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DNA methylation signatures associated with pathogenesis Crohn's disease-related genes

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Background: Epigenetic mechanism in Crohn's disease (CD) pathogenia is important for gene expression regulation, as complex interactions between genes and the environment occur. DNA methylation is an epigenetic mechanism that negative regulates DNA expression. However, little is known about the associations of DNA methylation and CD pathogenesis. The study of the level of methylation in CD-related genes may help to identify key elements in the pathology of CD, and to select new therapeutical targets. Therefore, we aimed to assess the DNA methylation changes on specific genes, previously related to the CD pathogenesis, and their possible associations with the pathology.

Methods: We included 31 subjects: 11 active CD (aCD) at the onset of disease and prior to any specific medication; 12 inactive CD (iCD) with clinical, analytical and morphologic remission; 8 healthy controls (CTR). DNA was obtained from peripheral blood and analysed by Sequenom. Gene-selection was based on the previous information regarding their role in CD. Candidate genes were: Catalase (CAT), α-defensin 5 (HNP-5), FasR, FasL, TNF, TNFRSF1A, TNFRSF1B, PPA2, ABCB1, NOD2, PPARγ, PKCζ. In addition, a prospective cohort of new patients and controls was recruited for the validation of results: 24 aCD; 24 iCD; 24 CTR. We used the elastic net algorithm for the statistical analysis and the R software (version 3.1.0).

Results: We studied a total of 280 CpGs from the selected genes. Only 16 CpGs showed differential methylation profiles between the three experimental groups (aCD, iCD and CTR). From these 16 CpGs, we selected for validation those with the higher differences between aCD, iCD and CTR: HNP-5 CpG_11 and CpG_13; CAT CpG_31.32; TNF CpG_4 and CpG_12; ABCB1 CpG_6.7.8. Results validated the genes HNP-5 and TNF with $p < 0.001$. HNP-5 showed increase in methylation, whereas TNF showed decrease in methylation. In both cases the level of methylation was maintained and did not change by the activity of CD (aCD vs. iCD). Subanalysis comparison between aCD and iCD showed significant differential methylation profile in the following CpGs: TNF CpG_10; FAS CpG_7.8.9; ABCB1 CpG_6.7.8, CAT CpG 6.8.9.31.32, TNFRS1BF CpG_10.11.12.

Conclusions: The identification of DNA methylation signatures associated with pathogenesis CD-related genes could help to improve the diagnosis and management of CD patients. The permanent increased methylation of HNP-5 gene and the permanent decreased methylation of TNF gene confer a signature for CD patients' identification. The differential profile of methylation between aCD and iCD could be used as an activity signature. New treatments focussed on modifying those methylation signatures could be explored for CD management.

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Heat shock protein GP96 is essential for maintaining intestinal epithelial architecture by supporting its self-renewal capacity

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Background: The intestinal epithelium is characterised by a remarkable self-renewal capacity and a high turnover of intestinal epithelial cells (IEC), which emerge from intestinal stem cells (ISC). Defects in proliferation and differentiation of ISC into mature IEC result in impaired barrier function, which is linked with systemic diseases, such as fatty liver disease and diabetes. Glycoprotein (GP)96 is a master chaperone for cell surface receptors including toll-like receptors (TLRs), integrins and the Wnt co-receptors LRP5/6. Wnt signalling is essential for the maintenance of the ISC niche, thus we here investigated how deletion of GP96 specifically in IEC affects intestinal homeostasis.

Methods: To study the role of GP96 in the intestinal epithelium, we used GP96-VillinCre-ERT2 mice which harbour a loxP flanked GP96 gene and Tamoxifen-inducible Cre expression specifically in IEC. To deplete GP96 from IEC, those mice were injected with Tamoxifen at five consecutive days (1 mg/day) and terminated on Day 5, 6, and 7 after the first dose to observe the changes in epithelial integrity over time. As a control, littermates with a loxP flanked GP96 gene without the VillinCre-ERT2 construct were injected with Tamoxifen.

Results: IEC-specific GP96 depletion resulted in rapid weight loss within 6 days after the first Tamoxifen injection. At Day 6, the intestine of GP96-VillinCre-ERT2 mice revealed visible signs of inflammation, characterised by a general shortening of the colon and the small intestine, as well as thickening of the colon wall observed by mouse endoscopy. Colon wall thickening was in sharp contrast to the transparent and fragile appearing small intestine and caecum wall. This was in line with a significant, successive reduction of IEC numbers upon depletion of GP96, as observed when harvesting IEC, and by histological analysis of small intestinal tissue sections. Additionally, the entire intestine was filled with bile-containing intestinal fluid, while solid food or faecal pellets were completely absent. Of interest, apoptosis was not affected upon GP96 depletion in IEC; however, proliferating cells as determined by Ki67 staining, at the crypt base were markedly reduced, indicating that loss of GP96 affects ISC proliferation/function.

Conclusions: Our results underline the importance of GP96 in maintaining homeostasis of the intestinal epithelial architecture. Elucidating molecular mechanisms that are decisive for the fate of the ISC niche will promote the understanding of the pathogenesis of inflammatory diseases associated with barrier defects. Given the pronounced phenotype upon deletion of GP96 in IEC, it might serve as a promising novel therapeutic target in diseases involving intestinal barrier defects.

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Adipose-derived stem cells ameliorate colitis by suppression of inflammasome formation and regulation of M1-macrophage population through prostaglandin E2

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