P034 The Hypoxia Adaptive Response Regulates Metallothionein Expression In Intestinal Epithelial Cells

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From year 2011

Aim Metallothioneins (MTs) are low weight cysteine rich proteins which are rapidly induced by a variety of stimuli including inflammation. As such, they are considered to be acute phase proteins eliciting a wide variety of protective cellular functions. In active intestinal inflammation, particularly in the gut epithelium, MT expression is down-regulated, however, the cause and functionality of this observation remains to be investigated. Hypoxia-inducible factor 1 α (HIF1A) is up-regulated during intestinal inflammation-dependent hypoxia. The interdependent role of HIF1A and MTs has been investigated in different inflammatory diseases and both proteins have independently been proposed in pathogenesis of Inflammatory Bowel Disease (IBD). In this study, we investigated the interdependent role of HIF1A and MTs in colonic epithelial cells.

Materials and Methods Dimethyloxalylglycine (DMOG) was used to subject colonocytes to hydroxylase inhibition and HIF1A stabilization in three experimental models (*in vitro*, *in vivo* and *ex vivo*). Small interfering RNA targeting HIF1A (SiRNA-HIF1A) and MT (SiRNA-MT) and zinc mediated MT induction was used in HT29 cells to study the interaction of HIF1A and MT. MT expression and HIF1A levels were measured using quantitative real-time PCR and ELISA respectively.

Results Hydroxylase inhibition down-regulated MT expression in cultured HT29 cells and in freshly isolated human colonocytes as well as in colonocytes isolated from mice treated with DMOG. SiRNA-HIF1A treated cells displayed significant higher basal MT levels ($p<0.05$) and an attenuated MT down-regulation after DMOG treatment. In turn, HIF1A stabilization was significantly lower in zinc treated control cells, displaying high levels of MT, compared to SiRNA-MT cells treated with DMOG ($p<0.05$).

Conclusion We, for the first time, demonstrated a HIF1A-mediated down-regulation of acute stress genes called metallothioneins in colonocytes. In turn, MTs were able to attenuate HIF1A stabilization, possibly through zinc deprivation. The observed reciprocity needs to be further explored for its role in intestinal inflammatory processes. Where HIF1A is over-expressed in IBD patients with protective properties in murine models of colitis, the low MT profile in IBD patients may point to a hypoxia-driven adaptive response in the course of gut inflammation.