Transglutaminase 2 is elevated in Crohn’s disease associated strictures and exerts profibrotic activities in myofibroblasts and experimental intestinal fibrosis

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Background: Intestinal fibrosis is a significant clinical problem in Inflammatory bowel disease (IBD). This may lead to intestinal strictures induced by excessive accumulation of extracellular matrix (ECM) produced by human intestinal myofibroblasts (HIMF). Transglutaminase (TG2) covalently cross-links ECM-associated proteins, increases ECM stiffness and has direct pro-fibrotic effects. We investigated the role of TG2 in intestinal fibrosis in human tissues, HIMF and transgenic mouse models.

Methods: TG2 was detected in human tissues from Crohn’s disease (CD) strictures (CDs; n=10), CD non-strictured (CDns; n=20), ulcerative colitis (UC; n=10) and non-IBD controls (NL; n=14) by immunofluorescence (IF), qPCR, in-situ hybridization (ISH), and biotinylated cadaverin incorporation (BCI). Relative contribution of TG2 was measured by BCI using a TG2-selective blocking antibody (TAb). The cellular source of TG2 was evaluated by IF. HIMF ECM production was measured using a novel ECM deposition assay and TG2-inhibitors. HIMF were exposed to anti-TG2 antibody or isotype control and bulk RNA-sequencing (RNAseq) analysis was performed. Tamoxifen-inducible globalCre/TG2 floxed conditional KO (cKO) mice were used in the DSS and TNBS fibrosis models.

Results: Total TG activity was increased in CDs and UC vs. NL in the submucosa (SM) and muscularis propria (MP; \( P \leq 0.04 \)). Strikingly 90-99% of TG activity were attributable to TG2 in all groups. TG2 activity correlated with fibrosis scores \( (P \leq 0.02) \), but not inflammation scores. TG2 amount was elevated in CDs patients vs. NL in qPCR and ISH \( (P \leq 0.05) \). IF revealed HIMF as the major source of TG2 in the intestine as indicated by colocalization with alpha-SMA. HIMF, in vitro, produced 82–97% of active TG2. Inhibition of TG2 by cell-permeable inhibitor or Tab reduced fibronectin (FN) and collagen (COL)1 and 3 deposition by HIMF. RNAseq showed TAb-treated cells expressed 432 downregulated and 402 upregulated genes. Functional enrichment analysis showed pathways associated with fibroblast activation/proliferation, ECM, and fibrosis-associated signaling (e.g. AKT) pathways to be downregulated in TAb-treated HIMF. Associated genes included nuclear protein (NUPR1) and sphingolipid signaling pathway proteins (SMPD1 and CTSD), which may be potential targets for TG2-mediated activity in HIMF. Deletion of TGM2 prior to inducing murine DSS or TNBS experimental fibrosis did not lead to any difference in inflammation, but decreased fibrosis (picrosirius red intensity, COL1/FN expression, wall thickness) in TG2 cKO mice vs. wild type \( (P \leq 0.05) \).

Conclusion: TG2 is involved in inflammation-independent progression of intestinal fibrosis and may represent a future anti-fibrotic target.