasconcellos JF, Sarkar D, Libermann TA, Fisher PB, Zerbini LF. GADD45 proteins: central players in tumorigenesis. *Curr Mol Med* 2012;**12**:634–51
7. Fornace AJ Jr., Nebert DW, Hollander MC, Luethy JD, Papathanasiou M, Fargnoli J, Holbrook NJ. Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. *Mol Cell Biol* 1989;**9**:4196–203
8. Butz K, Whitaker N, Denk C, Ullmann A, Geisen C, Hoppe-Seyler F. Induction of the p53-target gene GADD45 in HPV-positive cancer cells. *Oncogene* 1999;**18**:2381–6
9. 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**:165–7
10. Beadling C, Johnson KW, Smith KA. Isolation of interleukin 2-induced immediate-early genes. *Proc Natl Acad Sci U S A* 1993;**90**:2719–23
11. Zhang W, Bae I, Krishnaraju K, Azam N, Fan W, Smith K, Hoffman B, Liebermann DA. CR6: A third member in the MyD118 and Gadd45 gene family which functions in negative growth control. *Oncogene* 1999;**18**:4899–907
12. 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**:2765–76
13. 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**:176–84
14. Tian J, Huang H, Hoffman B, Liebermann DA, Ledda-Columbano GM, Columbano A, Locker J. Gadd45beta is an inducible coactivator of transcription that facilitates rapid liver growth in mice. *J Clin Invest* 2011;**121**:4491–502
15. 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**:2255–63
16. 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
17. 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**:327–38
18. Zhao H, Jin S, Antinore MJ, Lung FD, Fan F, Blanck P, Roller P, Fornace AJ Jr., Zhan Q. The central region of Gadd45 is required for its interaction with p21/WAF1. *Exp Cell Res* 2000;**258**:92–100
19. Chen IT, Smith ML, O'Connor PM, Fornace AJ Jr. Direct interaction of Gadd45 with PCNA and evidence for competitive interaction of Gadd45 and p21Waf1/Cip1 with PCNA. *Oncogene* 1995;**11**:1931–7
20. Mikule K, Delaval B, Kaldis P, Jurcyzk A, Hergert P, Doxsey S. Loss of centrosome integrity induces p38-p53-p21-dependent G1-S arrest. *Nat Cell Biol* 2007;**9**:160–70
21. Qiu W, David D, Zhou B, Chu PG, Zhang B, Wu M, Xiao J, Han T, Zhu Z, Wang T, Liu X, Lopez R, Frankel P, Jong A, Yen Y. Down-regulation of growth arrest DNA damage-inducible gene 45beta expression is associated with human hepatocellular carcinoma. *Am J Pathol* 2003;**162**:1961–74
22. 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
23. 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
24. 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
25. Zhang W, Li T, Shao Y, Zhang C, Wu Q, Yang H, Zhang J, Guan M, Yu B, Wan J. Semi-quantitative detection of GADD45-gamma methylation levels in gastric, colorectal and pancreatic cancers using methylation-sensitive high-resolution melting analysis. *J Cancer Res Clin Oncol* 2010;**136**:1267–73
26. 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**:2563–9
27. Zhan Q, Chen IT, Antinore MJ, Fornace AJ Jr. Tumor suppressor p53 can participate in transcriptional induction of the GADD45 promoter in the absence of direct DNA binding. *Mol Cell Biol* 1998;**18**:2768–78
28. Toledo F, Wahl GM. Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. *Nat Rev Cancer* 2006;**6**:909–23
29. Marhin WW, Chen S, Facchini LM, Fornace AJ Jr., Penn LZ. Myc represses the growth arrest gene gadd45. *Oncogene* 1997;**14**:2825–34
30. Zerbini LF, Libermann TA. Life and death in cancer. GADD45 alpha and gamma are critical regulators of NF-kappaB mediated escape from programmed cell death. *Cell Cycle* 2005;**4**:18–20
31. Jin S, Zhao H, Fan F, Blanck P, Fan W, Colchagie AB, Fornace AJ Jr., Zhan Q. BRCA1 activation of the GADD45 promoter. *Oncogene* 2000;**19**:4050–7
32. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;**144**:646–74
33. Tront JS, Hoffman B, Liebermann DA. Gadd45a suppresses Ras-driven mammary tumorigenesis by activation of c-Jun NH2-terminal kinase and p38 stress signaling resulting in apoptosis and senescence. *Cancer Res* 2006;**66**:8448–54
34. Tront JS, Huang Y, Fornace AJ Jr., Hoffman B, Liebermann DA. Gadd45a functions as a promoter or suppressor of breast cancer dependent on the oncogenic stress. *Cancer Res* 2010;**70**:9671–81
35. 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**:3859–71
36. 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**:2075–85
37. Yu C, Friday BB, Lai JP, Yang L, Sarkaria J, Kay NE, Carter CA, Roberts LR, Kaufmann SH, Adjei AA. Cytotoxic synergy between the multikinase inhibitor sorafenib and the proteasome inhibitor bortezomib in vitro: induction of apoptosis through Akt and c-Jun NH2-terminal kinase pathways. *Mol Cancer Ther* 2006;**5**:2378–87
38. Ou DL, Shen YC, Yu SL, Chen KF, Yeh PY, Fan HH, Feng WC, Wang CT, Lin LI, Hsu C, Cheng AL. Induction of DNA damage-inducible gene

GADD45beta contributes to sorafenib-induced apoptosis in hepatocellular carcinoma cells. *Cancer Res* 2010;**70**:9309–18
39. Yin F, Bruemmer D, Blaschke F, Hsueh WA, Law RE, Herle AJ. Signaling pathways involved in induction of GADD45 gene expression and apoptosis by troglitazone in human MCF-7 breast carcinoma cells. *Oncogene* 2004;**23**:4614–23
40. Jiang T, Soprano DR, Soprano KJ. GADD45A is a mediator of CD437 induced apoptosis in ovarian carcinoma cells. *J Cell Physiol* 2007;**212**:771–9
41. Barreto G, Schafer A, Marhold J, Stach D, Swaminathan SK, Handa V, Doderlein G, Maltry N, Wu W, Lyko F, Niehrs C. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature* 2007;**445**:671–5
42. Takeda S, Noguchi M, Matsuo K, Yamaguchi Y, Kudo T, Nishimura H, Okamoto Y, Amamoto T, Shindo M, Omiecinski CJ, Aramaki H. (-)-Xanthatin up-regulation of the GADD45gamma tumor suppressor gene in MDA-MB-231 breast cancer cells: role of topoisomerase IIalpha inhibition and reactive oxygen species. *Toxicology* 2013;**305**:1–9
43. Li Y, Hong X, Hussain M, Sarkar SH, Li R, Sarkar FH. Gene expression profiling revealed novel molecular targets of docetaxel and estramustine combination treatment in prostate cancer cells. *Mol Cancer Ther* 2005;**4**:389–98
44. Chiou SK, Hoa N. Up-regulation of GADD45alpha expression by NSAIDs leads to apoptotic and necrotic colon cancer cell deaths. *Apoptosis* 2009;**14**:1341–51
45. Arum CJ, Anderssen E, Viset T, Kodama Y, Lundgren S, Chen D, Zhao CM. Cancer immunoediting from immunosurveillance to tumor escape in microvillus-formed niche: a study of syngeneic orthotopic rat bladder cancer model in comparison with human bladder cancer. *Neoplasia* 2010;**12**:434–42
46. Yang J, Zhu H, Murphy TL, Ouyang W, Murphy KM. IL-18-stimulated GADD45 beta required in cytokine-induced, but not TCR-induced, IFN-gamma production. *Nat Immunol* 2001;**2**:157–64
47. Chi H, Lu B, Takekawa M, Davis RJ, Flavell RA. GADD45beta/GADD45gamma and MEKK4 comprise a genetic pathway mediating STAT4-independent IFNgamma production in T cells. *EMBO J* 2004;**23**:1576–86
48. Ju S, Zhu Y, Liu L, Dai S, Li C, Chen E, He Y, Zhang X, Lu B. Gadd45b and Gadd45g are important for anti-tumor immune responses. *Eur J Immunol* 2009;**39**:3010–8
49. Schneider G, Weber A, Zechner U, Oswald F, Friess HM, Schmid RM, Liptay S. GADD45alpha is highly expressed in pancreatic ductal adenocarcinoma cells and required for tumor cell viability. *Int J Cancer* 2006;**118**:2405–11
50. Kappler R, Hess I, Schlegel J, Hahn H. Transcriptional up-regulation of Gadd45a in Patched-associated medulloblastoma. *Int J Oncol* 2004;**25**:113–20
51. 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 U S A* 1999;**96**:3706–11
52. Zhang W, Hoffman B, Liebermann DA. Ectopic expression of MyD118/Gadd45/CR6 (Gadd45beta/alpha/gamma) sensitizes neoplastic cells to genotoxic stress-induced apoptosis. *Int J Oncol* 2001;**18**:749–57
53. 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**:6573–82
54. Liu B, Shuai K. Induction of apoptosis by protein inhibitor of activated Stat1 through c-Jun NH2-terminal kinase activation. *J Biol Chem* 2001;**276**:36624–31
55. Wang Y, Fisher JC, Mathew R, Ou L, Otieno S, Sublet J, Xiao L, Chen J, Roussel MF, Kriwacki RW. Intrinsic disorder mediates the diverse regulatory functions of the Cdk inhibitor p21. *Nat Chem Biol* 2011;**7**:214–21
56. Freund A, Patil CK, Campisi J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J* 2011;**30**:1536–48
57. Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer* 2009;**9**:537–49
58. Li L, Hu Y, Ylivinkka I, Li H, Chen P, Keski-Oja J, Hyytiainen M. NETRIN-4 Protects Glioblastoma Cells FROM Temozolomide Induced Senescence. *PLoS One* 2013;**8**:e80363
59. Chang BD, Xuan Y, Broude EV, Zhu H, Schott B, Fang J, Roninson IB. Role of p53 and p21waf1/cip1 in senescence-like terminal proliferation arrest induced in human tumor cells by chemotherapeutic drugs. *Oncogene* 1999;**18**:4808–18
60. Ewald JA, Desotelle JA, Wilding G, Jarrard DF. Therapy-induced senescence in cancer. *J Natl Cancer Inst* 2010;**102**:1536–46
61. Suzuki K, Mori I, Nakayama Y, Miyakoda M, Kodama S, Watanabe M. Radiation-induced senescence-like growth arrest requires TP53 function but not telomere shortening. *Radiat Res* 2001;**155**:248–253
62. Shimada H, Sakakima H, Tsuchimochi K, Matsuda F, Komiya S, Goldring MB, Ijiri K. Senescence of chondrocytes in aging articular cartilage: GADD45beta mediates p21 expression in association with C/EBPbeta in senescence-accelerated mice. *Pathol Res Pract* 2011;**207**:225–31
63. Furukawa-Hibi Y, Yoshida-Araki K, Ohta T, Ikeda K, Motoyama N. FOXO forkhead transcription factors induce G(2)-M checkpoint in response to oxidative stress. *J Biol Chem* 2002;**277**:26729–32
64. Zhang L, Yang Z, Ma A, Qu Y, Xia S, Xu D, Ge C, Qiu B, Xia Q, Li J, Liu Y. Growth arrest and DNA damage 45G down-regulation contributes to janus kinase/signal transducer and activator of transcription 3 activation and cellular senescence evasion in hepatocellular carcinoma. *Hepatology* 2014;**59**:178–89
65. Passos JF, Nelson G, Wang C, Richter T, Simillion C, Proctor CJ, Miwa S, Olijslagers S, Hallinan J, Wipat A, Saretzki G, Rudolph KL, Kirkwood TB, von Zglinicki T. Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Mol Syst Biol* 2010;**6**:347
66. Garcia R, Bowman TL, Niu G, Yu H, Minton S, Muro-Cacho CA, Cox CE, Falcone R, Fairclough R, Parsons S, Laudano A, Gazit A, Levitzki A, Kraker A, Jove R. Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. *Oncogene* 2001;**20**:2499–513
67. Xie TX, Wei D, Liu M, Gao AC, Ali-Osman F, Sawaya R, Huang S. Stat3 activation regulates the expression of matrix metalloproteinase-2 and tumor invasion and metastasis. *Oncogene* 2004;**23**:3550–60
68. Konnikova L, Simeone MC, Kruger MM, Kotecki M, Cochran BH. Signal transducer and activator of transcription 3 (STAT3) regulates human telomerase reverse transcriptase (hTERT) expression in human cancer and primary cells. *Cancer Res* 2005;**65**:6516–20
69. Bhattacharya S, Ray RM, Johnson LR. STAT3-mediated transcription of Bcl-2, Mcl-1 and c-IAP2 prevents apoptosis in polyamine-depleted cells. *Biochem J* 2005;**392**:335–44
70. Tkach M, Coria L, Rosemblit C, Rivas MA, Proietti CJ, Diaz Flaque MC, Beguelin W, Frahm I, Charreau EH, Cassataro J, Elizalde PV, Schillaci R. Targeting Stat3 induces senescence in tumor cells and elicits prophylactic and therapeutic immune responses against breast cancer growth mediated by NK cells and CD4+ T cells. *J Immunol* 2012;**189**:1162–72
71. Scuto A, Kirschbaum M, Buettner R, Kujawski M, Cermak JM, Atadja P, Jove R. SIRT1 activation enhances HDAC inhibition-mediated upregulation of GADD45G by repressing the binding of NF-kappaB/STAT3 complex to its promoter in malignant lymphoid cells. *Cell Death Dis* 2013;**4**:e635