Drug resistance and modifiers

300 POSTER

Novel bifunctional alkylating agents overcome multidrug resistant cancer cells

T.C. Lee[1], P.C. Lee[2], R. Kakadiya[1], T.L. Su[1]. [1]*Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan;* [2]*Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan*

Several bifunctional alkylating derivatives of 3a-aza-cyclopenta[*a*]inden (BO-1012, BO-1005, BO-1099, and BO-1101) were synthesized in our laboratory. In the present study, we explored their anticancer activity against tumor cells with multidrug resistance (MDR). Our results showed that 4 MDR cell lines, KBvin10, KBtax50, CEM/VBL, and MCF7/Adr, were more susceptible to these derivatives than their parental cell lines. By using xenograft model, we confirmed that BO-1012 significantly suppressed the growth of MDR KBvin10 cells in nude mice as compared to the parental KB cells. We have also investigated the mechanisms of action on the collateral sensitivity of BO-1012 to KBvin10 cells. We revealed that BO-1012 induced higher levels of autophagy in KBvin cells than KB cells. Furthermore, by using gH2AX as a marker of DNA double strand breaks, we demonstrated that the repair efficiency of BO-1012-induced DNA damage in KBvin10 cells was significantly lower than that in KB cells. Our present results showed that lower repair activity in KBvin10 cells was likely due to its defective in translocation of DNA-PK, a component of repair machinery of non-homologous end-joining, from cytosol into nucleus. By aid of DNA-PK inhibitor, we confirmed the roles of DNA-PK on repair of DNA damage induced by BO-1012 in KB cells. Taken together, we have revealed that the collateral sensitivity of MDR cancer cells to DNA damage agents, such as BO-1012, may be due to their impaired DNA-PK repair pathway. These results suggest that bifunctional alkylating derivatives of 3a-aza-cyclopenta[*a*]inden may serve as a promised anticancer agent against human cancer cells with MDR.

301 POSTER

Silencing of TCF7L2 sensitizes colorectal cancer cells to radiation therapy

M. Grade[1], E. Kendziorra[1], K. Ahlborn[1], M. Spitzner[1], J. Gaedcke[1], M. Rave-Fränk[2], H. Becker[1], B.M. Ghadimi[1], T. Pukrop[3], T. Ried[4]. [1]*University Medicine Göttingen, General and Visceral Surgery, Gottingen, Germany;* [2]*University Medicine Göttingen, Radiotherapy and Radiooncology, Gottingen, Germany;* [3]*University Medicine Göttingen, Haematology/Oncology, Gottingen, Germany;* [4]*National Cancer Institute, Genetics Branch, Bethesda, USA*

Background: The clinical response of locally advanced rectal cancers to preoperative chemoradiotherapy is very heterogeneous. To determine the molecular characteristics associated with this heterogeneity, we recently profiled a series of responsive and resistant rectal adenocarcinomas using gene expression microarrays, and identified a set of differentially expressed genes. One gene that was significantly overexpressed in the resistant tumors was TCF7L2, the main downstream effector of the Wnt signaling pathway. The aim of this study was to evaluate if RNAi-mediated silencing of TCF7L2 sensitizes tumor cells to radiation.
Material and Methods: We transfected three colorectal cancer cell lines (SW480, SW837 and HT-29) with two different shRNA-vectors targeting TCF7L2, and a non-silencing control, and subsequently established stable single cell clones. TCF7L2 protein levels after RNAi-mediated silencing were analyzed by Western blotting. For each vector, single cell clones were irradiated at 0, 1, 2, 4, 6 and 8 Gy, and survival fractions were calculated.
Results: The decrease in TCF7L2 protein levels ranged from ~60% to ~90%. RNAi-mediated silencing of TCF7L2 significantly reduced colony-formation after radiation: We observed dose reduction factors of ~1.55 and ~1.49 at 37% survival for SW480 and SW837, respectively. Interestingly, colony formation of HT-29 cells was only scarcely reduced.
Conclusions: TCF7L2 was overexpressed in resistant rectal carcinomas, and its RNAi-mediated silencing caused a significant radiosensitization in SW480 and SW837 cells, but not in HT-29 cells. Preliminary experimental evidence suggests that this diversity is based on differences in Wnt/beta-catenin signaling activity. This is now being investigated. Importantly, these data suggest TCF7L2 as a potential molecular target to sensitize a priori resistant tumor cells.

302 POSTER

Sulforaphane enhances effects of quercetin, sorafenib, and chemotherapy towards pancreatic cancer stem-like cells

I. Herr[1], G. Kallifatidis[1], V. Rausch[1], S. Labsch[1], W. Zhou[1], B. Baumann[2], J. Mattern[1], A.V. Salnikov[3]. [1]*Experimentelle Chirurgie, Allgemein- Viszeral-Transplantationschirurgie, Heidelberg, Germany;* [2]*Physiologische Chemie, Universität Ulm, Ulm, Germany;* [3]*Deutsches Krebsforschungszentrum, Translational Immunology Unit, Heidelberg, Germany*

Background: Despite intense efforts to develop treatments against pancreatic cancer, agents that cure this highly resistant and metastasizing disease are not available. Considerable attention has focused on broccoli compound sulforaphane, which is suggested as combination therapy for targeting of pancreatic cancer stem cells. However, there are concerns that anti-oxidative agents such as sulforaphane may interfere with cytotoxic therapy − as suggested e.g. for vitamins.
Material and Methods: The effects of sulforaphane upon combination with various standard chemotherapeutics, the dietary agent quercetin and the multi kinase inhibitor sorafenib were evaluated using in vitro and in vivo models of pancreatic tumor cells with stem-like phenotype. CSC-marker expression, ALDH1 activity, self-renewal potential, Notch signaling, migratory activity, apoptosis induction, viability, proliferation, NF-kB-signaling, and angiogenesis were analyzed.
Results: While each therapeutic agent alone diminished the stem-like characteristics, elimination of highly aggressive stem-like cells was not complete. However, combination with sulforaphane led to an additive effect of each single agent. This was due to inhibition of self-renewal activity and sensitization to apoptosis by inhibition of Notch, NF-kB, caspases, clonogenicity, spheroid-forming, migratory activity and downregulation of anti-apoptotic and EMT-related proteins. *In vivo,* combination treatment was most effective and totally abolished growth of cancer stem-like xenografts. No pronounced side effects were observed in mice. Our data suggest that sulforaphane increases the effectiveness of various cytotoxic drugs, sorafenib and quercetin against cancer stem cells without inducing additional toxicity in mice.
Conclusions: Our data suggest the combination sulforaphane with conventional or novel cancer therapeutics is safe and a promising new concept for targeting of pancreatic cancer stem-like phenotype.

303 POSTER

Hypoxia-inducible factor 1a promotes gastric cancer chemoresistance via modulation of p53 and NF-κB

N. Rohwer[1], C. Dame[2], A. Haugstetter[3], B. Wiedenmann[1], K. Detjen[1], C.A. Schmitt[3], T. Cramer[4]. [1]*Charité − Universitätsmedizin Berlin Campus Virchow-Klinikum, Medizinische Klinik m.S. Hepatologie und Gastroenterologie, Berlin, Germany;* [2]*Charité − Universitätsmedizin Berlin, Klinik für Neonatologie, Berlin, Germany;* [3]*Charité − Universitätsmedizin Berlin, Molekulares Krebsforschungszentrum, Berlin, Germany;* [4]*Charité − Universitätsmedizin Berlin, Medizinische Klinik m.S. Hepatologie und Gastroenterologie, Berlin, Germany*

Background: Reduced chemosensitivity of solid cancer cells represents a pivotal obstacle in clinical oncology. Hence, the molecular characterization of pathways regulating chemosensitivity is a central prerequisite to improve cancer therapy. The hypoxia-inducible factor HIF-1α has been linked to chemosensitivity while the underlying molecular mechanisms remain largely elusive. Therefore, we comprehensively analysed HIF-1α's role in determining chemosensitivity focussing on responsible molecular pathways.
Material and Methods: RNA interference was applied to inactivate HIF-1α or p53 in the human gastric cancer cell lines AGS and MKN28. The chemotherapeutic agents 5-fluorouracil and cisplatin were used and chemosensitivity was assessed by cell proliferation assays as well as determination of cell cycle distribution and apoptosis. Expression of p53 and p53 target proteins was analyzed by western blot. NF-κB activity was characterized by means of electrophoretic mobility shift assay.
Results: Inactivation of HIF-1α in gastric cancer cells resulted in robust elevation of chemosensitivity. Accordingly, HIF-1α-competent cells displayed a significant reduction of chemotherapy-induced senescence and apoptosis. Remarkably, this phenotype was completely absent in *p53* mutant cells while inactivation of p53 *per se* did not affect chemosensitivity. HIF-1α markedly suppressed chemotherapy-induced activation of p53 and p21 as well as the retinoblastoma protein, eventually resulting in cell cycle arrest. Reduced formation of reactive oxygen species in HIF-1α-competent cells was identified as the molecular mechanism of HIF-1α-mediated inhibition of p53. Furthermore, loss of HIF-1α abrogated, in a p53-dependent manner, chemotherapy-induced DNA-binding of NF-κB and expression of anti-apoptotic NF-κB target genes. Accordingly, reconstitution