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Calprotectin (S100A8/S100A9)-induced cytotoxicity and apoptosis in human gastric cancer AGS cells: Alteration in expression levels of Bax, Bcl-2, and ERK2

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Abstract

Calprotectin is a heterodimeric EF-hand Ca2+ binding protein that is typically released by infiltrating polymorphonuclear leukocytes and macrophages. This protein is a key player linking inflammation and cancer. Due to the increased levels of calprotectin in different inflammatory diseases and cancer, it is considered as a marker for diagnostic purposes. In this study, we evaluated the mechanism of cell viability and apoptotic-inducing effects of recombinant human calprotectin (rhS100A8/S100A9) on the gastric adenocarcinoma (AGS), the most common type of gastric cancer cell line. AGS cells were exposed to the different concentrations (5–100 μg/ml) of calprotectin for 24, 48, and 72 h, and cell viability was assessed through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Apoptotic-inducing effects of calprotectin were evaluated by sub-G1 cell cycle assay and Annexin V/propidium iodide double staining. Furthermore, real-time polymerase chain reaction and Western blot analysis were performed to evaluate the mechanism of action of calprotectin. Our findings indicated that calprotectin inhibits growth and viability of AGS cells in a time- and dose-dependent manner. The half-maximal inhibitory concentration values were measured as 85.77, 79.14, and 65.39 μg/ml for 24, 48, and 72 h, respectively. Additionally, we found that calprotectin downregulated the expression of antiapoptotic protein Bcl-2 and upregulated proapoptotic protein Bax in a time- and concentration-dependent fashion. Calprotectin also slightly upregulated the expression of extracellular signal-regulated protein kinase 2 (ERK2), while it significantly decreased the levels of phospho-ERK in a time-dependent manner. Overall, these findings indicated that calprotectin has cytotoxicity and apoptosis-inducing effects on AGS cell lines in high concentration by modulating Bax/Bcl-2 expression ratio accompanied by inhibition of ERK activation.

Keywords Calprotectin (S100A8/S100A9), apoptosis, Bax, Bcl-2, ERK, AGS cells