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OC-017 TRIM44: FROM PROGNOSIS TO THERAPY IN OESOPHAGEAL ADENOCARCINOMA AND BREAST CANCER

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Introduction The incidence of oesophageal adenocarcinoma (EA) has quadrupled in the last 30 years and outcomes remain poor. Unlike other epithelial cancers, targeted therapies for EA are at an early stage. Using gene expression profiling, we have previously identified TRIM44 as an independent prognostic gene in EA.

Methods The aims of this project were to (1) Explore the mechanism of dysregulation of TRIM44 and association with prognosis. (2) Examine the oncogenic potential of TRIM44 in EA and other epithelial cancers (3) Identify therapeutic options exploiting TRIM44 dysregulation.

Results Analysis of our EA expression microarray data (n=75), an independent matched aCGH and expression microarray of 997 breast cancers (BC) and an online database (Tumourscape n=1932, various epithelial tumours) revealed focal amplification of TRIM44 in 8% of EA, 6% of BC and 4% of epithelial tumours. Amplification in EA was validated using FISH on tissue microarrays (n=164). Expression of TRIM44 was copy number driven in both EA and BC and amplification conferred a poor prognosis in BC (p=0.037). Functional work demonstrated oncogenic addiction to TRIM44 in cell line models harbouring amplifications; siRNA knockdown in HSC39 (amplifications) and JIMT-1 (high expression) decreased proliferation of cells by twofold ($p<0.05$) and increased subG0 fraction on FACS (2.5-fold, $p<0.05$). In contrast, knockdown in OE19 (low expression) had no observed effect. Overexpression of TRIM44 in Hela cells using a Tet-inducible system increased proliferation (2.5-fold, p=0.0038) and invasiveness (twofold, $p<0.05$). Analysis of the microarray data (EA and breast) identified a potential link between TRIM44 and the mTOR pathway, and suggested sirolimus (mTOR inhibitor) as a therapeutic option. Validation of these findings were performed by IHC of amplified EA samples and showed exact co-localisation of TRIM44 and p-mTOR staining. In addition, treatment of HSC39 and JIMT-1 with RAD001 (mTOR inhibitor) showed that they were highly sensitive (IC50 <30 nm).

Conclusion TRIM44 is amplified in >5% of EA leading to increased proliferation and invasion in vitro. Our data suggest a mode of action of TRIM44 via the mTOR pathway. Evaluation of mTOR inhibitors in EA tumours is worthy of consideration and these are currently being evaluated in phase I/II oncology clinical trials in other epithelial cancers such as renal cell and lung cancer). Assessment of TRIM44 amplification status may allow selection of patients who are more likely to respond.

Competing interests None declared.

OC-018 VALIDATION OF TWO APC-DEPENDENT POTENTIAL BIOMARKERS OF COLORECTAL CARCINOGENESIS

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Introduction Most cases of sporadic colorectal cancer develop via the adenoma carcinoma sequence and *APC* mutation is a key early molecular event. APC regulates B-catenin function and the WNT signalling pathway to control intestinal homeostasis. However, mutation or loss of *APC* gene results in translocation of B-catenin into the nucleus, where it forms a heterodimeric transcriptional factor complex with TCF and results in altered cell fate. We have previously performed a proteomic analysis of changes which occur in murine intestinal epithelium following acute b-napthoflavone-induced deletion of Apc expression ($Apc^{fl/fl}$ mice). Several proteins showed increased abundance following intestinal Apc deletion and we hypothesised that some of these may represent potential biomarkers for the serological detection of the early stages of human colorectal cancer.

Aims To investigate whether two proteins which were demonstrated to be upregulated in $Apc^{fl/fl}$ intestinal epithelium by proteomic analysis, namely serine/arginine-rich splicing factor 2 (SRSF2) and ribosomal protein L6 (RPL6) show altered expression in murine and human intestinal tumours.

Methods The expression patterns of SRSF2 and RPL6 were assessed by immunohistochemistry and RTqPCR in intestinal and colonic tumour samples obtained from $Apc^{Min/+}$ mice aged 1, 3 and 6 months and 15 human subjects with colorectal cancer.

Results RTqPCR demonstrated a 3.0-fold increase in SFRS2 expression and a 2.8-fold increase in RPL6 expression in colonic polyp tissue from 6-month old $Apc^{Min/+}$ mice relative to colonic tissue from $Apc^{+/+}$wild-type mice of the same age. Immunohistochemistry showed nuclear rather than cytoplasmic localisation of both SFRS2 and RPL6 in intestinal and colonic polyps from $Apc^{Min/+}$ mice aged 3 months and 6 months. The alteration in subcellular localisation of SFRS2 and RPL6 appeared to occur in adenomatous cells which also displayed nuclear translocation of B-catenin. Statistically significant increases in the relative expressions of SFRS2 (1.2-fold) and RPL6 (1.9-fold) mRNAs were also observed in human colorectal cancer tissue samples relative to adjacent unaffected colonic tissue from the same patients.

Conclusion RPL6 and SFRS2 both showed altered expression in murine and human intestinal/colonic tumours. Both these proteins are involved in regulating cell cycle progression—RPL6 regulates the G1-S transition via cyclin E and SFRS2 affects the G2/M transition via CDC5, possibly through alternative splicing. The altered expressions of PRL6 and SFRS2 may therefore deregulate cell cycle control and promote cellular proliferation, a characteristic phenotype of the intestinal epithelium of $Apc^{fl/fl}$ mice as well as intestinal tumours.

Competing interests None declared.

OC-019 ALTERED TRAIL, CASPASE12, BAK AND FAS-L EXPRESSIONS ARE ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO RADIATION INDUCED INTESTINAL EPITHELIAL APOPTOSIS IN NF-κ B1-NULL AND NF-κ B2-NULL MICE

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Introduction The Nuclear Factor κ B (NFκB) family is composed of five members, RelA, c-Rel, NFκB1, RelB and NFκB2. The first three members signal via the classical pathway and the last two via the alternative pathway to regulate several cellular processes including apoptosis. NFκB1 has previously been shown to regulate radiation-induced apoptosis in the murine small intestine, but the underlying mechanisms have not been defined. The roles of other family members, particularly those involved in alternative pathway

signalling, in regulating intestinal epithelial apoptosis in vivo have not previously been investigated.

Aims To assess susceptibility to intestinal apoptosis and the associated molecular changes in mice with germline deletions of three individual NFκB family members.

Methods Intestinal apoptosis was induced in male c-Rel-null, NFκB1-null and NFκB2-null mice and their wild-type (C57BL/6) counterparts by 8Gy γ-irradiation (n=6 per group). Apoptosis was assessed on a cell positional basis from H/E stained sections. The mRNA expression of 10 key apoptosis regulating genes in the small intestine and colon (TRAIL, Caspase12, BAK, FAS-L, FAS, p53, BCL2, BCL-XL, c-IAP2 and XIAP) was assessed by real time PCR (n=4 per group). Statistical comparisons were by ANOVA with Bonferroni post-hoc tests.

Results Basal small intestinal crypt apoptosis was significantly increased in NFκB2-null relative to C57BL/6 mice. In addition, small intestinal and colonic crypt apoptotic indices were both significantly increased (up to threefold) in NFκB1-null and NFκB2-null mice 4.5 h after 8Gy γ-irradiation relative to wild-type and c-Rel-null mice. Untreated NFκB1-null and NFκB2-null small intestine showed reduced mRNA expression of the anti-apoptotic genes BCL2, BCL-XL, c-IAP2 and XIAP. Following irradiation, NFκB1-null mice showed significant increases in the mRNA of the pro-apoptotic genes TRAIL, Caspase12 (in both small intestine and colon) and BAK (small intestine only) compared to wild-type mice. Significant increases in the mRNA of the pro-apoptotic genes Caspase12 and FAS-L were also seen in irradiated NFκB2-null small intestine and colon relative to wild-type mice.

Conclusion c-Rel expression does not appear to regulate susceptibility to intestinal epithelial apoptosis in vivo. NFκB1 and NFκB2 deletion both caused increased susceptibility to intestinal apoptosis and this was associated with altered expression of TRAIL, Caspase12, BAK and FAS-L. These NFκB family members may therefore also regulate the susceptibility of intestinal epithelia to other consequences of DNA damage such as cancer.

Competing interests None declared.

OC-020 RECURRENCE AFTER RADIOFREQUENCY ABLATION FOR BARRETT'S RELATED HIGH GRADE DYSPLASIA IS DUE TO PERSISTENCE OF EPITHELIAL MUTATIONS

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Introduction Radiofrequency ablation (RFA) is a relatively new endoscopic method for ablation of high grade dysplasia (HGD) and intramucosal adenocarcinoma (IMC) in Barrett's oesophagus. Clinical trials have shown enduring eradication of these pathologies.[1] However, some patients develop recurrent dysplasia or cancer.[1] The reason for this is unknown. The aim of the study was to investigate whether this is related to the persistence of known cancer driving mutations after radiofrequency ablation.

Methods Patient records were searched for patients with recurrent HGD or IMC after RFA. Biopsies and endoscopic mucosal resection (EMR) specimens were available before and after RFA for each case. Whole biopsies underwent nested polymerase chain reaction (PCR) sequencing for mutations commonly implicated in progression to adenocarcinoma (*TP53, CDKN2A, K-ras*).

Results Tissue was obtained for six patients before and after RFA. All patients were male (mean age 67 SD±2). Samples from five patients contained detectable mutations. In 3/5 patients, the same mutation was found in material taken before RFA as after (all TP53 mutations). The indication for RFA in all three patients was HGD; two of the patients developed IMC after RFA. In these two cases laser microdissection was performed on the post-RFA EMR samples. PCR revealed the pre RFA mutation to also be present throughout the IMC specimen. Dysplastic tissue adjacent to the IMC contained a mixture of crypts that were either wild type for the mutation in the IMC or contained the mutation indicating that the IMC was a monoclonal outgrowth and that the persistent mutation was driving the development of the recurrence. Furthermore, in all three cases, PCR of a further specimens (performed at a later time point to the first post-RFA EMR), revealed the same mutation as the initial biopsy before RFA, and the first post-RFA EMR.

Conclusion The recurrence of dysplasia and cancer after RFA is likely to be due to failure to remove persistent, cancer driving mutations. Further work will need to be done to assess whether this is because of technical errors, or related to specific problems such as "buried Barrett's".

Competing interests None declared.

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OC-021 A NOVEL SUBSET OF FUNCTIONAL IL-10 SECRETING CD8 REGULATORY T CELLS INFILTRATE HUMAN HEPATOCELLULAR CARCINOMA

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Introduction Tumour specific effector T-cells can be detected in the blood and tumours of patients with hepatocellular carcinoma but fail to mount effective immune responses. Attempts to amplify anti-tumour immune responses using immunotherapy show promise, but are hampered by the presence of suppressive regulatory T-cells (Tregs) that inhibit anti-tumour immune responses. Tregs are crucial in the maintenance of immune homeostasis and in the prevention of auto-reactive immune response but in the context of cancer they can suppress beneficial anti-tumour immunity leading to tumour progression. A novel subset of CD8 expressing Tregs has recently been described and we now report the presence of such cells in human hepatocellular carcinoma and define their functional and homing properties.

Methods Fresh tissue from hepatocellular carcinoma and matched distal non-involved tissue was obtained from patients undergoing liver resection or transplantation at the Queen Elizabeth Hospital, Birmingham after informed consent. Liver-derived T-cells were isolated and phenotyped using multi-colour flow cytometry including intracellular cytokine staining. $CD8^+$Tregs were isolated using a Mo-Flow cell sorter for functional assays. Distribution of $CD8^+$Tregs was investigated by immunohistochemistry and immunofluorescence.

Results The percentage of $CD8^+$Tregs (defined as $CD8^+CD25^{high}CD127^{low}FoxP3^+$) infiltrating hepatocellular carcinoma tumours was significantly greater compared with matched non-involved liver. Tumour-derived $CD8^+$Treg isolated by Mo-Flo sorting suppressed allogeneic effectors cells in vitro and secreted interleukin-10 (IL-10). In contrast T-cell interferon-γ (IFN-γ) production was decreased within the tumour compared with matched non-involved liver. The chemokine receptor CXCR3 which is involved in T-cell recruitment to the inflamed liver was highly expressed on tumour-derived $CD8^+$Tregs.

Conclusion A novel subset of functional IL-10 secreting $CD8^+$Tregs may suppress anti-tumour immunity in hepatocellular carcinoma.