THE ROLE OF THE mRNA BINDING PROTEIN TRISTETRAPROLIN IN INFLAMMATORY BOWEL DISEASE AS A POTENTIAL THERAPEUTIC TARGET

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Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are chronic relapsing disorders of the gastrointestinal tract that are characterized by intestinal inflammation and epithelial injury. Cytokines seem to have a crucial role in the pathogenesis of progressive and destructive forms of IBD (Neurath, 2014). In particular cytokines production is under the control of the signaling cascades involving the MAPK-activated protein kinase 2 (MK2) that has also a role in mediating IBD (Broom et al., 2009).

The mRNA-binding protein tristetraprolin (TTP) binds to adenosine/uridine-rich elements in the 3' untranslated region of target mRNAs (Ross et al., 2016). TTP recognizes the conserved cis-acting pentameric sequence AUUUA and recruits the Ccr4/Caf1/Not deadenylation complex that shortens the poly (A) tail of target mRNAs resulting in suppression of translation and in a rapid degradation of the transcript. TTP target mRNAs are mostly pro-inflammatory cytokines, for example TNF, IL3, IL2, IL1b, IL6, IL8 and many others studied principally in macrophages of different tissues (Brooks et al., 2013). TTP has a complex regulation: the MK2 phosphorylates serines 52 and 178 of murine TTP (60 and 186 of human TTP), protecting it from destruction by the proteasome and impairing its ability to recruit deadenylases, resulting in a stabilization of its mRNA targets and in an increase of transcripts of most pro-inflammatory cytokines (Ross et al., 2016). TTP loss-of-function experiments indicate a role in controlling inflammation, indeed when the gene encoding TTP is disrupted in mice, the animals develop a profound inflammatory syndrome that includes cachexia, arthritis, conjunctivitis, dermatitis, myeloid hyperplasia, splenomegaly, and autoimmunity (Patial et al., 2016). In a recent work the expression of TTP protein was significantly elevated in synovial tissue of patients with rheumatoid arthritis compared with non-inflamed controls, as a result of a phosphorylated TTP (Ross et al., 2016).

The aim of this study is to investigate if the complex activity of TTP could influence also the pathogenesis of IBD.

Eleven IBD paediatric patients (5 UC and 6 CD) were enrolled at diagnosis at the Paediatric Clinic of IRCCS Burlo Garofolo in Trieste. For each patient, biopsy samples were obtained during a colonoscopy from inflamed and healthy mucosa. TTP protein was not detectable in the whole protein lysate from IBD patients using western blot (WB) analysis. Since TTP has been shown to be a very stable protein in macrophages (Cao et al., 2004), human macrophages, differentiated from peripheral blood mononuclear cells (PBMCs) of healthy donors from the Queen Elizabeth Hospital of Birmingham, were used as controls. Isolated macrophages were stimulated with 10 ng/ml bacterial lipopolysaccharide (LPS) for 60 and 120 minutes; WB results showed an increase of TTP expression after 60 minutes of stimulation respect to the control and a further increase after 120

minutes. These results confirm a role of the protein under LPS-induced inflammation and moreover suggest that isolation of macrophages from PBMC of IBD patients could be useful to clarify the role of TTP in the IBD. Immunofluorescence techniques will be used as a more sensitive method to colocalize TTP and macrophages cells directly on IBD patients' tissues.

If the role of TTP will be confirmed in the IBD, in the future it would be interesting to study the protein even in tissues obtained from patients treated with anti-TNF therapeutic antibodies, to better understand the TTP role in the regulation of TNF.

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