ESE-1/EGR-1 pathway plays a role in tolfenamic acid-induced apoptosis in colorectal cancer cells

Seong-Ho Lee¹, Jae Hoon Bahn¹, Chang Kyoung Choi¹², Nichelle C. Whitlock¹, Anthony E. English², Stephen Safe³ and Seung Joon Baek¹

¹ Laboratory of Environmental Carcinogenesis, Department of Pathobiology, College of Veterinary Medicine, and ² Department of Mechanical, Aerospace and Biomedical Engineering, College of Engineering, University of Tennessee, Knoxville, Tennessee and ³ Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas

Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are known to prevent colorectal tumorigenesis. Although antitumor effects of NSAIDs are mainly due to inhibition of cyclooxygenase activity, there is increasing evidence that cyclooxygenase-independent mechanisms may also play an important role. The early growth response-1 (EGR-1) gene is a member of the immediate-early gene family and has been identified as a tumor suppressor gene. Tolfenamic acid is a NSAID that exhibits anticancer activity in a pancreatic cancer model. In the present study, we investigated the anticancer activity of tolfenamic acid in human colorectal cancer cells. Tolfenamic acid treatment inhibited cell growth and induced apoptosis as measured by caspase activity and bioelectric impedance. Tolfenamic acid induced EGR-1 expression at the transcription level, and analysis of the EGR-1 promoter showed that a putative ETS-binding site, located at −400 and −394 bp, was required for activation by tolfenamic acid. The electrophoretic mobility shift assay and chromatin immunoprecipitation assay confirmed that this sequence specifically bound to the ETS family protein epithelial-specific ETS-1 (ESE-1) transcription factor. Tolfenamic acid also facilitated translocation of endogenous and exogenous ESE-1 to the nucleus in colorectal cancer cells, and gene silencing using ESE-1 small interfering RNA attenuated tolfenamic acid-induced EGR-1 expression and apoptosis. Overexpression of EGR-1 increased apoptosis and decreased bioelectrical impedance, and silencing of endogenous EGR-1 prevented tolfenamic acid-induced apoptosis. These results show that activation of ESE-1 via enhanced nuclear translocation mediates tolfenamic acid-induced EGR-1 expression, which plays a critical role in the activation of apoptosis. [Mol Cancer Ther 2008;7(12):3739–50]