

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 to 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 P13K 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 10 M 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

Robert C. Bertheau, Jagannath Pal, Jason Y. Wong, Mariateresa Fulciniti, Ramesh B. Batchu, Saem Lee, Kenneth C. Anderson, Immaculata De Vivo, David G. Beer, Nikhil C. Munshi, Raj K. Goyal, Masood A. Shammam

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 cytopins 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 effective 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

Aamer Qazi, Jagannath Pal, Ma'in Maitah, Mariateresa Fulciniti, Dheeraj Pelluru, Puru Nanjappa, Ramesh B. Batchu, Madhu Prasad, Christopher S. Bryant, Samiyah Rajput, Saem Lee, Kenneth C. Anderson, Sergei Gryaznov, David G. Beer, Donald W. Weaver, Nikhil C. Munshi, Raj K. Goyal, Masood A. Shammam

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 rhodamine 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 effect 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 ($P \leq 0.002$) 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 chemoprevention, 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

Nachman Mazurek, James C. Byrd, Yun Jie Sun, Robert S. Bresalier

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

Ying Jin, Masahiko Tsujii, Jumpei Kondo, Tomofumi Akasaka, Yoshito Hayashi, Takahiro Inoue, Sachiko Nakajima, Shinichiro Shinzaki, Satoshi Egawa, Tsutomu Nishida, Kenji Watabe, Hideki Iijima, Shusaku Tsutsui, Norio Hayashi

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 dimensional, 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