overlapping regions of TROP-2 demonstrating the importance of localizing the correct area within a target molecule. To further test this hypothesis, anti-TROP-2 antibodies were selected from a pool of antibodies shown to be non-functional in cell killing by the FunctionFIRSTTM screen. When these were tested in vivo, the non-functional anti-TROP-2 antibodies did not display tumor growth inhibition in the PL45 xenograft model, while the efficacy of AR47A6.4.2, currently being advanced to clinical trials, was confirmed. Thus, targeting the functional epitopes within a target is necessary to designing new therapies. The ability to screen monoclonal antibodies that to target relevant epitopes is a powerful tool for increasing efficiency in discovering and developing novel and effective drugs.

544 POSTER

Discovery of the 6F4 anti-tumor antibody targeting the tight junction molecule JAM-A. 2. Target expression on human tumors and in vitro and in vivo anti-cancer activity

L. Goetsch[1], J.F. Haeuw[1], A. Gonzalez[1], C. Beau-Larvor[1], L. Zanna[1], T. Wurch[1], C. Bailly[1], N. Corvaïa[1]. *[1]Institut de Recherche Pierre Fabre, Centre d'Immunologie Pierre Fabre, Saint-Julien-en-Genevois, France*

Biotherapeutic drugs in oncology are generating a great enthusiasm and tremendous amount of effort is made to identify new targets for the development of news therapeutics. Functional approaches using tumor cells as immunogens have been performed in order to select on the basis of their function, molecules that, targeted by monoclonal antibodies (Mabs), would induce inhibition of cell proliferation both in vitro and in vivo.
After immunization of mice with MCF-7 breast cancer cells, we generated a series of Mabs able to block MCF-7 cell proliferation in vitro. These Mabs were further tested in vivo for their ability to induce MCF-7 tumor regression in engrafted nude mice. Interestingly, one Mab named 6F4 was able to completely inhibit tumor growth in mice after i.p. treatment with 1 mg dose twice a week. A proteomic analysis using 6F4-tagged beads coupled to a MS analysis indicated that 6F4 Mab specifically recognized the human Junctional Adhesion Molecule A (JAM-A). The activity of 6F4 Mab was also observed after injection of 1 mg of the Mab twice a week, to mice xenografted with human epidermoid squamous carcinoma A431 cells.
Immunohistochemistry analysis on a panel of human tissues from normal and tumor origin revealed that the JAM-A molecule was strongly overexpressed on tumor tissues compared to normal ones. In order to get inside the mechanism of action of 6F4 Mab, ex vivo analysis was performed on MCF-7-xenografted mice. We observed a dose-dependent inhibition of cell proliferation together with a disappearance of JAM-A from the cell surface, in perfect agreement with the results obtained with cells in vitro. These results indicate that the 6F4 anti-JAM-A Mab is able to induce tumor growth inhibition and therefore suggest that JAM-A is a potential novel target in oncology. Further experiments are needed to better characterize the mechanism of action of the lead antibody. The results also demonstrate that a functional approach coupled to proteomic analysis can be successful to identify new antibody target molecules that lead to promising new antibody-based therapies against cancers.

Radiation interactive agents

545 POSTER

Enhancement of cell motility with radiation-induced VEGF in glioma

W. Kil[1], K. Camphausen[1]. *[1]National Cancer Institute/National Institute of Healths, Radiation Oncology, Bethesda, USA*

Background: Glioblastoma multiforme (GBM) is among the most lethal of all human tumors, with poor survival and frequent local recurrences after treatment. The mechanism accounting for such a recurrence pattern is unclear. It has classically been regarded as a local recurrence of treatment resistant cells. However, accumulating evidence suggests that a second mechanism may exist involving the migration of tumor cells or tumor stem cells from regions of the brain that received minimal RT. VEGF family members are well known as active mitogens and are up-regulated after RT. Here, we examine the effect of irradiation (IR)-induced VEGF on glioma cell motility.
Material and Methods: The U251 and LN18 human GBM cell lines were used to generate conditioned medium (CD). At 72 hours after various dose of IR, the supernatant of culture dishes were harvested and used as IR-CD. Actinomycin D (Act D) was used as a transcription inhibitor. ELISA was used to quantity the VEGF protein in conditioned medium. The expression level for VEGF mRNA transcripts was detected by RT-PCR. In vitro motility assay was done with chamber coated with/without Matrigel and CD as a cell motility enhancer. The VEGF antibody was used for neutralization of VEGF bioactivity in conditioned medium.
Results: ELISA showed the VEGF was increased in CD at 72 hours after IR (98.504±0.098pg/1000cells with 2 Gy v.s. 0.034±0.003pg/1000cell in control). RT-PCR revealed an increase of VEGF mRNA after IR, and the effect was mitigated by pre-RT exposure to Act D (432±53% and 75±14% of control, respectively). Cell motility (migration and invasion) was enhanced with the addition of IR-CD (174.9±11.4% and 334.2±46% of control, respectively). The enhanced cell motility measured with the addition of IR-CD was negated with the addition VEGF antibody to IR-CD (110.3±12.0% and 105.7±14.0% of control, respectively).
Conclusions: These results indicate that cell motility can be enhanced by conditioned medium from irradiated cells in vitro and suggest that this effect involves radiation-induced VEGF leading to an increase in glioma cell motility.

546 POSTER

GI261 brain tumor cells: responses to single or fractionated x-irradiation with the avβ3 integrin thyroxine receptor antagonist TETRAC (tetraiodothyroacetic acid)

A. Hercbergs[1], P.J. Davis[2], M. Cieslieski[3], F. Davis[4], J.T. Leith[5]. *[1]Cleveland Clinic Foundation, radiation oncology, Cleveland, OH, USA; [2]Ordway Research Institute Inc., Signal Transduction, Albany, NY, USA; [3]Roswell Park Cancer Institute, Neurosurgery, Buffalo, NY, USA; [4]Ordway Research Institute Inc., Signal Transduction, Albany, NY, USA; [5]Rhode Island Nuclear Science Center, Radiation Biology, Providence, RI, USA*

Background: The membrane integrin avβ3 has been shown to contain a receptor for thyroid hormone. TETRAC-a product of L-thyroxine, inhibits the proliferative/angiogenic effects of L-thyroxine and L-thyronine (T3, T4) initiated at the integrin receptor near or at the Arg-Gly-Asp (RGD) recognition site.. Since targeting the avβ3 integrin with cilengitide, an Arg-Gly-Glu tripeptide, results in increased cellular radiosensitivity, (Albert et al Int. J. Radiat. Oncol. Biol. Phys. 2006: 65: 1536–1543), we tested TETRAC as a radiosensitizing agent.
Methods and Materials: Glioma (GL261) cells were grown as exponential or plateau phase cultures and treated with 2 μM TETRAC for 1 h at 37ºC prior to exposure to 250 kVp x-rays. After irradiation, cells were removed from treatment dishes, counted and then re-plated in fresh medium (without TETRAC) for estimation of effect of TETRAC on immediate survival. Results were analyzed using the linear-quadratic (LQ) formalism. In other experiments, the time-dependent effects of TETRAC on sublethal and potentially lethal damage repair (SLDR and PLDR) were determined.
Results: LQ parameters for control cells were: a = 0.360 Gy-1 and β = 0.094 Gy-2. 2 Gy survival was 33.4%. For cells treated with TETRAC, LQ parameters were: a = 0.921 Gy-1 while β was zero. Survival at 2 Gy was 15.5%. The 2 Gy dose ratio yielded an enhancement factor of 2.2. TETRAC also reduced SLDR in exponential cells by 62%. In control exponential cells, there was little PLDR expression with an increase in survival of 1.5-fold seen at 8 h post-irradiation. TETRAC, however, completely removed PLDR expression in exponential cells. Control plateau phase cells reached an increase in survival of 4-fold 8 h post-irradiation. TETRAC decreased the expression of this PLDR by 75%.
Conclusions: TETRAC, which blocks the avβ3 integrin receptor from T3/T4 effects resulted in both radiosensitization and inhibition of SLDR and PLDR. This may involve occlusion of the RGD site which binds radioprotective FGF-2 and VEGF.

547 POSTER

Effect of the extract of *Taraxacum officinale* on inflammation induced by anti-cancer treatment

Y. Oh[1], S.H. Kang[1], M. Chun[1], E. Lee[1], E.H. Kim[1], J. Han[2], J. Yang[1]. *[1]Ajou Univ. Hospital, Radiation Oncology, Suwon, South Korea; 2. Ajou Univ. Hospital, Anatomical Pathology, Suwon, South Korea*

Purpose: Mucositis is a major complication associated with anti-cancer treatment. Inflammation is the main process of mucositis from both chemotherapy and radiotherapy. Many herbs including *Taraxacum officinale* (TO) have anti-inflammatory effect and we tried to evaluate that the extract of TO could reduce the inflammation from anti-cancer treatment.
Materials and Methods: Female Wistar rats for methotrexate (MTX) induced enteritis were orally administered with 3 different concentration of the TO extracts or saline for 12 days from 1 week before MTX injection and then MTX (2.5 mg/kg) were subcutaneously injected for 3 days to induce intestinal musocitis. The histologic severity of intestinal damage from MTX treatment was assessed semiquantitatively 5 days after injection. Rats for radiation induced proctitis received 17.5Gy of radiation on rectal mucosa. They were fed TO or saline for 7 days prior to radiation and continued for 10 days after irradiation. Rats were sacrificed 10 days and 6 weeks after irradiation for histologic evaluation of acute and chronic phase. For in vitro study, RAW 264.7 cells were treated with TO for 24 hours and then irradiated. We collected culture supernatant 48 hours after irradiation and

measured levels of inflammatory cytokines, IL-1 beta, IL-6, TNF-alpha and MCP-1 by ELISA.
Results: Histologic severity scores in jejunum-ileum after MTX treatment was 16±5.4 and 22±7.9 with TO and saline, respectively, although it was not statistically significant. TO showed no effect to reduce mucosal damage of rectum induced by radiation at acute phase. In chronic phase, there was a tendency to reduce the severity of proctitis. In vitro study, IL-1 beta, IL-6, TNF-alpha and MCP-1 were increased after irradiation and the dose dependent reduction of these values were observed in TO treatment group.
Conclusions: TO is effective to reduce the inflammatory cytokines induced by radiation and have a potential to ameliorate mucositis induced by anti-cancer treatment.

RNA and RNA based technologies

548 POSTER

Targeting non-coding promoter-associated RNAs in the c-myc gene with small interfering RNAs induce gene silencing and growth arrest in c-myc over-expressing cancer cells

C. Pastori[1], S. Napoli[1], M. Magistri[1], A. Sepe[1], F. Cavalli[1], G. Carbone[1], C. Catapano[1]. [1]*Oncology Institute of Southern Switzerland, Experimental Oncology, Bellinzona, Switzerland*

Background: Small interfering RNAs (siRNAs) can induce transcriptional gene silencing (TGS) when directed to sequences in gene regulatory regions. Promoter-directed siRNAs (p-siRNAs) can mimic natural pathways of transcriptional control and could be very effective tools to knock-down expression of cancer genes in therapeutic applications. SiRNA-directed TGS is associated with the induction of epigenetic changes in the target gene promoter. Furthermore, non-coding RNAs in gene promoters have been proposed to serve as docking sites for p-siRNAs and mediate the assembly of transcriptional silencing complexes. In this study we assessed the presence of non-coding promoter-associated RNAs (p-RNAs) in the c-myc promoter and the ability of p-siRNAs to inhibit c-myc transcription and affect growth and survival of c-myc over-expressing prostate cancer cells.
Methods: Transcriptional activity and epigenetic marks in the c-myc gene were examined by RT-PCR and chromatin immunoprecipitation. Cell growth and survival were assessed by cell counting, cell viability and clonogenic assays.
Results: Using strand-specific RT-PCR we identified p-RNAs overlapping the major TSS both in sense and antisense orientation and extending approximately 400 bp upstream and 120 bp downstream of the TSS. The level of p-RNAs was $\geqslant$50 fold lower than c-myc mRNA. Sense p-RNA transcripts were predominant and their expression correlated positively with the level of c-myc mRNA in different cell lines, with higher levels in c-myc over-expressing prostate cancer cells compared to normal epithelial cells and fibroblasts. siRNAs targeting distinct sites in the c-myc promoter region varied widely in their ability to inhibit c-myc transcription, with some being very effictive ($\geqslant$80% inhibition) and others having negligible effects. Inhibition of c-myc transcription was generally associated with knockdown of p-RNAs by the p-siRNA. Few exceptions were represented by p-siRNAs that affected p-RNAs but had limited effect on c-myc transcription. Silencing of the c-myc gene and knock-down of p-RNAs by p-siRNAs resulted in reduced survival and long-term inhibition of proliferation of c-myc over-expressing prostate cancer cells with minimal effects on normal cells, thus underscoring the therapeutic potential of this approach.
Conclusion: Our study shows that p-siRNAs targeting an oncogene like c-myc can effectively knock-down gene expression and elicit profound and long-lasting effects on the cancer cell phenotype. Our data suggest also that p-RNAs have important regulatory functions in cells, controlling gene transcription and perhaps yet undiscovered cellular processes. p-RNAs may be exploited as novel targets for discovery and development of effective cancer therapeutics.

549 POSTER

Quantitation/significance of MRD in standard risk adult ALL by RQ/PCR

N.K. Abousamra[1], D.M. Elghannam[1], D.A. Shahin[1], E.F. Goda[1], H. Azzam[1], E. Azmy[2], M. Salah El-Din[3]. [1]*Mansoura University Hospital, Clinical Pathology and Hematology, Mansoura, Egypt;* [2]*Mansoura University Hospital, Clinical Haematology Department, Mansoura, Egypt;* [3]*Mansoura University Hospital, Medical Oncology Department, Mansoura, Egypt*

Background: Modern treatment protocols lead to morphological complete remission in the majority of ALL patients, 40–55% ultimately relapse with disease that is highly refractory to current therapy.
Aim: The objective of this study is to apply real-time PCR for detection and quantitation of minimal residual disease (MRD) by immunoglobulin heavy chain gene rearrangement in standard risk adult acute lymphoblastic leukemia (ALL) after induction and during maintenance chemotherapy.
Subjects and Methods: Our study included 25 newly diagnosed standard-risk (SR) adult acute lymphoblastic leukemia, they were followed up for 12 months.
Results: Real-time PCR for all patients at diagnosis were done and revealed that 18/25 patients(72%) were positive for IgH1 (10 cases), and IgH2 (8 cases). Seven cases (28%) were PCR negative for the two probes. After induction chemotherapy MRD was positive in 44.4% of cases (8/18), negative in 55.6% (10/18). After consolidation MRD was positive in 40% (4/10), and negative in 60% (6/10). The Relation between positive MRD levels and relapse-free survival was significant. Relapse-free survival (12 months) was significantly increased in negative MRD compared to positive MRD.
Conclusion: From these study we can conclude that RQ PCR is powerful diagnostic tools for early detection and quantitation of minimal residual disease in adult acute lymphoblastic leukemia and with a sensitivity up to 10^4, 10^3 respectively. Monitoring of MRD in the follow up samples should be included in all current treatment protocols for adults with ALL, as it is informative and useful for early prediction of relapse and evaluation of treatment response.

550 POSTER

Designing nanovectors for siRNA delivery: coupled experimental/modeling investigations

S. Pricl[1], P. Posocco[1], G.M. Pavan[1], M. Fermeglia[1], G. Scocchi[2], A. Danani[2], A. Malek[3], S. Napoli[3], C.V. Catapano[3]. [1]*University of Trieste, MOSE – DICAMP, Trieste, Italy;* [2]*University of Applied Science of Southern Switzerland, Department of Innovative Technologies, Manno, Switzerland;* [3]*Oncology Institute of Southern Switzerland, Laboratory of Experimental Oncology, Bellinzona, Switzerland*

Background: Nucleic acid therapeutics, like small-interfering RNAs (siRNAs), have recently emerged as powerful new drug entities for treatment of human diseases and lead validation in the drug discovery process. The absence of efficient delivery systems for siRNA therapeutics, however, is identified as a major hurdle for clinical applications. Biocompatible polymers have been considered as potential non-viral carriers due to their safety for clinical use compared to virus-based carriers and despite their lower siRNA-transduction capacity. Improving specificity toward target cells and efficiency of intracellular delivery will be a prerequisite for successful clinical application of polymeric carriers. Multifunctional polymeric nanocarriers should be rationally designed at the molecular level for effective human siRNA-based therapy. However, systematic investigation of the structure of siRNA/polymeric nanocarrier complexes is hindered by the absence of appropriate experimental techniques.
Methods: We investigated the structure–activity relationship of a large set of siRNA/nanocarriers, including poly(ethylenimine)-poly(ethylene glycol) (PEI-PEG) copolymers of different size and degree of PEGylation, and poly(amido amine) based dendrimers (PAMAM). In silico simulation techniques were used to build the nanocarrier structures, simulate siRNA/nanocarrier assembly and analyze their biophysical properties. Data from computer simulations were confronted with experimentally determined parameters in cell culture-based and biochemical assays (e.g., gene knock-down efficiency, cellular uptake, in vitro toxicity, complex assembly and stability).
Results: In silico simulation and experimental data defined the structure of the polymeric nanocarriers, their mode of the interaction with siRNAs and critical parameters for optimization of the siRNA/nanocarrier interactions. The structure of PEI/PEG copolymers and their ability to assemble, protect and deliver siRNAs into cells depended on the size of PEI molecules, length and number of coupled PEG molecules. For PAMAM dendrimers, an important dependence on molecular generation and pH was found. The "proton sponge effect", which is at the base of endosomal escape and intracellular delivery of biologically active siRNA, was also verified by molecular simulations.
Conclusions: Our investigation identified a broad panel of PEI-PEG copolymers and PAMAM dendrimers capable of efficient delivery of siRNAs in cells and gene knock-down. This study provided information on the structure of the siRNA/nanocarrier complexes and analyzed properties driving complex assembly and stability. This combined experimental and computational approach could rapidly expand the portfolio of available synthetic nanocarriers for preparation of non-toxic, biocompatible delivery reagents for siRNA therapeutics.