Genistein-induced neuronal apoptosis and G2/M cell cycle arrest is associated with MDC1 up-regulation and PLK1 down regulation.


Abstract:

The aim of the present study is to investigate the effect of genistein on human neuroblastoma SK-N-MC cells. MTT proliferation assay, LDH cytotoxicity assay, flow cytometric analysis, real-time quantitative RT-PCR and western blotting were used to investigate the effect of genistein on cell survival, cellular toxicity, cell cycle progression, and mRNA and protein alterations of selected DNA damage-, cell cycle- and apoptosis-related genes in SK-N-MC cells. Genistein suppressed cell proliferation, increased LDH release and modulated cell cycle distribution through accumulation of cells at G2/M- and S-phase and sub-G0 (cell death) with a concurrent decrease of cells at G0/G1 phase. Genistein increased the MDC1 (Mediator of DNA damage Checkpoint protein 1), p53, p21(waf1/cip1), Cdc2 and Bax mRNA levels in a dose-dependent manner. However, PLK1 (Polo-Like Kinase 1) and Cyclin B1 mRNAs were down-regulated after genistein treatment. Furthermore, Genistein did not alter Chk2 (Checkpoint Kinase 2), Bcl-2 and Cdc25C mRNA levels. On western blotting analyses; genistein increased the protein level of MDC1, p53, p21(waf1/cip1), and Bax in a dose-dependent manner. Genistein also increased the phosphorylation of Chk2 and Cdc25C at Thr-68 and Ser-216, respectively. In addition, consistently with PLK1 down-regulation, the phosphorylation of Cdc25C at Ser-198 was markedly decreased after genistein treatment. Additionally, Chk2, Cdc25C, Cyclin B1, p-Cyclin B1 (Ser-147), and Cdc2 as well as Bcl-2 proteins were down-regulated after genistein treatment. Altogether, these results suggest for the first time the involvement of MDC1 up-regulation after genistein treatment in DNA damage-induced Chk2 activation- and PLK1 down-regulation-mediated apoptosis and cell cycle checkpoint pathways.

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