T1149

Risk of Upper Gastrointestinal Bleeding in Clopidogrel and Aspirin Users Receiving Concomitant Proton Pump Inhibitors

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Background and aims: Clopidogrel and aspirin (ASA) are wildly used in the prevention of cardiovascular and embolic events. However, these combined medications cause significant risk of peptic ulcers and bleeding complications. This case-control study was designed to evaluate the risk of upper gastrointestinal bleeding (UGIB) in clopidogrel and aspirin users who continue receiving standard dose of proton pump inhibitors (PPI). . Methods: Data for clinical information and endoscopic findings were collected during January 2009 and November 2009 from patients who used clopidogrel and/or ASA and continue receiving standard dose of PPI. Patients with history of prior UGIB or abdominal surgery were excluded. Clopidogrel or ASA user was defined as consumption of clopidogrel or ASA for at least 7 days period preceding the episode of bleeding. The UGIB was defined as overt bleeding (hematemesis, positive nasogastric aspirate, and melena) or fall in baseline hematocrit ≥ 5 points within 24 hours of admission. Ulcer was defined at endoscope by breaking mucosa > 3mm in diameter. Results:A total of 175 patients (82 men and 93 women, mean age of 65.3 years) were evaluated in this study including 54 patients (30.9%) with UGIB and 121 patients (69.1%) with dyspeptic symptoms. Male were significantly more common than female patients in bleeding group (61.1 % vs 38.9%: P=0.01). However, the underlying diseases of the patients including cardiovascular diseases, rheumatological diseases and diabetes mellitus were not different between these 2 groups. UGIB was significantly higher in current ASA (325mg) plus clopidogrel user than non-user (16.7% vs 5.8%; P=0.02). The multivariable model suggested that the probability of UGIB event increased with current ASA (325mg) plus clopidogrel use (OR= 2.3, 95%CI =1.2-3.9) in the patients receiving concomitant PPI. Summary: Risk of UGIB events still occur in ASA and clopidogrel users in spite of receiving concomitant PPI. Lower dose of ASA, concomitant higher dose PPI and carefully monitoring of UGIB should be considered in combined ASA and clopidogrel user patients.

T1150

Role of Cyclooxygenase-2 in Cancer Stem Cell Biology and Effect of COX-2 Inhibitors in Anti-Cancer Therapy Targeting Cancer Stem Cells

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Recent evidence has suggested the existence of cancer stem cells that are known to be capable of initiating and sustaining tumor growth, and exhibiting resistance to clinical cytotoxic therapies. Therefore, these cancer cells may be the most relevant target for cancer therapy. Cyclooxygenase-2 (COX-2) has been suggested to play an important role in carcinogenesis, and COX-2 inhibitors are recognized as effective chemopreventive drugs. In the present study, we investigated the involvement of COX-2 in the stem cell biology of colon cancer and the effects of COX-2 inhibitors on anti-cancer therapy targeting cancer stem cells. [Methods] To enrich stem cells, colon cancer cells (COX-2-non-expressing parental Caco-2, genetically engineered COX-2 expressing Caco-2, and COX-2-expressing colon cancer cells (LS174T, and WiDr)) were cultured in the shape of three dimensioned, non-adherent spheroids, because the ability to grow as spheres has come to be accepted as cancer-initiating stem cells. The ability to form spheres, cell proliferation, chemoresistance, transmembrane transport activity (pump out several prominent anticancer chemotherapeutic agents), intracellular reactive oxygen production (ROS), and oct-4(one of iPS cell-related genes) mRNA expression were investigated to estimate cancer cell stemness. [Results] Three dimensional non-adherent culture enhanced COX-2 expression, PGE2 production, and PKA activity in LS174T and WiDr. COX-2-expressing Caco-2, LS174T, and WiDr grew and form spheroid in three dimensioned culture, although Caco-2 did not. Spheroid forming cells showed accelerated chemoresistance and transporter activity and suppressed cell proliferation and ROS production in comparison with non-spheroid forming cells. PGE2 or forskolin (adenylyl cyclase activator) enhanced sphere formation, chemoresistance, transport activity, and oct-4 mRNA expression, and decreased cell proliferation and ROS production in not only spheroid forming COX-2-Caco-2, LS174T, and WiDr, but also parental Caco-2. COX-2 inhibitors (celecoxib and etodolac) or KT5720 (PKA inhibitor) inhibited spheroid formation, transporter activity, chemoresistance, and oct-4 mRNA expression, and enhanced cell proliferation, and ROS production in spheroid forming cells. [Conclusion] These results indicate that COX-2 plays an important role in cancer stem biology via PGE2 production/PKA activity pathway, and COX-2 inhibitors can be an effective anti-cancer drug in cancer therapy targeting cancer stem cell.

T1151

Chimeric Recombinant Anti-CD24 Antibody: A Novel Tool for Colorectal Cancer Therapy

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Background: We have shown that CD24, a cell surface molecule, is overexpressed early in the multistep process of colorectal neoplasia (Gastroenterology, 2006) hence, it can serve as a target for early detection and immunotherapy. The anti-CD24 murine SWA11 Mab (generous gift from Prof. Sthal, Zurich University) binds to CD24 and inhibits tumor cell growth, *In Vitro* and *In Vivo*, in a time- and dose-dependent manner (Cancer Research, 2008). However, murine Mabs have limitations because of their xenogeneic nature and due to the human anti-mouse antibody (HAMA) response. Aim: To produce an improved, chimeric form of the SWA11 Mab. Methods: Mammalian vector pMAZ-IgH for human γ1 heavy-chain and pMAZ-IgL for human κ light chain expression were designed. The variable region genes encoding the murine anti-CD24 SWA11 MAb were cloned for expression as chimeric gamma1/kappa antibody into the mammalian pMAZ-IgH and pMAZ-IgL expression vectors and co-transfected into HEK293 cells. Stable clones were screened and selected for IgG production, analyzed by whole-cell and antigen-based ELISA, and Western blotting. Results: The new chimeric IgG derivative of the anti-CD24 SWA11 Mab possesses similar affinity (Fig 1) and specificity (ELISA and Western blot), but has improved activity in inhibiting cell growth. The chimeric Mabs also have the ability to induce complement-dependent cytotoxicity, causing up to 60% cell lysis in the presence of complement (Fig 2). Conclusions: We had successfully generated a chimeric IgG derivative of the anti-CD24 SWA11 Mab that can serve as a novel therapeutic tool for cancer cells harboring CD24.

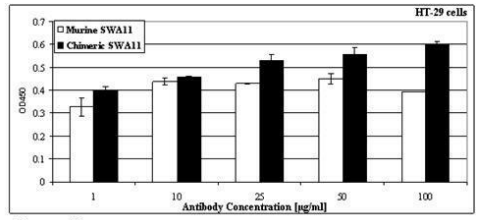

Figure 1

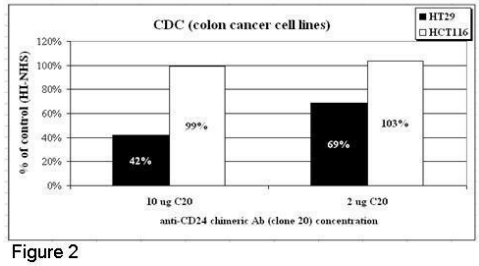

Figure 2

T1152

Novel use of Progastrin Binding to Extracellular Annexin2 on Epithelial Cancer Cells for Diagnosis/Treatment of GI Cancers

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Extracellular membrane receptors for peptide hormones are used for diagnosing and treating cancers. Annexin2 (Anx2), identified by proteomic methods, is over-expressed by epithelial tumors, including GI cancers. Extracellular Anx2 is not expressed by non-dividing cells, such as normal hepatocytes. Membrane-associated Anx2 functions as a high-affinity receptor for many ligands, including progastrin (PG). We additionally discovered that bound Anx2/PG rapidly internalizes and co-localizes within endosomes. Thus Anx2 represents a novel molecule which can be used for diagnosis/therapy of epithelial GI tumors, without collateral damage to normal tissues, especially liver. In the current studies we investigated the usefulness of labeled PG for diagnosing epithelial tumors, *In Vivo*. Fluorescense-PG peptide fragments were used. FITC-PG26 demonstrated equivalent binding affinity for Anx2 as full length 80AA PG peptide. Endocytotic internalization of FITC-PG26 demonstrated strong perinuclear co-localization with Anx2, within 15min of incubating PG responsive cells. Internalized FITC-PG26 was apparently degraded after 30-60 min, suggesting lysosomal degradation of the peptide, confirming our previous data with full length PG peptide. The homing potential of FITC-PG26 to Anx2 over-expressing tumors, growing as xenografts in nude mice, was next examined. Retention of endocytosed labeled PG by tumor lesions was imaged with *In Vivo* high resolution optical microscopy/ micro-CT/spect imaging. FITC-PG26 specifically homed to the site of a colon cancer xenograft, growing either sub-dermally or as a metastatic lesion in liver. FITC-PG26, injected intra-tumorally retained fluorescence for >30 mins (without demonstrating dye spread) confirming tumor specific retention of endocytosed FITC-PG26. FITC-PG26 successfully homed to tumor site after i.v. injection through the tail vein, within 5 min of injection, and was retained in the tumor for ~60min. Even small tumor lesions were detected after tail vein injection with FITC-PG26. Tail vein injected FITC-control peptide was not detected in tumors, confirming specific homing of FITC-PG26 peptide to tumors in situ. No other organ was labeled; excretion of FITC from kidneys and bladder was observed. Results from these experiments provide critical information required for further developing PG peptides/mimetics as diagnostic/treatment tools for negating growth of epithelial GI cancers (such as pancreatic/colon cancers), which over-express Anx2 by several fold, without collateral damage to liver and other normal tissues. Supported by NIH grants to PS (2R01 CA097959; 1 R01 CA114264) and a Sealy Smith Cancer Center grant to PS.

T1153

PI 3-Kinase Inhibitor (Wortmannin) Inhibits DNA Recombination, Genomic Instability, and Growth of Barrett's Adenocarcinoma Cells

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Introduction: Deregulated genetic recombination plays critical role in chromosomal translocation, gene amplification, and telomere maintenance, and may therefore underlie the chromosomal aberrations observed with high frequency in number of cancers. The purpose of this study was to monitor the effect of Wortmaninin (WM), a PI3 kinase inhibitor, on homologous (HR) and non homologous (nHR) recombination activities, ongoing genomic instability, and ability of Barrett's adenocarcinoma (BEAC) cells to grow as tumors in SCID mice. **Methods:** BEAC cell lines FLO-1 and OE33 were provided by Dr. David Beer. Cells

were cultured in the presence of WM at different concentrations over a period of 3 - 4 weeks. Cell viability was monitored by determining substrate attached viable cell number and confirmed by cell proliferation assays using Cell Counting Kit-8 (Dojindo Molecular Technologies, Inc), according the manufacturer's protocol. Expression of RAD51 was monitored by western blot analysis. HR and nHR activities were monitored by plasmid based assays in which the fluorescence of functional gene products created by these activities is measured by a plate reader. Impact of WM on evolution of genomic changes in BEAC cells was demonstrated by evaluating changes in copy number and heterozygosity throughout genome, using single nucleotide polymorphism (SNP) arrays (Affymetrix) and dChip software. **Results:** We showed that WM, a PI3K inhibitor, reduces the expression of recombinase (hsRAD51) and both HR and nHR activities in Barrett's adenocarcinoma (BEAC) cells within 24 hrs. Treatment of BEAC cells with 10μM WM led to 30% decrease in HR and >40% reduction in nHR, indicating that WM affects both HR and NHR pathways. Importantly, WM treatment markedly decreased the acquisition of new genomic changes in BEAC cells. Relative to control cells, the growth of BEAC cells in the presence of WM was associated with a 70% reduction in the appearance of new loss of heterozygosity loci throughout genome. Downregulation of these pathways by wortmannin was associated with a 20-25% reduced growth rate of BEAC cells in culture. To evaluate the impact of WM on *In Vivo* tumor growth, BEAC (FLO1) cells were injected subcutaneously in SCID mice and following appearance of palpable tumors, mice treated with WM at 0.75mg/kg, injecting daily intraperitoneally. Treatment with WM was associated with almost complete inhibition of tumor growth *In Vivo*. **Conclusions:** We conclude that WM inhibits both HR and nHR pathways, reduces genomic instability and proliferation rate and therefore is a potential therapeutic agent for prevention of disease progression and treatment of BEAC.

T1154

Role of Homologous Recombination in Telomere Maintenance and Evidence of ALT Pathway in Barrett's Adenocarcinoma Cells

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<p>**Introduction:** Telomere maintenance is one of the life lines of cancer cells. Telomere length in most cancers, including Barrett's adenocarcinoma (BEAC), is maintained by telomerase. However a subset of cancer cells lack telomerase and maintain telomere length by alternative mechanism (ALT), most probably involving homologous recombination (HR). We have found that recombinase (RAD51) expression and HR activity are constitutively elevated in BEAC cells. The purpose of this investigation was to study the role of elevated HR in telomere maintenance and growth, in telomerase positive BEAC cells. **Methods:** Studies were performed in BEAC cell lines, FLO-1 and OE33. FLO-1. ALT-associated PML bodies were detected by sequentially treating fixed cytospins with anti-PML rabbit polyclonal and Alexa Fluor 594-labeled goat anti-rabbit antibodies. HR was suppressed by treating BEAC cells with chemical inhibitors (wortmannin, nilotinib) or by transduction with lentiviruses producing control or recombinase-shRNAs. Telomerase activity was measured by modified "Telomeric Repeat Amplification Protocol" and telomere length by Real-Time PCR. For *In Vivo* studies, SCID-mice were subcutaneously inoculated with BEAC cells and following appearance of palpable tumors, injected with saline or HR inhibitor. **Results:** We found that ALT-associated PML bodies are highly expressed in BEAC cells and suppression of HR activity in these cells, by various treatments, consistently reduces telomere length by 15 – ≥40%. Moreover, transgenic upregulation of HsRAD51 and exposure to a recombinogen nickel chloride, lead to significantly elevated telomerase activity whereas suppression of RAD51 and HR caused a substantial reduction in telomerase activity in BEAC cells, indicating that HR proteins are also implicated in the regulation of telomerase activity. Consistently, the treatment with wortmannin was associated with significant reduction in tumor size in a subcutaneous mouse model of BEAC. We have also observed that combination of telomerase and HR inhibitors are significantly more affective in induction of growth arrest and apoptosis in BEAC cells *In Vitro*. **Conclusion:** These studies show a close association between recombinase(s) and telomeres in the nucleus. Elevated recombinase and HR may enhance telomere maintenance by upregulating the ALT pathway which along with the upregulated telomerase activity in BEAC provide a dual mechanism for telomere maintenance in BEAC. Therefore inhibitors of recombinase in combination with telomerase inhibitors may be highly effective in disrupting telomeres and thereby be highly effective in prevention and treatment of BEAC.

T1155

Anticancer Activity of a Broccoli Derivative, Sulforaphane, in Barrett's Adenocarcinoma: Potential use in Chemoprevention and as Adjuvant in Chemotherapy

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Introduction: Sulforaphane (SFN), an antioxidant contained in high concentrations in broccoli sprouts, has been shown to have antineoplastic properties. Esophageal Barrett's adenocarcinoma (BEAC) has clinically identifiable protracted precancerous state, namely dysplasia that makes it suitable for chemoprevention by nontoxic antineoplastic agents. We previously showed that SFN induces cell cycle arrest and apoptosis in BEAC cells. Purpose of the present investigation was to examine the effect of sulforaphane on: 1) Drug efflux in BEAC cells; 2) Efficacy as adjuvant in chemotherapy; and 3) Growth of tumors, derived by injecting BEAC cells subcutaneously in SCID mice. **Methods:** Effect of SFN on drug resistance/chemosensitivity was evaluated by rhodamine 123 efflux assay. BEAC cell lines (FLO1 and OE33) were treated with SFN alone or in combination with other antiproliferative agents including telomerase inhibitors, MST-312 and GRN163L, and live cell number determined. To determine the anticancer activity *In Vivo,* BEAC cells were injected subcutaneously in SCID mice and following appearance of palpable tumors, mice treated with SFN. **Results:** Rhodamine 123 is a substrate of multidrug resistance-associated protein and P-glycoprotein, a product of multidrug resistance gene, implicated in the extrusion of drugs outside the cell. A dose-dependent increase in the accumulation of intracellular rhodamin in FLO-1 cells treated with SFN was observed, indicating that SFN inhibits drug efflux. Consistently, the gene expression profile showed downregulation of multidrug resistance related proteins (MGC13170 and MGr1-Ag) in SFN-treated, relative to untreated cells. We previously showed that SFN enhances the antiproliferative affect of chemotherapeutic agent, paclitaxel. We extended these studies to telomerase inhibitors, MST-312 and GRN163L. The exposure of FLO-1 cells pretreated with MST-312 or GRN163L to SFN led to significant (P≤0.002) decrease in cell growth, relative to cells treated with telomerase inhibitors or SFN alone, indicating that SFN enhances the anticancer activity of chemotherapeutic and other antiproliferative agents. Finally, the efficacy of SFN was also demonstrated in a subcutaneous tumor model of BEAC, indicating anticancer activity *In Vivo*. **Conclusions:** SFN is a potent inhibitor of growth of BEAC cells *In Vitro* and *In Vivo*. SFN also downregulated multidrug resistance genes and reduces drug efflux in BEAC cells. These observations suggest that SFN, a natural product derived from broccoli may be useful in chemoprevetion, treatment, and as an adjuvant in cases resistant to other chemotherapeutic agents.

T1156

Cell-Surface Galectin-3 Confers Resistance to Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) by Impeding Trafficking of DR4 Death Receptors in Colon Cancer Cells

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Background: TRAIL is a potential cancer therapeutic agent, but TRAIL-surviving subpopulations of cells may contribute to therapeutic failure. Galectin-3, a beta-galactoside-binding mammalian lectin, can modulate apoptosis depending on its subcellular localization, nature of stimulus and cell type. Objective: To elucidate mechanisms responsible for TRAIL-resistance in colon cancer. Methods: Cell viability was assessed by the MTS assay and apoptosis by PARP degradation. Western blots and flow cytometry were used to study the underlying mechanism of cell death. Results: Exposure of LiM6 colon cancer cells to TRAIL resulted in apoptosis which plateaued at 65% cell death. The residual cells were propagated with periodic exposure to TRAIL to yield a TRAIL-resistant cell line, LiM6-TR. Components of apoptotic pathways downstream of caspase-8 activation were similar in LiM6 and LiM6-TR, but TRAIL failed to activate caspase-8 in LiM6-TR, suggesting that DISC formation is deficient. Total protein levels of constituents of the DISC, including DR4, DR5, FADD, FLICE and caspase-8, were not different in LiM6-TR compared to LiM6. Total protein levels of the apoptosis modulator galectin-3 were also similar in LiM6 and LiM6-TR. However, levels of cell-surface galectin-3 were 3-fold higher in LiM6-TR than in LiM6. TRAIL-resistant and parental cells had similar baseline levels of cell-surface DR4 and DR5. When LiM6 cells were treated with TRAIL, 36% of DR4 and 47% of DR5 were removed from the cell surface. When LiM6-TR cells were treated with TRAIL, only 12% of DR4 and 30% of DR5 were internalized. This suggested that binding of galectin-3 to N-linked and/or O-linked oligosaccharides on DR4 could interfere with the clustering of death receptors. Tunicamycin, an inhibitor of N-glycosylation, sensitized LiM6-TR to TRAIL-dependent apoptosis, and also further increased TRAIL-dependent apoptosis in LiM6. Tunicamycin had no cytotoxicity in the absence of TRAIL in either cell line. Benzyl-GalNAc, an inhibitor of O-glycosylation, had no effect on TRAIL-dependent apoptosis in either cell line. These results suggest galectin-3 binds to the N-linked oligosaccharide of DR4 (Asn156), rather than the O-linked oligosaccharides. Conclusions: Cell-surface galectin-3 can cause TRAIL-resistance in colon cancer cells by binding to N-linked carbohydrate on DR4, thus inhibiting the trafficking of death receptors and activation of caspase-8. Elevated levels of cell-surface galectin-3 may predict unfavorable outcome to TRAIL-based therapy of colon cancer patients, and small-molecule inhibitors of galectin-3 binding could overcome TRAIL resistance.

T1157

Potential of IL-6 Antibody as Promising Anti-Cancer Agent Targeting Colon Cancer Stem Cells

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Background and aims: Recent studies have shown that cancer stem cells can initiate and sustain tumor growth, and exhibit resistance to clinical cytotoxic therapies. Therefore, cancer stem cells are thought to be the main potential target for anticancer therapy. Morphologically, several surface markers of colon cancer stem cells have been developed. CD133+colon cancer cells can exhibit strong self-renewal properties, suggesting that the CD133 glycoprotein is a reliable cancer stem cell marker of colon cancer. Oct4, Klf4, c-myc (iPS cell-related genes) and Bmi1 (polycomb gene) are expressed in adult human stem cells, and these genes expressing cells are reported to be involved in the initiation and drug resistance of the carcinogenic process. On the other hand, high serum levels of IL-6 have been reported in colorectal cancer patients, suggesting IL-6 trans-signaling is involved in carcinogenesis. IL-6 promotes CSCs self-renewal in breast cancer, liver cancer, lung cancer, glioma, and neutralizing IL-6 suppresses breast cancer stem cell, glioma stem cell survival and tumor growth. In the present study, we investigated the involvement of IL-6 on stem cell biology and the effect of IL-6 antibody on chemo-resistance of colon cancer stem cells. Methods: The colon cancer cell line WiDr were cultured as spheroid-forming cells in serum free non-adherent condition, because three dimensioned, non-adherent spheroids are reported to enrich in stem cells, suggesting that spheroid formation is one of useful techniques to define cancer stem cells through functional assays. The expression of c-myc, Oct-4, KLF4, Bmi1, and IL-6 and surface marker CD133 were investigated by RT-PCR and FACS, respectively. The effects of IL-6 antibody treatment on spheroid formation, and the expression of these genes and chemoresistance were determined. Results: Spheroid forming WiDr cells were expressing higher levels of oct-4, klf4, Bmi1, IL-6, and CD133, and accelerated chemoresistance to 5FU, comparing with control cells cultured in adherent condition. IL-6 treatment enhanced forming spheroid sizes. IL-6 antibody treatment suppressed oct-4, klf4, and Bmi1 expression, decreased spheroid formation, and also significantly reduced chemoresistance in spheroid forming cells. Conclusion: These results indicate that IL-6 is involved in cancer