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P047
Osteopontin in colitis-associated carcinoma (CAC)

S. Manna[1], M. Sehn[2], D. Cardoso da Silva[1], C. Heldt[1], L. Horstkorte[1], S. Elezkurtaj[3], B. Siegmund[1], B. Weixler[2], M. Hummel[3], J. Gröne[2], M. Schumann[1]

[1]Charité Universitätsmedizin Berlin, Department for Gastroenterology-Rheumatology and Infectious Diseases, Berlin, Germany, [2]Charité Universitätsmedizin Berlin, Department of Visceral Surgery, Berlin, Germany, [3]Charité Universitätsmedizin Berlin, Institute of Pathology and Molecular Pathology, Berlin, Germany

Background: Inflammatory bowel disease (IBD) patients pose a lifelong risk of developing colitis-associated carcinoma (CAC). Current understanding and mechanisms of CAC development stem from murine CAC models while studies on human CAC carcinogenesis are scarce. Thus, we aimed to understand CAC carcinogenesis, using human samples from patients suffering from Crohn´s disease (CD), CD-associated CAC (CD-CAC), ulcerative colitis (UC), and UC-associated CAC (UC-CAC) and further validated the findings in vitro.

Methods: We obtained FFPE specimens from 40 patients that suffered from either one of the IBDs or CACs and furthermore inflammation-free healthy controls (10 patients per group). These samples were micro-dissected, followed by RNA isolation and Nanostring gene expression analysis. Our data revealed a strong upregulation of SPP1 (Secreted

Phosphoprotein-1) gene encoding for osteopontin (OPN) upon comparing heathy controls to IBD and to CAC. We then used in vitro-models to elucidate the mechanism of OPN-induced carcinogenesis. We stimulated intestinal epithelial cell lines HT-29/B6 and T84 cells with rhOPN (recombinant human OPN), following bulk RNAseq. We validated the findings using Seahorse assay for analysis of mitochondrial respiration (determining oxygen consumption rate, OCR). Western phospho-blots were done to unravel signal transduction events.

Results: Nanostring gene expression data revealed the following: (i) SPP1 gene encoding for OPN and epithelial-to-mesenchymal (EMT) transcription factors fibronectin-1 (FN1) and ZEB1 were strongly upregulated in UC-CAC and CD-CAC patients when compared to UC and CD. (ii) IL17-pathway genes were significantly downregulated in UC-CAC patients. (iii) Tight junction and polarity genes were altered in both UC-CAC and CD-CAC patients. rhOPN stimulation in vitro to HT29/B6 and T84 cells excluded OPN as the trigger of EMT but uncovered upregulated mitochondrial respiratory chain genes as a potential trigger for tumour cell proliferation. Validation of RNAseq findings by Seahorse assay revealed that after sequential injections of oligomycin, FCCP, rotenone and antimycin A both, HT-29/B6 and T84 cells, had higher basal and maximal OCRs in response to rhOPN (Fig. 1). T84, but not HT-29/B6 cells also showed an upregulated ATP production in response to rhOPN.

Conclusion: SPP1 expression correlates with CAC carcinogenesis. Putative underlying pathways rather include modulation of mitochondrial respiratory chain activity by differential gene expression than induction of EMT.