DOWNREGULATION OF TFFS ENCODING GASTROINTESTINAL HEALING PEPTIDES BY PROINFLAMMATORY CYTOKINES

Baus Lončar M. 1, Kayademir T.1, Marinović M. 2 Blin N. 3 (mbausloncar@yahoo.com)

1 Dept. of Medical Biology, School of Medicine, University of Osijek, Osijek, Croatia
2 German Institute of Human Nutritione, Bergholz-Rehbrücke, Germany
3 Inst. for Human Genetics,University of Tuebingen,Tuebingen, Germany

Introduction: Rapid repair of epithelial surfaces is essential for prevention of mucosal inflammation and in long term can reduce cancer risk (1). The three mammalian TFF (trefoil factor family) peptides (TFF1, TFF2, TFF3) are major secretory products of gastrointestinal (GI) epithelia and their expression is up-regulated in response to GI mucosal damage (2). TFFs promote cell migration, protect and heal the mucosa and may function as tumor suppressors (3). We assumed them to be regulated by the proinflammatory cytokines TNF-α, IL1β and IL6, which trigger the transcriptional factors NF-κB and C/EBPβ.

Methods: Gastrointestinal cell lines (KATO III and HT-29) were stimulated with TNF-α, IL1β, IL6 (20 ng/ml) and expression of TFFs was monitored by quantitative real-time PCR. D ct (threshold cycle) values were determined by subtracting the difference of the ct levels between TFF genes and the housekeeping gene (GAPDH) after incubation with stimulans or without (control) for the indicated times. Remained TFF expression relative to control is shown. Independent triplicate stimulation experiments were performed and analyzed by ANOVA. TFF3 expression was monitored by immunohistochemistry in TNBS induced colitis rat model. Animals were sacrificed at day 0 (control), 4, 11 and 21 after the TNBS injection. After autopsy, specimen were taken from the margin area of the treated colon segment. The samples were fixed in 5% paraformaldehyde and embedded in paraffin wax. After antigen demasking the slides were incubated with anti-TFF3 rabbit serum or with anti-NF-κB antibody detecting activated form of p65 subunit. Specific antibody binding was visualised by biotin-conjugated sheep anti-rabbit IgG, followed by a streptavidin-biotin-horseradish peroxidase complex and diaminobenzidine. Microscopy was done using a light microscope (E-1000, Nikon) with differential interference contrast (DIC).

Results: TNF-α, IL1β and IL6 caused a 3- to 11-fold reduction in TFF mRNA expression, displayed in real-time PCR. In vivo epithelial NF-κB expression coincided with strongly reduced TFF3 expression during the acute phase of experimental colitis.

Conclusions: Down-regulation of intestinal trefoil factor TFF3 due to transcriptional repression by TNF-α and IL1β through NF-κB as well as by IL6 through C/EBPβ activation in vitro reflects very likely the situation in vivo and may contribute to ulceration and decreased wound healing during inflammatory bowel disease.

References: