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Role of Gadd45 in Apoptosis

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ABSTRACT. *gadd45* is a p53-regulated growth arrest and DNA-damage-inducible gene that is also regulated in a p53-independent manner. Whether Gadd45 plays a direct role in apoptosis remains unclear. Microinjection of the exogenous *gadd45* expression vector into human fibroblasts has been shown to cause G_2 arrest but not apoptosis. Recent studies suggest that Gadd45 may mediate genotoxic stress or Brca1-induced apoptosis via activation of c-Jun N-terminal kinase (JNK) and/or p38 mitogen-activated protein kinase (MAPK). Analyses of *gadd45*-deficient mice and cells have revealed that Gadd45 appears to exhibit pleiotropic effects, including cell cycle arrest at G_2/M, DNA damage repair, and control of genomic stability, but is not required for radiation-induced apoptosis. Furthermore, stress-induced activation of JNK and p38 MAPK is not altered in *gadd45*-deficient embryonic fibroblasts, suggesting that the lack of Gadd45 may not affect the JNK and p38 MAPK activity. Thus, although the evidence from *gadd45*-null cells suggests that Gadd45 probably does not play a direct role in genotoxic stress-induced apoptosis, more in-depth studies are needed to firmly establish this contention. BIOCHEM PHARMACOL **59**;1:43–45, 2000. © 1999 Elsevier Science Inc.

KEY WORDS. Gadd45; p53; apoptosis; Brca1; MAP kinases; c-Myc

gadd45† is one of several growth arrest and DNA-damage-inducible genes that were originally cloned from hamster cells [1]. It is a p53-regulated gene [2] that encodes a small acidic protein of 21 kDa. *gadd45* has been shown to be regulated by a variety of genotoxic and non-genotoxic stresses in almost all the mammalian cells tested to date [1–3]. Although the exact function of Gadd45 remains unclear, evidence suggests that it may play an important role in regulation of cell growth. Gadd45 has been shown to interact with the products of two other p53-regulated genes, $p21^{WAF/CIP1}$ and *PCNA* (proliferating cell nuclear antigen) [4–6], and has been implicated in some aspects of nucleotide excision repair [5, 6]. c-Myc has been shown to down-regulate both basal and stress-induced *gadd45* expression [7, 8]. In *myc*-deficient rodent cells, *gadd45* expression has been found to be derepressed [9], suggesting that c-Myc may mediate its growth-promoting characteristics at least in part via repression of *gadd45*. Two recent studies have demonstrated that Gadd45 may be involved in the G_2/M checkpoint [10, 11]. Zhan *et al.* [10] have shown that Gadd45, through its association with Cdc2, appears to disrupt interactions between Cdc2 and CyclinB1 and thus may induce a G_2/M arrest. Wang *et al.* [11] have recently demonstrated that microinjection of the exogenous *gadd45* expression vector into human fibroblasts induced G_2 arrest but not apoptosis. Interestingly, microinjection of the p53 expression vector into the same cells did induce apoptosis, which highlights the specificity of Gadd45's function [11].

Evidence suggests that *gadd5* expression is enhanced during apoptosis induced by a variety of agents. For example, *gadd45* expression is up-regulated in a number of different cell types by genotoxic agents that are known to induce apoptosis [2, 12]. Recent studies have shown that *gadd45* is posttranscriptionally up-regulated during apoptosis induced by a synthetic retinoid in human breast and lung carcinoma cells [13, 14]. *gadd45* up-regulation is also coupled with apoptosis induced by agents that cause endoplasmic reticulum stress (our unpublished results). Thus, although Gadd45 regulation is correlated with induction of apoptosis, it remains unclear whether Gadd45 activates apoptosis or whether its up-regulation occurs as a consequence of stress response. Clearly, more direct evidence is needed to establish a role for Gadd45 in induction or protection from apoptosis.

Recently, two studies have shown that *gadd45* may play a role in apoptosis via activation of the JNK and/or p38 MAPK signaling pathways [15, 16]. MAP kinase signaling cascades involving JNK and p38 MAPK are important mediators of cellular response to genotoxic or non-genotoxic stress [17–19]. Activation of JNK and p38 MAPK involves phosphorylation via upstream MAPKK that are in turn activated by another group of kinases collectively known as MAPKKK [17–19]. Evidence suggests that although distinct sets of kinases appear to activate JNK or p38, there are common upstream kinases that can activate both JNK and p38 [20, 21]. MTK1 is a MAPKKK (also

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† *Abbreviations: gadd45*, growth arrest and DNA-damage-inducible gene; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; and MTK1 (MEKK4), mitogen-activated protein kinase kinase kinase.

known as MEKK4) that is believed to activate both JNK and p38 [20, 21]. Takekawa and Saito [15] have recently reported that Gadd45 and the two other similar proteins MyD118 (Gadd45β) and CR6 (Gadd45γ) appear to physically interact with MTK1, and the ensuing interactions result in the activation of MTK1 [15]. Activated MTK1 is thought to further activate its downstream targets JNK and p38 MAPK [15]. JNK and p38 MAPK are activated by methyl methanesulphonate, UV, and to some extent by ionizing radiation [22–25], and the same stress-inducing agents also activate p53, up-regulate Gadd45 expression, and induce apoptosis. Thus, it appears an attractive possibility that Gadd45 may mediate stress-induced apoptosis via its interactions with MTK1 [15]. In another study, Harkin *et al.* [16] have reported that Brca1-induced apoptosis depends on JNK activation and is coupled with up-regulation of *gadd45* expression. *BRCA1* is a tumor suppressor gene that exhibits germline mutations in women genetically predisposed to breast and ovarian cancers [26]. Brca1 appears to function as a transcription factor that is regulated during the cellular response to genotoxic stress [27, 16, and refs. therein]. Based on their findings, Harkin *et al.* [16] have concluded that Gadd45 appears to reside downstream of Brca1 and may play an important role in Brca1-induced p53-independent apoptosis [16].

Although the up-regulation of *gadd45* expression by a variety of genotoxic and non-genotoxic agents that elicit apoptosis has been demonstrated, the role of Gadd45 in mediating apoptosis remains unclear. Based on their findings, Takekawa and Saito [15] have proposed the following model to implicate Gadd45's role in apoptosis: p53 is activated in response to genotoxic stress, it then causes transcriptional up-regulation of *gadd45*, and Gadd45 interacts with MTK1 to set into motion the JNK/p38-mediated apoptotic pathway(s). The proposed model [15] implicates p53 to reside upstream of JNK/p38 pathways and suggests transcriptional and translational dependence for JNK and p38 kinase activation. The role of JNK and p38 MAPK in mediating apoptosis remains controversial [26–30], and the model proposed by Takekawa and Saito [15] does not address a number of issues. For example, evidence suggests that new protein synthesis is not required for JNK activation following UV irradiation.* Furthermore, recent studies have demonstrated that p53 can be activated to induce growth arrest and/or apoptosis following genotoxic stress-induced JNK and p38 kinase-mediated phosphorylation [31–33]. Thus, it remains unclear whether stress-mediated induction of p53 leads to Gadd45-dependent activation of JNK and p38 MAPK or whether stress-mediated activation of JNK and p38 MAPK occurs first, inducing p53 phosphorylation and activation. Genotoxic stress-induced ATM (ataxia-telangiectasia, mutated) and DNA-PK-dependent phosphorylation and activation of p53 makes the issue even more complex [34, 35].

Generation of *gadd45*-null mice has allowed us to address the role of Gadd45 in apoptosis [36]. Just like *p53*-null mice, *gadd45*-deficient animals develop normally but are more prone to exhibit radiation-induced carcinogenesis [36]. In addition, *gadd45*-null cells display the characteristic features of genomic instability similar to those noted in *p53*-null cells, suggesting that Gadd45 may be important in mediating some effects of p53 [33]. Unlike *p53*-null thymocytes, however, the *gadd45*-deficient thymocytes are proficient to undergo ionizing radiation-induced apoptosis, suggesting that Gadd45 may not be required for DNA damage-induced p53-dependent apoptosis, at least in thymocytes [36]. Stress-induced activation of JNK and p38 MAPK is not altered in *gadd45*-deficient embryonic fibroblasts [37], suggesting that the lack of Gadd45 may not affect the JNK/p38 MAPK activity. Furthermore, the kinetics of JNK and p38 MAPK activation and *gadd45*, *myd118*, and *cr6* up-regulation in response to genotoxic stress has revealed that both JNK and p38 MAPK are activated prior to obvious up-regulation of *gadd45* and related genes [37]. However, it is still a formal possibility that Gadd45 homologous proteins, such as MyD118 and CR6, may play a compensatory role in the absence of Gadd45 to activate JNK and p38 in an MTK1-dependent manner. In the case of ionizing radiation, it is clear that only *gadd45* is regulated while *CR6* and *myd118* are not, and since the lack of Gadd45 does not alter ionizing radiation-induced apoptosis, it appears less likely that CR6 and/or MyD118 would play a compensatory role. Clearly, our understanding of the biological functions of Gadd45 is still incomplete and more in-depth studies are needed to investigate (i) the temporal relationship between Gadd45 up-regulation and p53 and JNK/p38 activation and (ii) the exact role, if any, of Gadd45 in p53-dependent and -independent apoptosis.

* Karin M, personal communication.

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