GADD45

Growth arrest and DNA-damage-inducible protein GADD45 alpha is a protein that in humans is encoded by the GADD45A gene.

Function

This gene is a member of a group of genes, the GADD45 genes, whose transcript levels are increased following stressful growth arrest conditions and treatment with DNA-damaging agents (mutagens). The DNA damage-induced transcription of this gene is mediated by both p53-dependent and -independent mechanisms. The protein encoded by this gene responds to environmental stresses by mediating activation of the p38/JNK pathway via MTK1/MEKK4 kinase.

Applications

The fact that expression of this gene is an indicator of DNA damage has been exploited to construct an in vitro test for mutagenicity, the GADD45a-GFP GreenScreen HC assay.

This assay consists of a cell line which has been engineered so that expression of GADD45A will lead to expression of green fluorescent protein, which can easily be detected. To test a substance for mutagenicity, it is applied to these cells and fluorescence is measured.

GADD45A was found to be up-regulated by TMZ in both cell cycle and apoptosis arrays. Furthermore, GADD45A knockdown (GADD45Akd) enhanced the cell growth arrest and cell death induced by TMZ, even in natural (T98) and adapted (TR-U373) TMZ-resistant cells. Interestingly, GADD45Akd decreased the expression of O6-methylguanine-DNA methyltransferase (MGMT) in TMZ-resistant cells (T98 and TR-U373). In MGMT-deficient/TMZ-sensitive cells (U87 and U373), GADD45Akd decreased TMZ-induced TP53 expression. Thus, in this study, we investigated the genes influenced by TMZ that were important in GBM therapy, and revealed that GADD45A plays a protective role against TMZ treatment which may through TP53-dependent and MGMT-dependent pathway in TMZ-sensitive and TMZ-resistant GBM, respectively. This protective role of GADD45A against TMZ treatment may provide a new therapeutic strategy for GBM treatment [1)].

Alterations of the TP53 tumor suppressor gene occur in ~30% of primary glioblastoma (GBM) with a high frequency of missense mutations associated with the acquisition of oncogenic “gain-of-function” (GOF) mutant (mut)p53 activities. PRIMA-1MET/APR-246, emerged as a promising compound to rescue wild-type (wt)p53 function in different cancer types. Previous studies suggested the role of wtp53 in the negative regulation of the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT), a major determinant in resistance to therapy in GBM treatment. The potential role of MGMT in expression of p53 and the efficacy of PRIMA-1MET with respect to TP53 status and expression of MGMT in GBM remain unknown. We investigated response to PRIMA-1MET of wtp53/MGMT-negative (U87MG, A172), mutp53/MGMT-positive U138, LN-18, T98/Empty vector (T98/EV) and its isogenic MGMT/shRNA gene knockdown counterpart (T98/shRNA). We show that MGMT silencing decreased

expression of mutp53/GOF in T98/shRNA. PRIMA-1MET further cleared T98/shRNA cells of mutp53, decreased proliferation and clonogenic potential, abrogated the G2 checkpoint control, increased susceptibility to apoptotic cell death, expression of GADD45A and sustained expression of phosphorylated Erk1/2. PRIMA-1MET increased expression of p21 protein in U87MG and A172 and promoted senescence in U87MG cell line. Importantly, PRIMA-1MET decreased relative cell numbers, disrupted the structure of neurospheres of patient-derived GBM stem cells (GSCs) and enabled activation of wtp53 with decreased expression of MGMT in MGMT-positive GSCs or decreased expression of mutp53. Our findings highlight the cell-context dependent effects of PRIMA-1MET irrespective of p53 status and suggest the role of MGMT as a potential molecular target of PRIMA-1MET in MGMT-positive GSCs [2].

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Wang HH, Chang TY, Lin WC, Wei KC, Shin JW. GADD45A plays a protective role against temozolomide treatment in glioblastoma cells. Sci Rep. 2017 Aug 18;7(1):8814. doi: 10.1038/s41598-017-06851-3. PubMed PMID: 28821714.

2)
Patyka M, Sharifi Z, Petrecca K, Mansure J, Jean-Claude B, Sabri S. Sensitivity to PRIMA-1MET is associated with decreased MGMT in human glioblastoma cells and glioblastoma stem cells irrespective of p53 status. Oncotarget. 2016 Aug 11. doi: 10.18632/oncotarget.11197. [Epub ahead of print] PubMed PMID: 27533246.