and Periodic acid–Schiff (AB-PAS) staining. The expression of interested genes and proteins was detected by real-time quantity PCR and western blot, respectively. Results AB-PAS staining showed that VitD deficiency and VDR deletion ($VDR^{-/-}$) induced an obvious colonic mucus decrease compared to the control mice, respectively. Meanwhile, the number of goblet cells were also decreased in VitDd mice and $VDR^{-/-}$ mice by detecting the expression of mucin 2 (MUC2, $P < 0.05$) and Trefoil factor 3 (TFF3, $P < 0.05$). In addition, decrease in other important colonic mucus components FGCPB, CLCA1 and CLCA2 were also found in VitDd ($P < 0.05$) and $VDR^{-/-}$ mice ($P < 0.05$) when compared with the control mice, respectively. VitD supplement obviously increased the mucus secretion and the number of goblet cells ($P < 0.05$) when compared with the VitDd mice. In LS174T cells, siVDR transfection remarkably decreased the expression of MUC2 ($P < 0.05$), whereas paricalcitol treatment significantly induced MUC2 expression ($P < 0.05$). In the following mechanistic experiments, we further found that siVDR transfection induced significant increase in HES1 ($P < 0.05$) and decrease in Math1 ($P < 0.05$) at mRNA and protein levels. The expression of Math1 were also found decrease in VitDd mice ($P < 0.05$) and $VDR^{-/-}$ mice ($P < 0.05$) when compared with the control mice, respectively. In addition, the suppressive effects of VDR knockdown on MUC2 were blocked by LY411575 exposure ($P < 0.05$). Conclusion The VitD/VDR pathway could regulate colonic mucus barrier by modulating mucins secretion. The potential mechanism is associated with goblet cell differentiation which is dependent on notch signaling pathway. Overall, our findings would provid a novel understanding on the pathogenesis of colitis. Key word: Vitamin D; VDR; mucus; goblet cell; Notch

Su1104

ALPHA-TOCOPHERYLQUINONE ACTIVATES ARYL HYDROCARBON RECEPTOR AND ENHANCES INTESTINAL EPITHELIAL TIGHT JUNCTION BARRIER FUNCTION

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The aryl hydrocarbon receptor (AhR), a member of the basic HLH/Per-Arnt-Sim protein family, is a highly conserved ligand-activated transcription factor which acts as an environmental sensor. AhR binds to the dioxin-responsive element (DRE) in the DNA sequence and control the expression of target genes. Recent studies have demonstrated that AhR may play an anti-inflammatory role in Inflammatory Bowel Disease (IBD). Alpha-tocopherylquinone (TQ), the major oxidation product of vitamin E, was previously identified to activate AhR through chemical screening AhR assay, using AhR-responsive green fluorescence protein (GFP) reporter. The aim of the present study was to investigate the mode of action of AhR activation by TQ and understanding its mechanism of enhancing the intestinal epithelial tight junction (TJ) barrier in human intestinal Caco-2 cells and mouse model. We found that TQ-mediated increase in Caco-2 TER and reduction in paracellular flux of urea and inulin was associated with AhR activation. TQ treatment significantly increased luciferase activity in DRE luciferase vector transfected Caco-2 cells. The TQ-mediated increase in DRE luciferase vector activity was inhibited in the presence of AhR inhibitor CH-223191. In cytoplasm-nuclear fractionation study, TQ treatment caused significant shift in AhR protein expression from cytoplasm (inactive) to the nucleus (activated). TQ-mediated nuclear translocation of AhR in Caco-2 cells was also evident in confocal microscopy. TQ increased mRNA expression of barrier forming claudins CLDN-1, CLDN-3 and CLDN-4 and decreased pore forming CLDN-2 mRNA and protein expression. Since AhR is a DRE binding protein, the effect of TQ on CLDN-3 (promoter contains DRE in both human as well as mouse) promoter activity was tested. The CLDN3 promoter activity was found to be significantly increased after TQ treatment. AhR siRNA knockdown prevented TQ-induced increase in claudin-3 expression. In mice, administration of TQ significantly enhanced baseline colonic TJ barrier function (increase in TER and decrease in urea and inulin flux in Ussing chamber studies) along with an increase in colonic epithelial mRNA expression of barrier forming claudins CLDN-3, and CLDN-4 and decrease in pore forming CLDN-2 expression. In mouse DSS and TNBS colitis models, TQ-mediated reduction in severity of DSS and TNBS colitis was associated with reduction in DSS and TNBS-induced colonic TJ permeability and preservation of claudin-3 protein levels. Thus TQ activates AhR and enhances colonic TJ barrier function via differential regulation of claudins. Furthermore, TQ treatment increased AhR expression in Caco-2 cells and mouse colonocytes. TQ also rescued reduction in AhR expression during DSS and TNBS colitis. TQ offer a naturally occurring, non-toxic tool for enhancement of intestinal TJ barrier and IBD therapeutics.

Su1105

TRIM 21 PROTECTS FROM ULCERATIVE COLITIS AND COLITIC CANCER VIA SMAD7 INHIBITION

Ki Baik Hahm

Proteins of the tripartite motif-containing (TRIM) superfamily are critical in a variety of biological processes in either innate immunity or eliminating invading pathogens, by which had been implicated in pathogenesis of autoimmune diseases including inflammatory bowel diseases. The typical structure of TRIM proteins contains a RING motif in the N-terminal end, followed by a B-box motif, a coiled-coil domain, and a B30.2/PRYSPRY region in the C-terminal end led to the regulation of TGF-b anti-inflammatory cytokines, by which TRIM21 has been reported to regulate IBD negatively through inhibiting Th1/Th17 cell differentiation. Since antisense oligonucleotide targeting smad7 was withdrawn from clinical trial due to insufficient efficacy, in this study, we generated TRIM21 overexpessed cell lines to study the binding of TRIM21 to smad7 as well as the regulation of consequent TGF-b receptor. As result, TRIM21 significantly binds to smad 7 as well as repressed levels of TGF-b type I/II receptor. SBE-luc and 3TP-luc assay showed significantly decreased activities under TRIM21 + TGF-b. Since TRIM21 contains ubiquitin ligase, PRYSPRY, TRIM21 with TGF-b significantly decreased TGFRII via UPL. These *in vitro* evidences that TRIM21 significantly repressed TGF-b after binding smad7 were validated with DSS-induced colitis and colitic cancer model. TRIM21 was significantly decreased in DSS-induced ulcerative colitis, whereas ameliorated colitis showed significant restoration of TRIM21, leading to conclusion that loss of TRIM21 led to significant bout of IBD.

Su1106

KRÜPPEL-LIKE FACTOR 4 MODULATES GOBLET CELL DIFFERENTIATION AND PLAYS A RADIO-PROTECTIVE ROLE IN THE BMI1$^+$ INTESTINAL RESERVE STEM CELL LINEAGE

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Background: Krüppel-like factor 4 (KLF4) is a zinc-finger transcription factor, expressed in villus cells, that promotes cellular differentiation and tissue homeostasis. Previous studies suggest that BMI1$^+$ cells represent secretory progenitors with reserve intestinal stem cell (rISC) activity. Studies also indicate that KLF4 contributes to crypt regeneration originated from BMI1$^+$ rISC lineage following γ-irradiation (IR). **Aim**: To investigate the mechanism by which KLF4 contributes to BMI1$^+$ rISC lineage during homeostasis and after IR injury. **Methods**: *Bmi1CreER;Rosa*^{EYFP} (*Bmi1*^{Ctrl}) and *Bmi1CreER;Rosa*^{EYFP};*Klf4*$^{fl/fl}$ (*Bmi1*$^{\Delta}$*Klf4*) mice were injected with tamoxifen to label BMI1$^+$ cells and to delete *Klf4*. EYFP$^+$ cells were FACS-isolated from proximal small intestine for organoid culture at 48 h after tamoxifen injection. Organoids were collected 0, 24, 48, 72 and 96 h post IR. The regeneration rate was calculated as number of organoids 7 days post IR/number of organoids 0 h. **Results**: During homeostasis, MUC2$^+$ goblet cells appeared in the BMI1$^+$ cell lineage at 3 and 7 days after tamoxifen administration. Sixty-six percent eYFP$^+$MUC2$^+$ cells stained positive for KLF4 in *Bmi1*^{Ctrl} mice ($p<0.01$, Spearman's rank correlation). KLF4$^+$ MUC2$^+$ cells in eYFP$^+$ cells were significantly lower in *Bmi1*$^{\Delta}$*Klf4* mice than *Bmi1*^{Ctrl} mice (n=3, $p<0.01$). Organoids derived from single eYFP$^+$ cells of *Bmi1*^{Ctrl} mice contained MUC2-expressing cells that co-expressed KLF4. However, organoids derived from *Klf4*-deleted eYFP$^+$ cells from *Bmi1*$^{\Delta}$*Klf4* mice did not contain MUC2-expressing cells. To determine the effect of *Klf4* deletion from BMI1$^+$ cells on tissue regeneration, organoids derived from FACS-isolated eYFP$^+$ cells were irradiated with 6-12Gy at culture day 4. Similar to apoptotic phase post IR *in vivo*, organoids shrunk and underwent apoptosis within first 48 h following IR. Irradiated organoids started to regenerate between 48 and 96 h post IR. The regeneration rate of organoids from eYFP$^+$ cells obtained from *Bmi1*^{Ctrl} mice was significantly higher than *Bmi1*$^{\Delta}$*Klf4* mice (*Bmi1*^{Ctrl} vs. *Bmi1*$^{\Delta}$*Klf4*, 62.4±12.2% vs. 58.5±11.9%, 36.0±9.6% vs. 21.0±13.5%, 16.6±6.9% vs. 6.8±3.6% and 1.9±1.7% vs. 0.6±1.0% for 6Gy, 8Gy, 10Gy and 12Gy, respectively; n=5, $p<0.05$). By immunostaining for *Bmi1*^{Ctrl} derived organoids, expression of p21 increased and peaked after 48-72 h, and then decreased at 96h. Most of p21$^+$ cells were co-expressed with KLF4. Inversely, EdU positivity decreased at 48 h and then increased at 96 h. **Conclusion**: KLF4 is positively correlated with goblet cell differentiation in BMI$^+$ rISC-derived lineage. The organoid irradiation model reconstitutes the characteristics of *in vivo* response following IR and suggest that KLF4 plays a radio-protective role in cooperation with p21$^{Waf1/Cip1}$.

Su1107

CYTOKINE-TRIGGERED STAT5 ACTION PROMOTES METASTASIS THROUGH HIGH COLONIC CANCER STEM CELL ACTIVITY

Ruixue Liu, Haifeng Li, Juan Cai, Richard Moriggl, Xiaonan Han

Background and Aims: Lgr5 intestinal stem cells (ISC) are significantly amplified in colorectal cancer (CRC), but the mechanism is unclear. Cytokine-STAT5 action is essential for Lgr5 ISC stemness, and enhanced STAT5 phosphorylation can restrict Lgr5 ISCs into secretory fate through controlling WNT signaling. We investigate too much or too little cytokine-STAT5 action in various experimental CRC based on genetics and phenotyping. **Methods:** To generate the inducible deficient or constitutive active cytokine-STAT5 signaling in Lgr5 ISCs, Stat5 (Stat5$^{f(l)}$ or constitutive active STAT5 (icS5) floxed mice were crossed with Lgr5CreER plus Rosa26-stop-LacZ mice (LacZ) (thereafter called Lgr5-RsLacZ) mice. Colitis or Apc$^{Min/+}$-associated intestinal tumor models were used to evaluate the effects of cytokine-induced STAT5 action on inflammation or gene mutation-induced colon cancer severity, progression or metastasis. *Apc*^{Min}-engineered murine organoids were performed to study the effects of depletion of activation of STAT5 signaling in ISCs on the cancer phenotypes. RNA-seq was used to investigate the altered pathways in the *Apc*^{Min} and/or *Stat5* genetically edited organoids. **Results:** *Stat5* deletion in Lgr5 ISCs led to colitis-associated CRC progression while increasing metastasis in Apc$^{Min/+}$ mice. Furthermore, prolonged pYSTAT5 activation in Lgr5 ISCs promoted CRC distant organ metastasis in Apc$^{Min/+}$ mice, but not primary CRC severity. Finally, using *Apc*^{Min}-engineered murine organoids, *Stat5* depletion or icS5 activation increased cancer organoid phenotypes coincident with aberrant Wnt, Notch, NFkB and MAPK gene signatures. **Conclusions:** STAT5 is a key tumor suppressor for colon cancer establishment, but its activation enforces cancer ISC metastasis. STAT5 blockade could be important to block CRC metastasis.

Su1108

THE THYROID HORMONE NUCLEAR RECEPTOR TRα1 PLAYS AN ESSENTIAL ROLE IN INTESTINAL EPITHELIUM STEM CELL PHYSIOPATHOLOGY

Maria Virginia Giolito, Leo Claret, Frau Carla, Michelina Plateroti

Background. Thyroid hormones (THs) control several aspects of gut development and homeostasis. They act through the nuclear receptors TRs, that are T3-modulated transcription factors. The paradigm is the amphibian metamorphosis, where they are responsible for gut remodeling and emergence of the stem cells (SC). In previous studies we showed that TH/TRα1 play a fundamental role in regulating the balance between cell proliferation and cell differentiation of murine intestinal crypt cells. In accordance with this function, targeted expression of TRα1 in the epithelium (*vil*-TRα1 mice) is sufficient to induce aberrant and

hyper-proliferative crypts and confers increased susceptibility to *Apc*-mutation dependent tumorigenic program (*vil*-TRα1/Apc mice); the reversal effect was observed in TR α-KO background (TRα$^{0/0}$/Apc mice). Finally, in human colorectal cancer (CRC) from patients we observed increased TRα1 expression and a significant correlation between TRα1 levels and Wnt activity. This last result proved the translational potential of our fundamental studies to human CRC. ***The aim of this work*** was to study TH/TRα1-dependent control of SC physiopathology. ***Methods.*** We used the *Lgr5*-EGFP-Cre^{ERT2} mice crossed with tamoxifen inducible TRα1 loss-of-function animals (TRα1-LOF) in a WT or an *Apc*-mutated background. To link TRα1 expression, TH levels and cancer SC biology we settled up a 3D culture system of spheroids from human colon adenocarcinoma Caco2 cells with altered TRα1 expression (TRα1 GOF/LOF). ***Results.*** Induction of TRα1-LOF in mice *in vivo* resulted in decreased Wnt and Notch pathways as well as of SC and proliferation markers expression. 3D organoids growth *ex vivo* was also strongly impaired in TRα1-LOF mice, indicating altered SC activity. In parallel, experiments on Caco2 cells showed that TRα1-GOF increased while TRα1-LOF decreased the size of spheres, strongly acting on their growth capacity. In addition, the altered growth is linked to alteration of SC markers. ***In conclusion,*** our results show that modulating TH/TRα1 has a strong effect on normal crypt SCs and on cancer SCs. Given the extremely high number of patients displaying thyroid dysfunctions and receiving deprivation/replacement therapies, even all along their life (12% of US population estimated by the American Thyroid Association), our work opens a new perspective in the study of TH/TRα1-dependent signal on the physiopathology of the intestinal SCs.

Su1110

P53-DREAM DIFFERENTIALLY CONTROLS TRANSCRIPTION OF CELL CYCLE REGULATORS IN ILEUM AND COLON EPITHELIA

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Background: Distal ileum and proximal colon are two alimentary tract segments separated only by the ileocecal valve and cecum. However, numerous physiologic and pathophysiologic studies have demonstrated distinct differences not only in the function of the two segments, but importantly, in susceptibility to specific diseases. The incidence of cancer in right colon is high in comparison to ileal tumors. The goal of our study was to identify the transcriptional interactome specific for each of the two segments and to determine how differences in the transcriptome may contribute or predispose the epithelia to cancer. **Methods**: Enteroids derived from normal human distal ileal and proximal colonic biopsies from 3 consented subjects per segment were made into epithelial monolayers. Differentiated distal ileal (DFDI) and proximal colonic (DFC) epithelial monolayers were subjected to RNAseq analysis. Heat map analysis showed the homogeneity of samples within the gut segments. Only DFPC/DFDI above 2-fold were considered for further analysis. The validity of transcriptional differences between DFDI and DFPC were further verified by the expression of established ileum- or colon-specific markers. STRING protein interactome and gene ontology (GO) analyses were used to identify the DFPC-specific interactome complexes. **Results**: More than 400 transcripts were differentially express between DFPC and DFDI. The highest number of interacting transcripts differentially expressed between DFPC and DFDI were under control of the p53-DREAM complex. The p53–DREAM pathway controls more than 250 cell cycle-associated genes, and can serve as a transcriptional activator or repressor depending on DREM complex protein composition. In DFPC, the DREAM complex appears to act as a transcriptional repressor, directly inhibiting expression of 88 proteins, which then interact with at least other 30 proteins found to be downregulated in DFPC compared to DFDI (Figure 1A). Surprisingly, several DREAM-controlled histone transcripts were upregulated in DFPC. Interactome and GO pathway analyses for these histones show that they are involved in negative regulation of gene expression (Figure 1B), further promoting the main p53-DREAM function of transcriptional repression in DFC. In contrast, in DFDI the changes in composition of DREAM complex due to substantial elevation of two proteins, MYBL2 and FOXM1 lift the translational restriction. **Summary/Conclusion:** Regulation of genes by the p53-DREAM complex in DFPC is inversed to DFDI. p53-DREAM controls a plethora of cell cycle genes, and p53 mutations in colon are known to contribute to uncontrollable proliferation and colon cancer development. Based on our data, similar p53 mutagenesis in ileum would likely have less effect on cell proliferation because the downstream DREAM complex does not work as a transcriptional repressor in ileum.

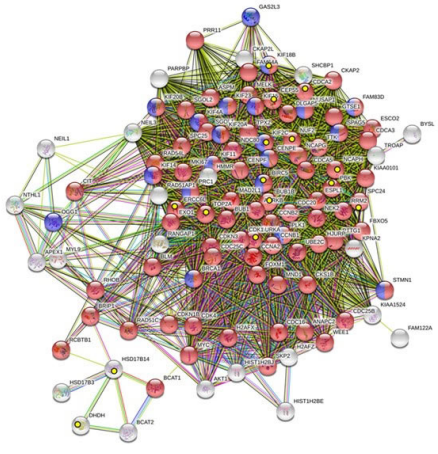

Figure 1A: 112 interacting transcripts that are downregulated above 2-fold in DFPC downstream of p53-DREAM complex. Red nodes represent 86 transcripts from GO:0007049 "cell cycle" controlled by DREAM. Blue nodes represent 20 transcripts from GO:0015631 "tubulin binding" controlled by DREAM. Yellow nodes represent transcripts downregulated more than 20-fold in DFPC.

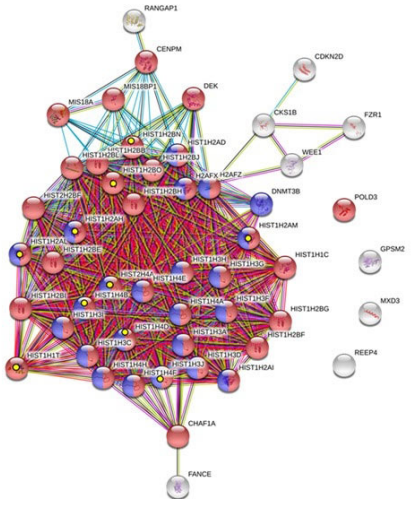

Figure 1B: 51 interacting transcripts that are upregulated above 2-fold downstream of p53-DREAM complex in DFPC. Red nodes represent 41 transcripts from GO:0051276 "chromosome organization". Blue nodes represent 23 transcripts from GO:0015631 "negative regulation of gene expression, epigenetic". Yellow nodes represent transcripts upregulated more than 20-fold in DFPC.

Su1111

CLOCK GENE EXPRESSION LEVELS CAN DISTINGUISH BETWEEN ULCERATIVE COLITIS AND CROHN'S DISEASE

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Background: Pathophysiological mechanisms active in inflammatory bowel disease (IBD), such as mucosal barrier repair, innate and adaptive immune responses, intestinal motility and gut microbiome, all exhibit diurnal variations. Chronic disruption of the molecular clock augment inflammatory response. We have previously shown that newly diagnosed, naïve to treatment, young IBD patients present reduced clock gene expression in both inflamed and non-inflamed intestinal tissues and in peripheral White Blood Cells (WBC). This reduction correlated with disease activity. Our aim in the current study was to determine whether certain clock genes correlate with disease activity scores or inflammatory markers in Crohn's disease (CD) vs. Ulcerative Colitis (UC). **Methods:** 14 CD and 11 UC patients, 8-22 years old, were recruited. Patients were evaluated upon diagnosis and during medical treatment. Disease activity scores, C-reactive protein (CRP) and fecal calprotectin (Fcal) levels were measured and WBC were analysed for clock gene (*CLOCK*, *BMAL1*, *CRY1*, *CRY2*, *PER1* and *PER2*) expression. Clock gene expression levels were corelated to disease activity scores (clinically active vs. remission), CRP levels (<5 mg/l vs. >5 mg/l) and Fcal levels (< 250 µg/mg vs. >250 µg/mg) in CD (21 samples) and UC patients (20 samples). **Results:** In UC, *CLOCK* (p<0.025), *CRY1* (p<0.033) and *PER1* (p<0.038) showed decreased expression when Fcal levels were > > 250 µg/mg. When compared with the clinical status only *BMAL1* showed reduced expression (p<0.019). CRP levels showed no correlation with clock gene expression. In CD, CRP as well as the clinical status correlated with clock gene expression. Whereas *CLOCK* was reduced (p<0.019), *PER2* was induced (p<0.005) when CRP levels were > 5 mg/dl. Furthermore, *CLOCK* (p<0.0005), *PER1* (0.017) and *PER2* (0.044) were reduced in CD patients with an active disease. However, Fcal did not correlate with clock gene expression. **Conclusions:** Altered levels of certain clock genes were demonstrated in young CD and UC patients in relapse vs. remission and correlated with Fcal in UC patients and clinical status as well as CRP levels in CD patients.

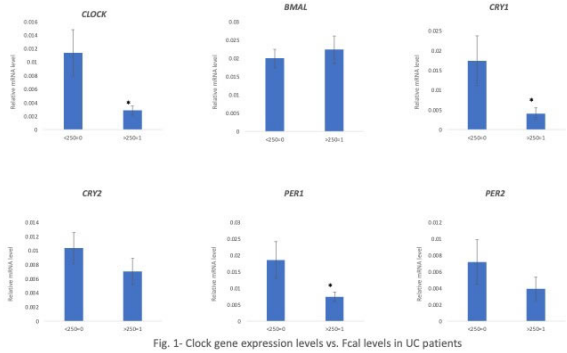

Fig. 1- Clock gene expression levels vs. Fcal levels in UC patients