Comparing densitometry with iTRAQ fold change.

Figure 1. Graphical representation of K8 Western immunoblot densitometry and iTRAQ fold change. H - high butyrate, L - low butyrate, AD - adenoma, CO contralateral, MS - Midsigmoid, N - normal

Figure 2. western immunoblot for keratin 8

Abstract PWE-167 Figure

associated with these dysplastic crypts show a differentiated epithelium. The genotypic relationship between the BCDA and the differentiated surface epithelium is unclear.

Methods We obtained 17 examples of BCDA: the lower crypts and upper crypts and surface epithelium were differentially laser-microdissected from formalin-fixed, paraffin embedded sections and mutations were sought in tumour suppressor genes frequently associated with progression in Barrett's oesophagus.

Results Two patients showed a c.C238T mutation in the p16 (CDKN2A, p16Ink4A) gene and where the precise microanatomical relationships could be discerned: this clonal p16 mutation was present in both the BCDA at the crypt base and in the upper crypt and surface epithelium. This shows that the surface epithelium is derived from the dysplastic crypt epithelium: the dysplastic phenotype is therefore not fixed and can be reversed.

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Conclusion The mechanism of this change is unclear: dysplastic cells may, probably at an early stage in their progression, respond to differentiation signals. We are some way from a definition of the genotypic correlates of the dysplastic phenotype, and from an understanding of its plasticity.

Disclosure of Interest None Declared.

PWE-169 DNA METHYLATION AND EXPRESSION OF TNF, IFNG AND FOXP3 IN COLORECTAL CANCER

doi:10.1136/gutjnl-2013-304907.457

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Introduction Colorectal cancer (CRC) progression is associated with suppression of host cell mediated immunity (CMI) and local immune escape mechanisms (1,2). The aim of the study was to assess immune function in terms of the DNA methylation and expression of TNF, IFN- γ and FOXP3 and to identify potential targets for immunotherapy in CRC.

Methods 60 CRC patients and 15 matched controls were recruited. TaqMan quantitative PCR (qRT-PCR) was performed for relative quantitation of expression of TNF, IFNG, FOXP3 in the PBMC and tumour. Methylation specific PCR was performed to determine the methylation status. MSI status was determined using microsatellite primers BAT25 and BAT26.

Results TNF expression was suppressed in CRC tumours (median fold change 0.48). IFN- γ was found to be suppressed in the PBMC (median 0.34) of CRC patients. Tumours showed enhanced expression of FOXP3 (median 2.2). Expression of FOXP3 in tumours was significantly higher when the size of the tumour was more than 50mm ($p = 0.005$). Methylated *TNF* promoter and *FOXP3* cpg correlated significantly with suppression of TNF and FOXP3 respectively. Significant TNF suppression ($p = 0.002$) was noted in the PBMC of patients with MSI. No correlation was observed between the MSI status and DNA methylation status of our study genes.

Conclusion Our study identified changes in protein expression which correlated with the methylation status. It demonstrates the influence of DNA methylation on gene expression that could act as a biomarker for future immunotherapy with possible role for demethylating agents.

Disclosure of Interest None Declared.

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