attenuates L-OHP induced anti-apoptotic protein expression in HCT116 cells and increases the sensitivity of the cells to L-OHP. RNAi-mediated suppression of CXCR1 and CXCR2 expression also results in increased sensitivity of these cells to L-OHP.
Conclusions: These studies indicate that constitutive and drug induced IL-8 signalling contributes to an increased survival of CRC cells in response to L-OHP treatment. Inhibition of IL-8 signalling may be an appropriate intervention to sensitise CRC cells to L-OHP treatment.

499 POSTER

The effects of hypoxia on the sensitivity of glioma cells to gemcitabine treatment

R. Foster[1], S. Mead[1], K. Grimshaw[1]. *[1]Hypoxium Ltd, Cell Biology, Cambridge, United Kingdom*

It has become well recognised that hypoxia can play an important role in the resistance of tumours to a variety of chemotherapeutic agents. Cell models are commonly used for drug discovery research, yet the responses of cancer cell lines to chemotherapeutic agents under hypoxia are not routinely evaluated. A better understanding of these responses may help to identify chemotherapeutic agents that will not only be effective *in vitro*, but also efficacious *in vivo*. Gemcitabine is a deoxycytodine analogue that is widely used to treat pancreatic cancer and is under investigation for the treatment of glioma. Work has previously demonstrated that hypoxia increases the resistance of pancreatic cancer cells to gemcitabine-induced apoptosis via the PI3K/Akt/NF-kB pathways[1]. However, to date the effects of hypoxia on glioma cell sensitivity to gemcitabine-induced apoptosis have not been investigated.
We have characterised the response of glioma cell lines grown under hypoxic conditions, by monitoring protein expression of known hypoxia-inducible proteins, such as HIF1 alpha. Further studies were carried out to investigate the sensitivity of glioma cell lines to gemcitabine under varying oxygen concentrations by measuring cellular proliferation and apoptosis.
Our results have demonstrated that under low oxygen concentrations, glioma cells are more resistant to the anti-proliferative effects of gemcitabine. Moreover, the resistance to gemcitabine is inversely correlated to oxygen concentration, with increased resistance seen at 0.1% oxygen, compared to 1% oxygen.
We present for the first time, data demonstrating that oxygen concentration is indeed an important determining factor in the sensitivity of glioma cells to gemcitabine. Work to investigate the mechanisms and pathways involved in hypoxia-induced cellular resistance to gemcitabine is ongoing. A better understanding of these mechanisms within glioma cells will aid future research into therapeutic intervention for this disease.

References

[1] Yokoi and Fidler, 2004 Clinical Cancer Research 10: 2299–2306.

500 POSTER

Molecular and cellular consequences of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) direct interaction with the S23906−1/DNA adduct

G. Lenglet[1], S. Depauw[1], D. Mendy[1], A. Pierré[2], M.H. David-Cordonnier[1]. *[1]Inserm U837 Centre de Recherches Jean-Pierre Aubert, "Molecular and Cellular Targeting for Cancer Treatment" and IFR114-IMPRT IRCL Place de Verdun, Lille, France; [2]Institut de Recherches Servier, Département Cancérologie, Croissy sur Seine, France*

S23906−1 is a DNA alkylating compound bonding DNA in the minor groove on N2 group of guanine residues and subsequently induces a local opening of the double helix [1,2]. Based on its high antitumor potency on a wide variety of pre-clinical models, this acronycine derivative entered phase 1 clinical trial. At the cellular level, exposure to S23906 led to an accumulation of DNA double strand breaks (DSB) and apoptosis. The precise molecular mechanism leading to the formation of DSB[3], which are thought to be the major lethal DNA lesions induced by S23906−1, is not identified. Therefore, the investigation of the mechanism by which S23906−1 DNA adduct interferes with the nuclear machinery would help understanding the way this compound exerts its cytotoxic activity.
Using a proteomic approach, we identified the glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) as a protein specifically interacting with S23906−1 DNA adduct. Electromobility shift assays confirm the strong potency of GAPDH to specifically recognize S23906−1/DNA adduct. Interestingly, GAPDH did not interact with ET-743 adducts, another drug alkylating the N2 position of guanine but which, in contrast to S23906−1, stabilizes the DNA helix, suggesting different downstream cell consequences. GAPDH is a well known glycolysis enzyme which was also shown to be involved in DNA binding, repair and apoptosis processes[4]. Binding of GAPDH to DNA was observed using both double-(dsDNA) and single-stranded DNA (ssDNA) as observed for DNA alkylation by S23906−1[2]. Moreover, S23906−1 destabilizes alkylated-dsDNA thus generating alkylated-ssDNA suggesting that locally alkylated-ssDNA could be generated in cells. Therefore, we evaluate the ability of GAPDH to recognize S23906−1 DNA adduct within ssDNA. EMSA evidenced interactions between GAPDH and S23906−1 adduct on radiolabeled ss-DNA, suggesting that binding of GAPDH to the locally destabilized DNA helix bearing a S23906−1 adduct could have an important role in the S23906−1 cytotoxicity.
GAPDH is implicated in the cytotoxicity of the natural bis-quinone alkaloid saframycin A (SafA), a compound structurally related to ET-743, and to be translocated to the nucleus upon treatment with SafA[5]. We therefore looked at sub-cellular localisation of GAPDH following exposure of cells to S23906−1 using transfected GAPDH-GFP fusion vector. Using a siRNA approach, we evaluated the relationship between GAPDH level and S23906−1 cytotoxic effect. In contrast to SafA, S23906−1 cytotoxic effect was increased upon decrease of GAPDH protein expression.
In conclusion, GAPDH might play a role in S23906−1/DNA adduct recognition in the nucleus.

References

[1] David-Cordonnier et al., 2002, Biochemistry, 41, 9911–9920.
[2] David-Cordonnier et al., 2005, Mol. Cancer Ther., 4, 71−80.
[3] Léonce et al., 2006, Cancer Research, 66, 7203–7210.
[4] Sirover, 2005, J. Cell. Biochem., 95, 45−52.
[5] Xing et al, 2004, PNAS, 101, 5862–5866.

501 POSTER

MicroRNA expression profiling in paclitaxel-resistant ovarian cancer cell line: miR-31 is involved in the acquired resistance to paclitaxel

M. Hassan[1], H. Watari[1], A. Abdel Kader[2], T. Mitamora[3], N. Sakuragi[3]. *[1]Hokkaido University Graduate School of Medicine, gynecology, Sapporo, Japan; [2]South Valley University Aswan Faculty of Science, Biological Science, Aswan, Egypt; [3]Hokkaido University Graduate School of Medicine, gynecology, Sapporo, Japan*

Background: MicroRNAs (miRNA) represent a novel class of genes that regulate the gene expression. This class of genes have been recently implicated in development, carcinogenesis and apoptosis. Here we show the difference of microRNAs expression profile between paclitaxel (TX)-sensitive and TX-resistant ovarian cancer cell line to map out novel candidates regulators involved in the resistance mechanism.
Material and Methods: We used serous ovarian cancer cell KF and its Paclitaxel resistant counterpart. The microRNA profile was compared between both cells using mirVana microRNA bioarray system. The down-modulated micro-RNAs after development of resistance to TX were listed. We then focused on studying the role of miR-31 in chemoreisitance and its ability to re-sensitize KF-TX cells in cultures. We established stable clones expressing, exogenous, miR-31 precursor from KF-TX cells. Vaiability test, FACS analysis and Annexin V staining were used to study the effect of TX on the different clones.
Results: The miRNA bioarray indicated that miR-31, miR-93 miR-181 d, and miR-183 were down-modulated in KF-TX cells, ten folds less, when compared with parental KF cells. Northern blot of both parental and TX-resistant cells verified the bioarray results. We then introduced a line of evidence that exogenous expression of miR-31 precursor in KF-TX cells re-sensitized cells to paclitaxel. Moreover, in reverse, transfection of anti-miR-31 into parental cells was performed to confirm its involvement in the resistance mechanism.
Conclusion: Our data indicate involvement of miRNAs modulation in the acquired resistance mechanism of ovarian cancer. Specifically, miR-31 was evident to contribute the development of TX-resistance. Thus, targeting miR-31 could be a novel therapeutic tool to enhance or restore chemo-sensitization of the resistant serous ovarian cancer cells.

502 POSTER

Modification of cisplatin administration schedule in FLEP preoperative chemotherapy improved response to the chemotherapy in patients with locally-advanced esophageal cancer

T.A. Bogush[1], B.E. Polotskiy[2], E.A. Bogush[2], I.A. Pokataev[3], E.O. Ignatova[1], S.A. Tjulandin[3], M.I. Davydov[2]. *[1]N.N. Blokhin Russian Cancer Research, Laboratory of Medical Chemistry, Moscow, Russian Federation; [2]N.N. Blokhin Russian Cancer Research, Surgical Department, Moscow, Russian Federation; [3]N.N. Blokhin Russian Cancer Research, Department of Chemotherapy, Moscow, Russian Federation*

Background: We have demonstrated previously that cisplatin and carboplatin are effective inhibitors of multidrug resistance mechanism