including malignant and non-malignant lung tissue, obtained surgically or at bronchoscopy, and blood.
Materials and Methods: A tissue collection team was established comprising thoracic surgeons, pathologists and the laboratory research team. Project implementation required (i) approval from local Ethics and Hospital R&D Committees; (ii) preparation and implementation of standard operating procedures (SOPs) for tissue collection, processing and storage; and (iii) the establishment of dedicated databases for recording clinical and pathological patient data. Donors were selected on the basis of having a radiological lung abnormality; planned rigid bronchoscopy or lung surgery; age $\geqslant$18 years; and written informed consent. Patients were not excluded if clinical suspicion of malignant histology was low. Surgical tissue samples were snap frozen, bronchial biopsies frozen in OCT medium, and blood samples fractionated, buffy coat and plasma separated and then frozen. Frozen material was cryopreserved at −80ºC pending molecular analysis.
Results: Over a 12 month period we have collected samples from 81 donors whose characteristics include: M/F, 54/46%, median age 64 years, current or former smokers 88%, previous asbestos exposure 12%. In 60% of donors malignancy was confirmed, including NSCLC 45%, metastatic colorectal cancer 5% and carcinoid 2%; benign conditions included pleural fibrosis 5%, adenochondroma 2%, and sarcoidosis 2%. Lung tissue, bronchial biopsies and peripheral blood have been collected from 48%, 68% and 88% of donors, respectively. Twenty-one lung cancer tissue specimens and paired non-cancerous lung tissue specimens or peripheral blood have been collected so far. No adverse events were associated with the study procedures.
Conclusions: We have established a successful lung tissue bank. Challenges that had to be overcome included obtaining consent in busy clinical environments, co-ordination of sample collection with changes in theater lists and outside normal working hours, and the need for a designated pathologist to process tissue samples. A designated person to act as tissue collector, and good links with clinicians, pathologists and operating theater staff were identified as vital to the success of establishing a tissue repository.

1118 POSTER

Diet-induced obesity modulates signaling through the Akt/mTOR pathway in colon and colon cancer xenografts

M.B. Flores[1], G.Z. Rocha[1], M.M. Dias[1], J.A. Saad[1], J.B. Carvalheira[1]. [1]*Faculty of Medical Science – Unicamp, Internal Medicine, Campinas, Brazil*

Obesity has recently been linked to mortality from the majority of cancers. For colon cancer, in particular, several epidemiological studies have supported the concept that high energy intake, obesity, and/or hyperinsulinemia are the major risk factors for its incidence and severity. However, the exact molecular mechanism linking obesity and colon cancer is not fully understood. Insulin, via activation of insulin receptors expressed ubiquitously in normal and neoplastic cells as well as IRS-1, Akt, mTOR and p70 activation may enhance the anabolic state necessary for cell growth. By the other hand, markers of chronic inflammation like IKKβ and COX-2 are in the centre of colon carcinogenesis by promoting cell proliferation, inhibiting apoptosis and stimulating angiogenesis.
Western blotting assay was performed to evaluate IR, IRS-1, Akt, mTOR, p70S6K, COX2 and IKKβ in colon from diet-induced obesity (DIO) wistar rats and *ob*/*ob* mice as well as tumor extract from DIO SCID mice xenografted with HT-29 colon cancer cells.
Insulin-stimulated phosphorylation of IR, IRS-1, Akt, mTOR and p70S6K in colon tissues were enhanced in diet induced obesity (DIO) rats and *ob*/*ob* mice compared with lean counterparties. In contrast, the insulin signaling pathway demonstrated reduced phosphorylation rates in the muscle of insulin stimulated DIO and ob/ob animals compared to lean controls. DIO increased the activation of mTOR pathway in vivo and induced tumor growth in colon cancer cell xenografts. We also observed an increased in protein expression of COX-2 and phosphorylation of IKKβ in the colon or colon cancer xenografts extracts from obese animals compared to that observed in lean controls.
This study provides direct measurements of insulin signaling in colon and colon cancer xenografts, and documents an increased sensitivity to insulin, despite the activation of inflammatory signaling pathways, in colon and colon cancer xenografts.

Drug development − Preclinical and phase I

Oral presentations (Wed, 23 Sep, 09:00−11:00)
Drug development − Preclinical and phase I

1200 ORAL

Drug responses and predictive markers for sensitivity in colorectal cancer cell lines

K. Bracht[1], A.M. Nicholls[1], O. Dikomitou[1], J. Wilding[1], W.F. Bodmer[1]. [1]*Weatherall Institute of Molecular Medicine, Cancer Immunogenetics, Oxford, United Kingdom*

Background: Colorectal cancer (CRC) is one of the main causes of death from malignant disease and new strategies have to be found to target therapy better to patients. This study uses a large panel of 85 CRC cell lines and correlation analysis to detect markers for drug response and to elucidate the mechanism of action of drugs.
Material and Methods: Drug response is tested using serial dilutions of various drugs in a 96-well format and assayed using the SRB method. An algorithm is used to group cell lines objectively into clearly distinct categories of response. Subsequently, correlations with genetic, epigenetic and protein expression data are detected by statistical analysis to find significant associations. Combination treatment with different drugs as well as 3D assays, InCell Western and Western blotting are used for further investigation.
Results: We were able to find both well known and new associations. For example, response to 5-fluorouracil (5-FU) correlates with replication error (RER) status and mutations in kras and TGFβRII: insensitive cell lines tend to be RER positive (replication error defective) ($p = 0.0027$, Fisher's Exact test) and to have mutations in those two genes ($p = 0.0173$ and 0.0198, respectively). Sensitivity against a novel, specific MEK1/2 inhibitor (MEKi; GSK1120212) was found to be associated with mRNA levels and the degree of methylation of LY75 (CD205): resistant cell lines show high levels of methylation of LY75 and in concordance with this, very low message levels. But response to MEKi does not correlate with the degree of inhibition of Erk phosphorylation, its direct downstream target in the MAP kinase pathway. This suggests that resistance is caused further downstream in the pathway or by an altogether different mechanism.
Conclusions: CRC cell lines show differential responses to a variety of drugs. These correlate with other cell line characteristics and could therefore be used as predictive markers for a certain cell line/drug combination. Large cell line panels as used for this study prove to be powerful tools for finding associations, as demonstrated by the confirmation of clinical data published for 5-FU.

1201 ORAL

New fluorine-18 radiolabeled Cdk4/6 inhibitors: potential radiotracers for tumour imaging by positron emission tomography

F. Graf[1], L. Koehler[2], B. Mosch[1], J. Pietzsch[1]. [1]*Forschungszentrum Dresden-Rossendorf e.V. Institute of Radiopharmacy, Radiopharmaceutical Biology, Dresden, Germany;* [2]*Forschungszentrum Dresden-Rossendorf e.V. Institute of Radiopharmacy, PET Tracer, Dresden, Germany*

Background: Cyclin-dependent kinases 4 and 6 (Cdk4/6) are important components of cell cycle activation in G_1 phase and play critical roles in dysfunction of growth control during cancerogenesis. The aim of our study was the evaluation of new fluorine containing pyrido[2,3-*d*]pyrimidin-7-one derivatives (CKIC, CKID and CKIE) concerning their efficacy and suitability as Cdk4/6 inhibitors and, after fluorine-18 radiolabeling, as radiotracers for imaging of tumors by positron emission tomography (PET).
Materials and Methods: Small molecule inhibitors CKIC, CKID and CKIE were analyzed concerning their biological and radiopharmacological properties in human tumor cell lines HT-29, FaDu and THP-1. Cell cycle distribution of cells was determined by flow cytometry DNA analysis and effects on cell growth were measured. Phosphorylation of retinoblastoma protein (pRb) at Ser^{780} was analyzed by Western blotting. mRNA expression of the pRb affected genes E2F-1 and PCNA was measured with quantitative RT-PCR. Stability and radiotracer uptake studies with [^{18}F]CKIE were performed.
Results: Cell cycle analyses showed a concentration-dependent (50 nM to 10 μM) increment of percentage of tumor cells in G1 phase after 24 h of incubation with CKIC, CKID and CKIE, with CKIE to be more potent than CKIC and CKID. Cell growth studies indicated reduced tumor cell numbers after 48 h of treatment with 1 μM (<75%) and 10 μM (<30%) CKIE and 10 μM (<70%) CKIC or respectively CKID. Cdk4 specific phosphorylation at pRb-Ser^{780} is decreased in a concentration dependent manner after