CDX2 regulates ICAM2 expression. The effects of CDX2 induction of ICAM2 are not cell-autonomous, as *In Vitro* colony formation is not affected. However, CDX2 induction of ICAM2 promotes cancer cell-endothelial cell interactions and angiogenesis.

W1975

Genetic Inhibition of Telomerase Leads to Induction of ALT in Immortalized Human Esophageal Epithelial Cells and in Generated Human Squamous Cancer Cells

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Introduction: During malignant transformation telomere maintenance is important for immortalization. Telomere maintenance is either mediated through activation of the enzyme telomerase, which contains an RNA-template (hTER) and the core protein (hTERT) or through a recombination based alternative mechanism (ALT). Little is known about the regulation of these two mechanisms in a single cell. We investigated whether ALT can be induced in genetically defined immortalized cells or malignant transformed cancer cells by specific genetic telomerase inhibitors. Methods: We generated immortalized human esophageal epithelial cells overexpressing Cyclin D1 or hTERT, respectively (EPC D1 and EPC hTERT) as well as human oral squamous cancer cells by overexpression of Cyclin D1, d.n.p53, EGFR and c-myc (OKF6 D1/d.n.p53/EGFR/c-myc) using retroviral transduction. To genetically inhibit telomerase, all cell types were transduced with mutated versions of hTER, anti-hTER, siRNA or a combination of both by lentiviral mediated gene transfer. Transduced cells were sorted by GFP-coexpression. Growth behavior, telomerase activity (TRAP-assay) and telomere length (PFGE-TRF and Q-FISH) were assayed. Indirect immunofluorescence for telomere related proteins (APBs) was performed. Results: Overexpression of MT-hTER, anti-hTER siRNA and the combination of both rapidly inhibits cell growth in transduced immortalized cells but not in transduced generated cancer cells. A reduction of telomerase activity could be observed in all hTER-inhibited cell types, whereas TRF analysis revealed telomere elongation, which is characteristic for ALT. Q-FISH analysis suggested a heterogeneous pattern of telomere length on a chromosomal level. In contrast, control cells displayed a robust telomerase activity and a homogenous telomere length in immortalized as well as in malignant transformed cells. Additionally, in indirect immunofluorescence ALT-associated PML bodies (APBs) were observed in a higher frequency in immortalized hTER-inhibited cells. Summary: Genetically defined immortalized human esophageal epithelial cells and the generated cancer cells are capable to elongate their telomeres using both telomere maintenance mechanisms, namely ALT or telomerase activation. ALT can be induced by the inhibition of telomerase with mutant template telomerase RNA, anti-telomerase siRNA and the combination of both. These findings suggest that immortalized and malignant transformed cells and eventually all telomerase positive cancer cells, treated with telomerase inhibitors as potential anti-cancer strategy might find alternative ways to maintain their telomeres.

W1976

Opposing Effects of Deoxycholic Acid and Ursodeoxycholic Acid On Golgi Structure; Implications for Colon Cancer Progression

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Deoxycholic acid (DCA) is a hydrophobic bile acid implicated in colon cancer. We have observed that DCA causes fragmentation of the Golgi, an organelle responsible for protein processing in the cell. The Golgi packages and transports proteins by a process called membrane fission which involves activation of the enzymes PKCη and PKD. Hyperactivation of this process has important implications as disruption of the Golgi can lead to abnormally glycosylated proteins which are inherent in malignant cells. The aim of this study was to investigate the mechanism of DCA-induced Golgi fragmentation and the effect of UDCA, previously shown to be cytoprotective and antagonistic to DCA, on this process. HCT116 colon carcinoma cells were treated with 300 μM DCA and Golgi were visualised using a fluorescently labelled Golgi (GM130) antibody. Golgi fragmentation was assessed by High Content Analysis. DCA was shown to induce Golgi fragmentation in 54.40 ± 1.19% cells compared to 33.28 ± 2.21% in untreated cells ($p<0.05$). DCA induced this Golgi fragmentation via activation of the PKCη - PKD pathway as demonstrated by Western blot analysis using phospho-specific antibodies. Pre-treatment of cells with 300 μM UDCA decreased DCA-induced Golgi fragmentation (42.87 ± 4.36%, $p<0.05$). UDCA inhibited DCA-induced PKD activation and also inhibited Golgi fragmentation induced by constitutively active PKCη. As UDCA has previously been shown to bind the glucocorticoid receptor (GR) we investigated whether UDCA-mediated inhibition of DCA-induced Golgi fragmentation was via activation of the GR. UDCA could no longer overcome DCA-induced Golgi fragmentation when cells were treated with the GR antagonist Mifipristone or following knockdown of the GR using siRNA. This demonstrates that UDCA mediates its protective effects via the GR in this system. (Fragmentation: DCA: 54.40 ± 1.19%, UDCA+DCA: 42.87 ± 4.36%, UDCA+DCA+Mifipristone: 57.43 ± 3.68%, UDCA+DCA+ siRNA: 51.66 ± 2.54%). We conclude that DCA-induced Golgi fragmentation is due to overactivation of the membrane fission process via activation of PKCη and phosphorylation of PKD. These effects were reversed by UDCA which acts through the GR. This study identifies DCA as the first physiological inducer of Golgi fragmentation, and suggests UDCA may be used as a chemopreventative agent. This study also gives an insight into mechanisms of bile-acid signalling which may be involved in neoplastic progression in the colon.

W1977

Trastuzumab Induced CTL Responses Against Esophageal Cancer Are Inhibited By Down-Regulation of the Antigen Processing Machinery Component Tap2

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Introduction: Esophageal adenocarcinoma (EAC) is a disease with an extremely poor prognosis. In a previous study we demonstrated that Dendritic Cell (DC) therapy is a promising treatment option for EAC. We earlier found high HER-2 gene amplification in up to 50% EACs. HER-2-derived peptides are naturally processed as cytotoxic T cells (CTL) epitopes that can be recognized by CTL. The humanized antibody against HER-2, Trastuzumab, has potent inhibitory effects on HER-2 overexpressing tumors. One of the mechanisms of action of Trastuzumab is the enhancement of the CTL response through improvement of HER-2 epitopes presentation via MHC-Class I molecule on tumor cells that may mediate a HER-2 specific CTL response and tumor cell lysis. It is known that deficiencies of components of the antigen processing machinery (APM) are responsible for reduced antigen presentation by MHC-Class-I molecules, which is an important immuno-surveillance-escape mechanism of cancers. We previously demonstrated that by treating HER-2 positive EAC cell lines with Trastuzumab, there is enhanced CTL response against the cell line OE33, but this effect was not observed in the cell line OE19. Aim: to investigate whether down regulation of the APM is responsible for inhibition of a Trastuzumab mediated CTL response in OE19 and in EAC in general and if so, to investigate whether a deficient APM can be restored by interferon gamma (INF-gamma). Methods: RT-PCR and immunohistochemistry (IHC) were applied to study the expression of the three most important components of the APM: Transporters Associated with antigen Processing 1 and 2 (TAP1 and 2), and Tapasin, in three EAC cell lines, OE33, OE19 (HER-2 positive) and Bic-1 (HER-2 negative) and in tumor biopsies taken from EAC patients. In case of TAP deficiencies, IFN-gamma, known as a potent inducer of TAP expression, was used to investigate whether TAP deficiencies could be restored. Results: Both at RNA and protein level, expression of TAP1 and Tapasin were observed in all the three EAC cell lines, and in 88% of the patient tumor biopsies. Interestingly, expression of TAP2 was lost in the EAC cell line OE19, and in 41% of the tumor biopsies. Moreover, by treating the cell lines with IFN-gamma, the TAP2 function could be restored in OE19. Conclusions: The unresponsiveness of OE19 to Trastuzumab mediated CTL response may be due to deficient APM. Down regulated expression of TAP2 is a frequent event in EAC, and may also determine unresponsiveness of HER-2 positive EAC patients to Trastuzumab. However, the unresponsiveness to Trastuzumab might be overcome by treatment with INF-gamma that directly restores the APM by up-regulating TAP2.

W1978

B7-H1 Is Highly Expressed in Gastric Carcinoma and Suppresses Intratumor T Cell Immune Response

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Background & Aims: B7-H1 is expressed in antigen presenting cells, and functions as one kind of co-stimulatory molecules associated with PD-1 expressed in T lymphocytes. It plays an important role in immune regulation through inhibiting T cell activation and differentiation. B7-H1/PD-1 interaction has been found to involve in cancer immune evasion from the host immune surveillance system in several malignancies, while the relevance to carcinogenesis in gastric carcinoma is still elusive. In this study, we investigated the expression of B7-H1 in human gastric carcinoma and its functional role in regulating T cell activation in anti-tumor immune response. **Materials and Methods**: B7-H1 expression in gastric tissue and gastric carcinoma cell lines (SGC7901, SGC/VCR, BGC823) was determined by immunohistochemistry, RT-PCR and flow cytometric analysis. Cytokines were detected by ELISA. **Results**: Immunohistochemical analysis revealed that B7-H1 was highly expressed in 64.4% (47/73) of gastric carcinoma specimens, and PD-1 was present in 43.8% (32/73) of gastric carcinoma specimens examined. However, no B7-H1 and PD-1 expression were detected in normal gastric tissue by immunohistochemistry. Alpha-naphthyl acetate esterase staining demonstrated that B7-H1 expression was inversely correlated with tumor infiltration lymphocyte (TIL) infiltration, particularly CD8+ T cells in gastric carcinoma. B7-H1 mRNA was also found to be increased expression in all gastric carcinoma tissue compared with healthy controls ($P < 0.05$). Additionally, all 3 gastric carcinoma cell lines constitutively expressed B7-H1 mRNA and protein. To elucidate the functional relevance of B7-H1 molecule to the induction of intra-tumor T cell immune response, T cells (1×10^6/well) isolated from gastric carcinoma tissue were stimulated *In Vitro* with B7-H1 fusion protein (5 μg/ml) in the presence of immobilized anti-CD3 mAb (5 μg/ml) for 48h. Interestingly, the results demonstrated that T cells isolated from gastric carcinoma tissue produced higher levels of interleukin-10 and lower levels of IFN-γ compared with healthy controls ($P < 0.05$). **Conclusion**: B7-H1 is highly expressed in gastric carcinoma, and closely associated with the inhibition of T-cell anti-tumor activities. This work highlights the important role of B7-H1 in immune surveillance to gastric carcinoma growth and represents a novel mechanism by which gastric carcinoma cells evade immune recognition and destruction.

W1979

Telomerase Activity and Telomere Length in Lcm Purified Barrett's Esophageal Adenocarcinoma Cells

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INTRODUCTION: The aim of this study was to assess telomere length and telomerase activity in normal and Barrett's adenocarcinoma (BEAC) cells purified by laser capture microdissection (LCM). METHODS: Epithelial cells were identified according to standard histopathological criteria and purified by LCM from tissue samples of surgically resected normal, Barrett's, and BEAC esophagi. Telomerase activity was assessed by an improved version of the original Telomeric Repeat Amplification Protocol assay whereas telomere

length evaluated by quantitative real time polymerase chain reaction. RESULTS: Firstly, we compared telomerase activity levels in LCM purified cells with those in tissues extract of the same samples. Telomerase activity was ~ 10-fold higher in LCM derived cells relative to that in tissue extracts of the same samples. Moreover, a significant elevation (p=0.004) of telomerase activity was detected in BE when epithelial cells were purified by LCM; whereas no significant difference in telomerase activity was seen in tissue extracts of BE and normal esophagi. We then measured telomerase activity in LCM purified cells from normal, BE, BEAC, and commercially obtained primary normal esophageal epithelial cells. Telomerase activity in LCM purified normal esophageal epithelial cells from three different donors was 41±8 TPG units. Consistent with this, the activity in commercially obtained primary normal HEEC was also low (37±3 TPG units). A markedly elevated telomerase activity was detected in BE (ranging from 133 to 200 TPG units) and BEAC (275±20 TPG units). Relative to normal esophageal epithelial cells, the activity in BEAC was 7.0±0.5-fold elevated (p=0.004). Secondly, we analyzed telomere length in a panel of LCM purified normal and BEAC cells using real time quantitative polymerase chain reaction. T/S ratio, indicating relative telomere length, ranged from 10.3 to 14.3 in normal and 6.2 to 9.2 in BEAC cells, indicating that telomeres in all BEAC specimens were shorter than those in normal samples. Average telomere length was reduced by 38% (P=0.001) in BEAC relative to normal epithelial cells. CONCLUSIONS: We have shown that telomerase activity assays conducted in tissue extracts can produce false negative results whereas the assays conducted in LCM purified specimens are more accurate and reliable. Our data also show that telomerase activity is significantly elevated, whereas telomeres are significantly shorter in BEAC relative to normal esophageal epithelial cells; thus providing a rationale for investigating the importance of telomerase as a target for the treatment of Barrett's esophageal adenocarcinoma.

W1980

Immortalization and Characterization of a Human Colonic Epithelial Cell Line

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Long-term culture of normal adult human colonic epithelial cells (HCEC) has historically been exceptionally difficult. The lack of an established method for the sustained culture of HCECs has been a major impediment for progress in studying gastrointestinal development and colorectal cancer (CRC). Current *In Vitro* models for studying CRC are based on established colon cancer cell lines that contain many of the key mutations responsible for initiating and progressing normal cells into tumors. Prospectively introducing relevant mutations into normal human colonic epithelial cells would be highly informative for confirming the mutation's mode of action at the molecular level and determining the biologic changes imparted to the cell via tumorigenicity assays. We here describe conditions for the long-term proliferation of HCECs by immortalization with cyclin-dependent kinase 4 (*Cdk4*) and the catalytic component of telomerase (*hTERT*). Immortalized HCEC (HCEC CTs) expressing *Cdk4* and *hTERT* have continued to replicate for well beyond nine months. HCEC CTs show strong Cdk4 expression, telomerase activity, express cytokeratin 18 and vimentin proteins, stain negatively for markers of pericryptal fibroblasts (α-smooth muscle actin) and endothelial cells (CD31), and do not make colonies is soft agar. After exposing sub-confluent cultures to 30mM lithium chloride, HCEC CTs stop replicating and display a cuboidal monolayer phenotype. Cells in this differentiated state have positive membrane staining for tight junctions (ZO-1) and components of adherens junctions (β-catenin). Our goal is to use these cells as a model for CRC cancer initiation and progression, focusing on progressively transforming the cells by adding key mutations known to be involved in CRC tumorigenesis. We are currently using stable shRNAs to knockdown APC, p53, and SMAD4 function, and have expressed mutant K-ras. Soft agar, matrigel invasion, and growth in nude mice assays will then be used to validate the tumorigenic effect of these genetic alterations individually and in combinations. Immortalized HCECs should be useful in validating current paradigms of CRC initiation and progression and may provide new insights into this disease.

W1981

Lysyl Oxidase Like Protein 2 (Loxl2) and Loxl3 Expression Is Up-Regulated in Colorectal Carcinoma

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Introduction: Lysyl oxidase (Lox) is a copper dependent amine oxidase that oxidizes lysine-rich proteins such as collagen and elastin resulting in formation of covalent crosslinks between protein subunits. Recently, additional Lox gene family members (Loxl1, Loxl2, Loxl3 and Loxl4) were identified all characterized by a conserved Lox-like copper-binding catalytic domain. Expression of Lox was reported in fibrogenesis and metastases-associated fibrosis and desmoplasia. Lox, Loxl2 and Loxl4 expression was reported in breast, head and neck cancer, and, recently, in esophageal and colorectal carcinoma (CRC). No study examined Lox family proteins expression in adenomatous polyps. No study examined Loxl3 expression in CRC. Aim: to study the expression of Loxl2 and Loxl3 in adenomas and CRC. Methods: Immunohistochemistry was applied on paraffin-embedded surgical sections from patients with CRC (n=18), and adenomatous polyps (n=20: 10 tubular adenomas, 5 villous adenomas with mild dysplasia, 5 villous adenomas with moderate or severe dysplasia) removed endoscopically. Normal colonic margins of the surgical resection (where available, n=14) were also examined for loxl2 and loxl3 staining. Immunostaining was performed with highly-specific anti-Loxl2 and Loxl3 polyclonal antibodies. Staining intensity was classified by an expert pathologist as (0): none; (1): weak; and (2): strong. Results: Staining for Loxl2 and Loxl3 was observed in 15/18 (83%) and 12/18 (67%) of CRC specimens, respectively. Normal colonic margins exhibited mostly negative staining for Loxl2 and Loxl3. Only 1/14 (7%) stained positively for loxl2 and loxl3, a difference that was highly significant ($p<0.05$). Adenomas tended to stain much less than carcinomas. Only 8/20 (40%) and 11/20 (55%) of adenomas stained positively for Loxl2 and Loxl3, respectively. Strong staining was observed only in adenomas with moderate or severe dysplasia (4/5). Loxl2 staining was located in the cytoplasm. Surprisingly, Loxl3 stained mainly the cell nuclei. Conclusions: Loxl2 and Loxl3 expression is up-regulated in CRC and to a much lesser extent in adenomatous polyps suggesting a role for these enzymes in tumor progression. Loxl3 localization to cell nuclei suggests that it may oxidize and modulate the activity of nuclear proteins such as transcription factors.

W1982

Malignant Transformation of Rat Gastric Epithelial RGM-1 Cell Line Induced By Carcinogen N-Methyl-N'-Nitro-N-Nitrosoguanidine (Mnng) Treatment Was Accompanied with the Ectopic Expression of H+,K+-Adenosine Triphosphatase (ATPase)

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[Background and Aims]: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) induces gastric cancer in animal models. We presented at last year's DDW the establishment of an MNNG-induced mutant of the rat murine RGM-1 gastric epithelial cell line, which we named RGK-1. This cell line showed signs of neoplasia and transformation, in that it lost contact inhibition and formed tumors in nude mice. Interestingly, the mutant cells also expressed parietal cell-specific H+,K+-adenosine triphosphatase (H+,K+-ATPase), which parent RGM-1 did not. [Methods]: RGK-1, a MNNG-induced transformed cells, were examined with immunohistrochemistry and Western blot analysis using anti-H+,K+-ATPase alfa and beta subunits. The acid secretory activity of histamine-treated the mutant RGK-1 cells and the parent RGM-1 cells was assessed by 14C-dimethyl-aminopyrine accumulation. The ultrastructures of RGK-1 and parent RGM-1 cells were examined with electron microscope. [Results]: Immunohistrochemistry and Western blot analysis confirmed that RGK-1 cells expressed both H+,K+-ATPase alfa and beta subunits proteins. Both RGM-1 and RGK-1 cells did not show the accumulation of 14C-dimethyl-aminopyrine despite histamine stimulation. Electron microscopic examination demonstrated that increased homogenous, dense secretory granules and strongly-deformed mitochondria in RGK-1 cells. Although RGK-1 cells expressed H+,K+-ATPase, no intracellular canaliculi, which are characteristic of parietal cells, were observed. RGM-1 cells showed no secretory granules, normal shaped mitochondria and the abundance of polyribosomes. [Conclusion]: We demonstrated that the induction of H+,K+-ATPase in the transformed RGK-1 cells, suggesting the differentiation of parent RGM-1 cells, which reportedly regarded as mucous pit cells or mucous neck cells, into parietal cell-like cells. Electron microscopic examination and 14C-dimethyl-aminopyrine accumulation assay, however, indicated that RGK cells did not show the characteristic features of mature parietal cells morphologically and physiologically. Since the ultrastructures of RGM-1 suggested that RGM-1 cells are undifferentiated, proliferating mucous progenitor cells which observed in rodent gastric glands, we assume that progenitor RGM-1 cells might differentiate into pre-parietal cell-like RGK cells and express H+,K+-ATPase through the malignant transformation.

W1983

Identification of Putative Functional Domains of Human Trefoil Factor Family-3 (TFF3)

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Background: TFF3 is a gastrointestinal peptide that shares with other TFFs the distinct structural fold of the trefoil domain, a 3-loop architecture connected by disulfide bonds between 6 conserved cysteines. However, in contrast to TFF1, which acts as a tumor suppressor, TFF3 seems to promote a more aggressive behavior of GI cancer cells. We previously undertook a structure-based investigation of the homologous protein, TFF1, and discovered phenotype-determinant amino acid residues in the Loop 1 region that, when mutated, converted the TFF1 tumor suppressor function to a tumor promoter (Gastroenterology 130:1696,2006). Aim: We applied similar structural- and computational-based modeling to inform the identification and testing of functional mutants of TFF3 that would help reveal the mechanisms and interactions by which it functions as a tumor promoter. Methods: A conservation analysis of trefoil domain sequences from the TFF peptide family was performed. Bioinformatics tools and computational structure analysis were then used to select mutants that would alter the properties of putative interaction surfaces of the TFF3 protein without significantly disrupting its global fold. The selected mutants were constructed with site-directed mutagenesis. Results: Conserved residues were mapped onto the known 3D structure of TFF3 (Biochemistry 40:9552,2001). This identified the location of a highly conserved patch as the surface of a pocket in the Loop 2-Loop 3 region (composed of >95% conserved residues). This conserved patch is largely hydrophobic, and this conservation pattern suggests a functional role for this region, possibly essential for protein-protein interactions. Thus, we sought to design mutants to alter the properties of this Loop 2-Loop 3 pocket. Computational analysis of putative mutations that will not interfere with global folding of the protein identified several putative mutants of interest, including Y23A and H25G. These mutants have been produced in a recombinant *E. coli* expression system. Their identity was verified by Western blot, and they were purified for NMR analysis and functional assay of cancer-related activities. Studies are ongoing to examine differences in functional properties of these mutants compared to wild-type TFF3. Conclusions: The design and production of these mutants represent the first report of site-directed mutagenesis experiments for TFF3 (other than cysteine substitution) that seek to probe the mechanisms responsible for the specific function of this trefoil factor, in a structural context that addresses both the structure-function elements and the possible involvement in signal transduction.

W1984

Depletion of the Colonic Epithelial Precursor Cell Compartment Upon Conditional Activation of the Hedgehog Pathway

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Introduction Members of the Hedgehog family of morphogens are expressed throughout the epithelium of the gastrointestinal tract. The study on the role of Hedgehog signaling in the adult gut has been hampered by the embryological phenotype of Hedgehog mutant mice. Here we examined the role of Hedgehog signaling in the colon of adult mice with a tamoxifen inducible deletion of the Hedgehog receptor and transcriptional target Patched (Ptc). Loss of Ptc leads to loss of inhibition of the Hedgehog signaling receptor Smoothened