GADD45 Deregulation in Cancer: Frequently Methylated Tumor Suppressors and Potential Therapeutic Targets

Commentary on Ying et al., p. 6442

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In this issue of Clinical Cancer Research, Ying et al. (1) have investigated the hypothesis that GADD45γ, a member of the GADD45 family originally identified as a growth arrest– and DNA damage–inducible gene, could serve as a functional tumor suppressor gene, and moreover, as a therapeutic target. DNA methylation is an important regulator of gene transcription, and its role in carcinogenesis has been a topic of considerable interest in the last few years. Epigenetic alterations, such as abnormal DNA methylation patterns, are associated with many human tumor types and are now recognized as one of the most common molecular alterations in human cancer and a cause of oncogenesis (Fig. 1; ref. 2). Among epigenetic modifications, hypermethylation, which represses transcription of the promoter regions of tumor suppressor genes leading to gene silencing, has been most extensively studied. New techniques have been developed to perform genome-wide screening for alterations in DNA methylation patterns not only to identify tumor suppressor genes but also to find patterns that can be used in diagnosis and prognosis and may influence future treatment regimens (2).

The studies of Ying et al. provide strong evidence that GADD45γ is frequently epigenetically inactivated in various types of cancers and tumor cell lines and remarkable findings emerge: first, the authors identified CpG islands in the GADD45γ gene as tumor-specific, rarely mutated, but commonly hypermethylated target sequences; second, GADD45γ expression as well as stress-induced activation is reduced or silenced due to its promoter hypermethylation. Using demethylating agents, they showed that methylation is directly responsible for silencing of the GADD45γ promoter, a process that is pharmacologically reversible. Furthermore, ectopic overexpression of GADD45γ results in reduced tumor growth and colony formation supporting the notion that GADD45γ is a tumor suppressor gene.

These data confirm and extend previous data about reduced expression of GADD45γ in several other types of cancer. Furthermore, we (3) recently focused on a similar question with regard to GADD45γ regulation by nuclear factor-κB (NF-κB) in human cancer. We reported that the transcription factor NF-κB, which is deregulated and activated in many tumors and a critical regulator of cell survival, mediates repression of GADD45α and GADD45γ gene expression, and NF-κB-mediated repression of these two GADD45 genes is essential for escape from programmed cell death (3, 4). Together, these findings show the importance of GADD45γ for the development and progression of various cancer types pointing out different mechanisms to inactivate GADD45γ functions in cancer.

Epigenetics, DNA Methylation, Prognostic, and Cancer

Methylation of cytosines is an evolutionary conserved mechanism of gene regulation that plays a critical role during normal development and genetic defects associated with methylation result in multiple developmental diseases. Because cancer cells many times express various features of undifferentiated or dedifferentiated cells, it is not surprising that methylation of genes is frequently observed in cancer, although a loss of global methylation is observed concomitant with methylation of specific CpG islands. A vast amount of information has been gained about aberrant methylation patterns in human cancers. Tumor-specific methylation changes in different genes have been identified and the potential clinical applications of these data had an effect on cancer diagnosis, prognosis, and therapeutics. Consequently, several DNA methylase inhibitors such as 5-azacytidine, decitabine, and MG98 are currently in clinical trials for various types of cancer. For example, a phase III clinical trial of 5-azacytidine in myelodysplastic syndrome patients resulted in significantly improved response rate, quality of life, and enhanced overall survival (5).

What is unexpected and thus far unexplained is the tumor-specific methylation of specific subsets of genes, in particular, tumor suppressor genes such as GADD45γ in the current study. The precise mechanisms are not known, although some increase in DNMT1 (2, 6) expression has been observed in some cancers. Epigenetics can be described as a stable alteration in gene expression potential that takes place during development and cell proliferation, without any change in gene sequence. DNA methylation is one of the most common epigenetic events in the mammalian genome. Although heritable, this process is reversible, making it an interesting therapeutic target. Because cancer is a result of aberrant gene expression, it is no surprise that methylation plays a crucial role.

DNA methylation is a chemical modification, resulting in the addition of a methyl group at the carbon 5 position of the cytosine ring. Although most cytosine methylation occurs in the sequence context 5’ CpG3’, it can also engage CpA and CpT
The role of GADD45 is complex and poorly understood. It has been shown to play a role in cell cycle arrest, DNA repair, and apoptosis. GADD45α and GADD45β are involved in p53-dependent mechanisms, while GADD45γ is a direct downstream target of p53. The role of GADD45 in cell cycle arrest has been well established, whereas the role of GADD45 in apoptosis remains unclear. Overexpression of GADD45 in normal human fibroblasts causes G2/M arrest but not apoptosis. Nevertheless, genotoxic stress or Brca1-induced apoptosis seem to involve GADD45α-mediated activation of the stress-responsive c-jun NH2-terminal kinase and/or p38 mitogen-activated protein kinase (13, 14). NF-κB-induced cell survival has been proposed to be mediated by induction of GADD45α.

Fig. 1. A model for the molecular mechanisms by which DNA methylation may result in transcriptional silencing of tumor suppressor genes in cancer. Most CpGs in the genome are methylated in the normal cellular state, whereas CpGs in CpG islands are normally unmethylated, regardless of the transcription state of the gene. During tumorigenesis, CpG islands in the promoter regions of tumor suppressor genes become hypermethylated, which spread through the promoter and silence the gene.
GADD45β expression and down-regulation of c-jun-NH₂-kinase activity (15, 16). A yeast two-hybrid screen revealed direct interaction of all three GADD45 family members with the upstream kinase MTK1/mitogen-activated protein kinase kinase kinase kinase 4 that activates both p38 and c-jun-NH₂-kinase in response to environmental stresses. All three GADD45 members activate MTK1 kinase activity leading to p38/c-jun-NH₂-kinase activation and apoptosis. Recent findings established the GADD45 family as a critical mediator of apoptosis in cancer cells (3). NF-κB-mediated cell survival in cancer cells is absolutely dependent on two GADD45 family members, GADD45α and GADD45γ (Fig. 3). NF-κB-mediated repression of GADD45α and γ is sufficient for cancer cell survival and repression of the GADD45α and GADD45γ genes is for a large part the result of NF-κB-mediated up-regulation of c-myc, another oncogene frequently overexpressed in a variety of cancers. Whether NF-κB-mediated repression of GADD45α and GADD45γ gene expression in cancer may involve also changes in methylation is not known. Inhibition of NF-κB in cancer cells results in GADD45α- and GADD45γ-dependent induction of apoptosis and inhibition of tumor growth (3) correlating well with the findings of Ying et al. (1) that ectopic expression of GADD45γ in cancer cells inhibits tumor growth.

GADD45α and GADD45γ induction has been shown to be essential for c-jun-NH₂-kinase activation and apoptosis in cancer cells, highlighting the notion that GADD45α and GADD45γ repression via methylation or other mechanisms plays an unambiguous and universal role in the ability of certain types of cancer to escape from programmed cell death (3). Changes in GADD45α and GADD45γ expression are likely to play a role in the proapoptotic functions of various anticancer drugs as well. Whether these drugs act via inhibition of NF-κB or changes in methylation is not clear.

Although members of the GADD45 family seem infrequently mutated in cancer as far as currently known, reduced expression of the three GADD45 family members due to promoter methylation has been frequently observed in several types of human cancer. In resectable invasive pancreatic ductal carcinomas, GADD45α is frequently mutated, and mutation combined with the p53 status correlates with survival of invasive pancreatic ductal carcinoma patients (17). This is the only type of cancer where mutation of a GADD45 gene has been observed thus far. GADD45α expression is also reduced in ovarian cancer, although the precise mechanism has not been elucidated. The GADD45α promoter is methylated in the majority of breast cancers resulting in reduced expression when compared with normal breast epithelium (18). It is thus interesting to notice that in the current publication no differences in GADD45γ methylation were observed in breast cancer cell lines suggesting specific methylation of the GADD45α gene in breast cancer. In pituitary adenomas, silencing of the GADD45γ gene in 67% of patients is primarily associated with methylation of the GADD45γ gene and reversal of this epigenetic change results in re-expression (19). GADD45γ is also down-regulated in anaplastic thyroid cancer and in 65% of hepatocellular carcinomas due to hypermethylation of the GADD45γ promoter. Interestingly, the GADD45β gene is methylated and silenced in hepatocellular carcinoma as well, indicating a strong linkage between at least two GADD45 genes and liver cancer. Our observation that activated NF-κB leads to repression of GADD45α and GADD45γ expression in various types of cancer together with the frequent constitutive activation of NF-κB in cancers, furthermore, suggests that there are at least two mechanisms whereby GADD45 genes become repressed in cancer. Thus, repression of GADD45 gene expression in various types of cancer via methylation or NF-κB activation seems a critical step in oncogenesis and highlights the role of the GADD45 gene family in regulating DNA damage, cell cycle, and cell survival. Because activation of NF-κB is a critical step for many cells to escape programmed cell death and this is dependent on GADD45α and GADD45γ repression, methylation of the GADD45γ gene as reported by Ying et al. may result in a similar phenotype (i.e., helping cancer cells to survive and become resistant to stress and DNA damage).

The current publication by Ying et al. (1) analyzes the methylation status of two regions in the GADD45γ promoter in a total of 75 cell lines as well as primary tissues and tumors. They show that promoter hypermethylation is frequently detected in tumors 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 but not in immortalized normal epithelial cell lines, normal tissues, or peripheral blood mononuclear

![Fig. 2. Alignment of the amino acid sequences of the human GADD45 genes.](image-url)
GADD45 gene family expression and function may be critical events as tumor biomarkers can provide useful information for early diagnosis, cancer risk assessment, and prognosis (2). Methylated CpG islands are associated with virtually every type of tumor (2), may be grouped into tumor-specific marker panels (2), and can be detected with a high degree of sensitivity (2). The mapping of hundreds to thousands of CpG island–associated tumor suppressors and the discovery of the repressive properties of methylation have had a great effect on disease prevention and treatment (2). Differences in methylation patterns among tumors seem associated with patient outcome or other clinical responses and can be used as markers to classify tumors in association with more effective treatments for patients. Most importantly, the results provided by Ying et al. (1) establish the GADD45\(\gamma\) gene as a major target for methylation in various types of cancer and further support the notion that modulation of GADD45 gene family expression and function may be critical steps in cancer development and progression. These results also imply that therapeutic strategies targeting the epigenetic repression of the tumor suppressor GADD45\(\gamma\) have the potential to result in strong anticancer effects.

References
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