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The role of telomeric repeat-binding factor (TRF1) in inflammatory bowel diseases – diagnostic and therapeutic potential

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Introduction

Inflammatory bowel diseases (IBD) are chronic relapsing disorders of the gastrointestinal (GI) tract with an unknown pathophysiology. The association between telomeric proteins, i.e. telomeric repeat-binding factor 1 and 2 (TRF1, TRF2), and the GI diseases has been poorly studied; however, changes in TRF1 expression (at mRNA level) were found in IBD patients as compared to the controls. Furthermore, some in vitro and in vivo studies have demonstrated that chronic inflammation may be associated with cellular senescence, and may lead to telomere dysfunction. For example, telomere length was hypothesized to be affected by stress-induced premature senescence (SIPS) in IBD patients.

Numerous studies are currently focused on finding drugs that can modulate senescence. It is possible that lactoferrin, a glycoprotein derived from milk, which displays anti-inflammatory, anti-cancerogenic, and antioxidant properties, may be considered as a potential modulator of senescence. In this study, we aimed to identify the role of senescence and selected telomeric proteins in IBD etiology and analyze the potential effects of LF in vitro and in vivo.

Materials and Methods

For the in vitro part of the study, two colorectal cancer lines (HCT116, SW-480) have been chosen. In order to induce senescence, the cells were incubated with doxorubicin (100 nM DOX) or LF (0.625-2.5 mg/mL) according to the protocols available. As part of the research to assess senescence, the activity of the lysosomal enzyme-galactosidase associated with aging (SA-β-gal) was evaluated. Further, we investigated the relationship between senescence and expression of TRF1, and TRF2 proteins, and LF's effects on senescence and expression of selected telomeric proteins.

The in vivo part of the experiment was performed on BALBc mice (male). The dextran sulfate sodium (DSS)-induced model of colitis was used in the research. The mice were randomly distributed among 4 different experimental groups: group 1, no treatment; group 2, treated with DSS; group 3, treated with DSS + 250 mg/kg b.w. LF (p.o., once daily, from day 3 to 6); group 4, treated with DSS + 500 mg/kg b.w. LF (p.o., once daily, from day 3 to 6). Groups 2-4 were given 3% DSS in tap water from day 0 to 5. Following the removal of DSS, all animals were given tap water on days 6 and 7. Animal body condition, general health, and disease progression were monitored daily. After euthanasia, the tissues were isolated and then assessed in macroscopic, histopathological, and molecular examinations. Western blotting was used to detect the expression of proteins such as TRF1 and TRF2 in the colons harvested from the DSS-induced mouse model of colitis. All animal protocols were approved by the Local Ethical Committee for Animal Experiments (ŁB/229/2022).

Results

DOX significantly increased the relative number of SA-β-gal-positive cells in HCT116 ($p < 0.0001$) in comparison with the control group (Fig. 1). Moreover, treatment with DOX resulted in a marked increase of the size of cells and their nuclei (data not shown). By contrast, LF did not significantly affect the change in cell size in both cell lines. However, the concentrations of 1.25 and 2.5 mg/mL LF significantly increased the number of SA-β-gal-positive cells in HCT-116 ($p < 0.05$ and $p < 0.0001$, respectively) compared to control.

In both cell lines, there was a significant change in the expression of TRF1 and TRF2. In HCT116 cells, DOX significantly reduced mRNA and protein expression of TRF1 and TRF2 vs. the control group (Fig. 2). At lower concentrations of LF (0.625 and 1.25 mg/mL LF, respectively) mRNA and protein expression of TRF1 and TRF2 did not change significantly in comparison with the control, while 2.5 mg/mL LF decreased mRNA and protein expression of TRF1 and TRF2. Likewise, 100 nM DOX significantly reduced TRF1 and slightly diminished TRF2 mRNA and protein expression in SW480 cells. In turn, the effects of 0.625 and 1.25 mg/mL LF were non-significant, however, 2.5 mg/mL LF significantly reduced TRF1 and TRF2 mRNA levels.

In the DSS-only treated mice, the expression of TRF1 and TRF2 proteins was significantly augmented in comparison to control (Fig. 3). Similarly, 250 mg/kg LF upregulated TRF1 ($p < 0.001$) and TRF2 ($p < 0.0001$) protein expression, whereas 500 mg/kg LF caused a statistically significant increase only in TRF2 ($p < 0.01$). Noteworthy, 500 mg/kg LF significantly reduced TRF1 expression ($p < 0.05$) compared with the DSS-only treated group. Also, TRF2 expression was reduced in 500 mg/kg LF-treated mice compared to the DSS-only treated group, but the difference was not statistically significant.

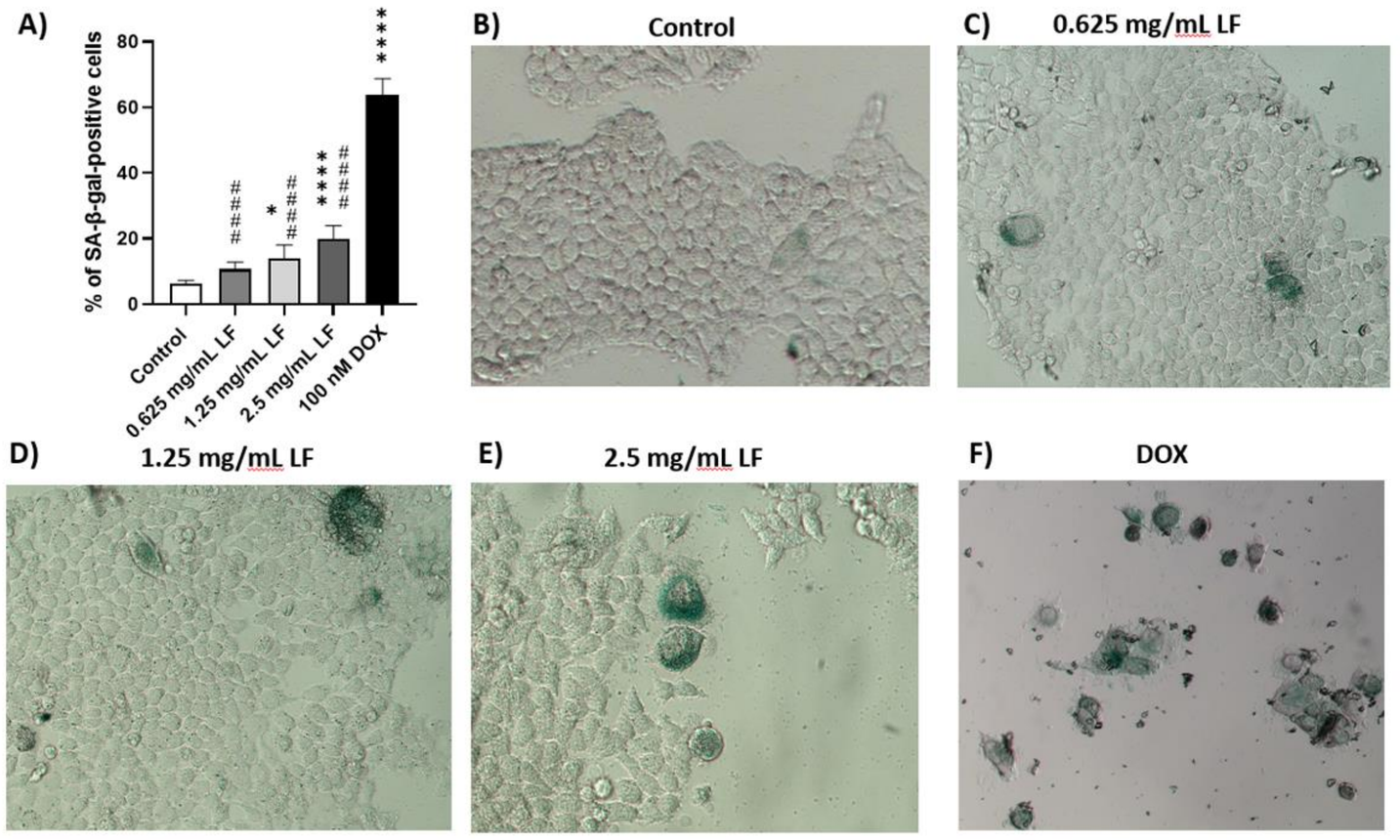

Figure 1. Quantification of SA-β-gal-positive HCT116 cells in response to LF and DOX. Results are expressed as a mean of at least 3 independent experiments ± SEM. **** $p < 0.0001$, * $p < 0.05$ vs. control; #### $p < 0.0001$ vs. DOX.

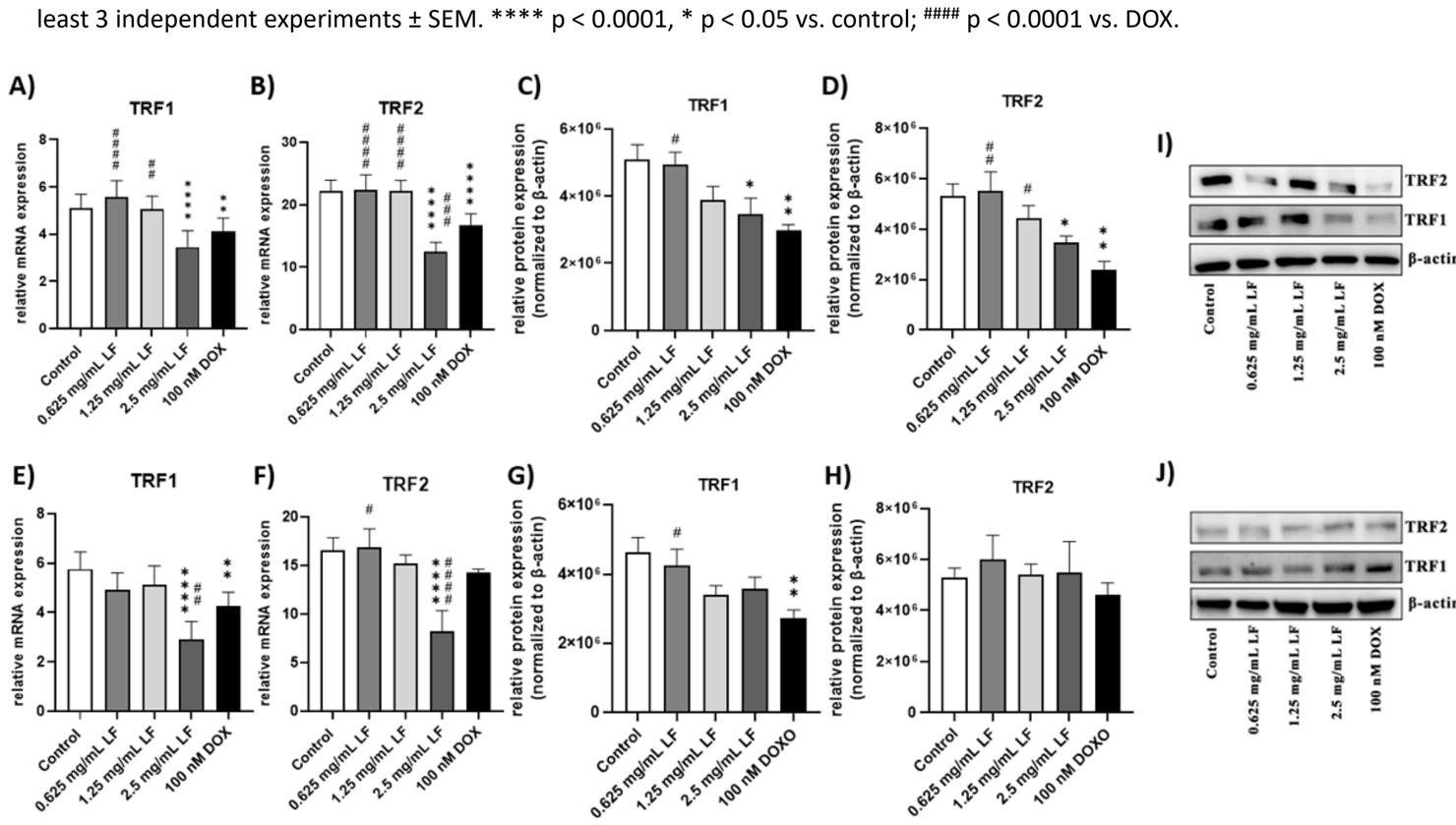

Figure 2. Changes in relative mRNA and protein expression of TRF1 and TRF2 in HCT116 (A-D) and SW480 (E-H) cells in response to LF and DOX. Representative Western blot analysis of TRF1 and TRF2 in HCT116 (I) and SW480 (J). Data represent mean ± SEM. **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$ as compared with untreated control; #### $p < 0.0001$, ### $p < 0.001$, ## $p < 0.01$, # $p < 0.05$ as compared with DOX.

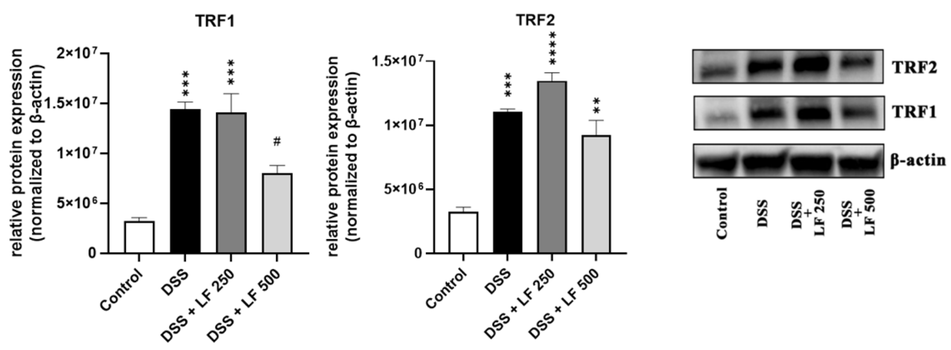

Figure 3. Changes in protein expression of TRF1 (A) and TRF2 (B) in the colon of control, DSS-only treated mice (DSS), and mice with DSS-induced colitis treated with LF in two doses: 250 mg/kg (DSS + LF 250) and 500 mg/kg (DSS + LF 500). Representative Western blot analysis of TRF1 and TRF2 (C). Data represent mean ± SEM. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$ as compared with the control; #$p < 0.05$ as compared with DSS-only treated mice.

Conclusions

- A model of in vitro senescence was validated.
- It was observed that senescence may contribute to IBD pathogenesis by causing chronic inflammation.
- A change in TRF1 and TRF2 expression may occur as part of senescence, but its role in IBD still needs to be determined.
- LF regulates cell senescence markers expression in vitro and in vivo and may modulate senescence in a concentration-dependent manner.