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

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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.

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500 POSTER

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

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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.

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501 POSTER

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

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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

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

(MDR) associated with expression of energy-dependent transport proteins, extruding MDR-drugs out of the cells. In fact, this is an explanation of the well-known clinical phenomenon of increased efficacy of platinum agents in combination with inactive MDR-drugs in treatment of MDR tumors. It is obvious then that to maximize platinum inhibition of MDR-transporter function the sequence of drug administration "platinum-MDR-drug" should be maintained during the entire chemotherapy duration. Our own clinical experience may be a positive example demonstrating efficacy of this approach.
Design of the investigation: Patients with locally-advanced esophageal cancer received preoperative chemotherapy with cisplatin, etoposide, 5-fluorouracil, leukovorin (FLEP); cisplatin being administered by different modes, i.e. by standard schedule: cisplatin on day 1, or by modified schedule: cisplatin on days 1 to 3. The remaining drugs were always given on a daily basis, with etoposide administered after cisplatin. Response was assessed after 2 three-day cycles with a 3-week interval. The 36 patients enrolled in the two arms were fairly homogeneous in terms of major clinical characteristics.
Results: The number of cases demonstrating decreased severity of dysphagia after chemotherapy completion was greater in the modified schedule group (43% vs. 24% of cases), though there were more patients with higher dysphagia intensity in this group at baseline (19% vs. no patients with grade III dysphagia). More patients receiving modified FLEP regimen as compared with the standard regimen group demonstrated decrease (60% vs. 48%) or no change (40% vs. 26%) in disease extent by x-ray after chemotherapy completion. Complete responses (20% of cases) were shown in the modified regimen group only, and no patients had progressive disease vs. 26% of cases with progressive disease in the standard regimen group. And finally, more patients receiving modified FLEP survived 1 year of follow-up (79% vs. 65%).
Conclusion: Although these findings are but interim, we nevertheless believe that modification of cisplatin administration schedule alone may improved response to chemotherapy even in this very serious and a priori resistant patient category.
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503 POSTER

ABCG2 transporter gene expression in childhood rhabdomyosarcoma

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Background: Multidrug resistance (MDR) to cytotoxic drugs can be caused by increased expression of one or multiple genes belonging to ATP-binding cassette (ABC) superfamily, which function as drug efflux transporters. In childhood rhabdomyosarcoma (RMS), ABCB1 (MDR1) and ABCC1 (MRP1) genes have been shown to be expressed and their role in determining MDR and therapeutic failure has been described. ABCG2 (BCRP) is the third ABC gene primarily related to MDR. This study was aimed at investigating if this gene is expressed in childhood RMS and the possible associations with clinicopathological features.
Materials and Methods: Primary tumor samples were obtained and snap frozen from 26 pts (14 male/12 female), aged 5–183 months (median, 59), with newly diagnosed RMS. Primary site was favourable (orbit and genitourinary non-bladder/prostate) in 5 pts and unfavourable (head and neck parameningeal and non-parameningeal, genitourinary bladder or prostate, extremity and others) in 21. Pts were staged according to the IRS post-surgical grouping system and assigned as group I (n. 2), II (n. 6), III (n. 16) or IV (n. 2). Histological subtype was embryonal in 20 pts and alveolar in 6. ABCG2 mRNA expression in RMS samples, normal skeletal muscle (constitutive low expression) and normal ovary (constitutive high expression) obtained from healthy voluntary donors (5 for each tissue) was measured by quantitative real-time PCR. Institutional written informed consent from the patient's parents and ethical approval according to local institutional guidelines were obtained.
Results: ABCG2 mRNA levels significantly higher than the mean level in normal skeletal muscle were found in all 26 RMSs, with 9/26 (35%) tumors expressing high levels, i.e., levels in the range ($\pm$20%) of the mean level in normal ovary. No associations between ABCG2 mRNA levels and well-established clinicopathological features such as age at diagnosis, sex, primary site, and size of primary were demonstrated. A non significant trend was identified for tumors with high levels of ABCG2 expression to have embryonal histology: 8/20 (40%) of embryonal cases vs. 1/6 (17%) of alveolar cases ($p = 0.7$).
Conclusions: ABCG2 mRNA expression in childhood RMS is widely increased compared to its normal counterpart, with a substantial part of tumors expressing high levels, i.e., levels physiologically significant. The role of ABCG2 in determining MDR in RMS deserves further investigations in a larger series.

Monoclonal antibodies and targeted toxins/nuclides

504 POSTER

GA101, a therapeutic glycoengineered CD20 antibody recognizing a type II epitope mediates outstanding anti-tumor efficacy in Non-Hodgkin lymphoma xenograft models and superior B cell depletion

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Background: GA101 is the first humanized, glycoengineered CD20 antibody recognizing a type II epitope. GA101 was derived by humanization of the murine B-Ly1 antibody and is characterized by high binding affinity, type II mode of CD20 binding with reduced CDC but strong direct cell death induction compared to classical type I CD20 antibodies. The glycoengineered Fc region binds with enhanced affinity to FcgRIIIa on immune effector cells leading to enhanced ADCC.
Material and Methods: We studied the dose-dependent effects of GA101 on the growth of s.c. and orthotopic NHL xenografts in SCID beige mice; both as single agent and in combination with chemotherapeutic agents and Bcl-2 inhibitors in direct comparison to rituximab. Depletion of non-malignant B cells was studied in hCD20 transgenic mice and in cynomolgus monkeys.
Results: In various NHL models GA101 demonstrated outstanding anti-tumor efficacy. Specifically, complete tumor remission was induced in SU-DHL4 DLBCL xenografts. By contrast, rituximab at equal or higher doses was only able to slow down tumor progression. Treatment with GA101 increased the median and overall survival in an orthotopic disseminated Z138 MCL model compared to rituximab. Combination studies showed that GA101 works in a synergistic and superior manner in combination with chemotherapeutic agents such as vincristine or cyclophosphamide as well as with novel targeted therapeutic agents such as Bcl2 inhibitors. In hCD20 transgenic mice, GA101 demonstrated superior depth of B cell depletion. The increased B cell depletion extended into the peripheral lymphoid compartments and to the range of B cell subsets targeted. Analogous findings were observed in cynomolgus monkeys where the efficacy of GA101 in depleting B cells in lymphoid tissues was compared with that of non-glycoengineered GA101 and rituximab. These studies showed that the enhanced anti-tumor efficacy and depth of depletion observed with GA101 treatment is influenced by its unique binding mode and the induction of CD20-dependent cell death.
Conclusions: In summary, the data demonstrate that GA101 represents a novel class of CD20 antibodies with outstanding efficacy compared to classical type I CD20 antibodies. GA101 is currently in Ph I clinical trials. It is anticipated that the combination of the type II epitope recognition with improved ADCC potency exclusive to GA101 will translate into superior clinical efficacy establishing GA101 as best in class anti-CD20 therapy.

505 POSTER

Preliminary results of a phase II clinical trial of the anti EGFR monoclonal antibody Nimotuzumab in combination with whole brain radiation therapy in patients diagnosed with advanced non-small cell lung cancer tumors unresectable brain metastases

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Brain metastases are the most common intracranial tumor of adults. Lung cancer is the main primary tumor given rise to brain metastases.