HLA_DR expression was evaluated. CD86 expression is considered the most relevant CD28 ligand. Furthermore CD86 is constitutively expressed in all activated DCs, showing a faster induction and reaching higher expression.

Materials: The L1 and L2 coding sequences were PCR-amplified from a plasmid containing the whole HPV-16 genome and cloned in a pENTR vector by means of the pENTRR /SD/D-TOPOR (InVitrogen). The expression cassette containing L1 or L2 coding sequences were subcloned into the pHRLVGateway (GatewayR Invitrogen). GFP-L1 or GFP-L2 expressing plasmids were co-transfected with plasmids coding for HIV gag/pol. HekFT cells were used as packaging cell lines. Lentiviral supernatants were titrated by quantitating infection of Jurkat cells. Peripheral Blood Mononuclear Cells (PBMC) were purified by density-gradient centrifugation with Lymphocyte separation medium (Eurobio, Les Ulis, France). CD11c+ cells were purified from PBMC with CD1c (BDCA-1)+ bound magnetic beads using the Dendritic Cells Isolation Kit (Miltenyi Biotech GmbH) according to manufacturer's instructions. CD11c+ cells were seeded in 24-well culture plates at 1x105 cells/ml and stimulated with 1 μg/ml LPS (Sigma). DCs were cultured with lentiviral supernatans and transduction efficiency was evaluated by FACS analysis (Dako Cytomation). More than 60% of DCs were efficiently transduced.

Results: A further, though slight increase of CD80 + CD86, mostly due to CD86 expression, is observed when GFP-L1 or GFP-L2 expressing cells are compared to cells expressing GFP alone which could be due to the costimulatory boosting effect of HPV capside proteins. Only minor differences in HLA-DR expression in cells transduced with either GFP-L1 or GFP-L2 being even more apparent in GFP-L2 expressing cells. Thus differences in HLA-DR expression could not explain the functional defects observed in HPV infected individuals.

Conclusions: Either GFP-L1 and/or GFP-L2 infected DCs express costimulatory surface molecules and HLA-DR, showing no significant differences with control DCs expressing GFP alone.

210 Poster

Liver cancer caused by long-term infection with liver fluke, Opisthorchis viverrini in experimental hamster

J. Prasongwatana[1], N. Songserm[1]
[1]Faculty of Medicine Khon Kaen University, Parasitology, Khonkaen, Thailand

To elucidate cholangiocarcinogenesis caused by liver fluke infection, histopathology of the liver at different time point intervals up to 20 months post infection were studied in the experimental hamster infected with O.viverrini. Not only bile duct was tremendous affected during the infection but hepatocyte as well. Lymphocytic aggregation was found not uncommon in the liver section. Sclerosing cholangitis, liver fibrosis and cirrhosis were demonstrated. A small nodule of clear cell type liked hepatocellular carcinoma were found in hamster with 13 months post infection. Both benign dilated peribiliary cyst and high grade of dysplastic changes of peribiliary gland harmatoma were demonstrated. Two of infected hamsters could survived up to 20 months post infection, one was found to harbour a small solitary modules of well-differentiated intrahepatic cholangiocarcinoma. The other was found to harbour high grade dysplasia and carcinoma in situ of peribiliary gland harmatoma. Immuno-histochemical staining are needed to confirm.

Animal subjects used in this study has been obtained with permission from Animal house under regulation of Faculty of Medicine, KKU

211 Poster

Cell cycle proteins in squamous cell carcinoma of oral cavity

M. Buim[1], C.P. Nagano[2], S.V. Lourenço[2], J.H. Fregnani[3], A.L. Carvalho[4], F.A. Soares[1]
[1]Hospital A.C. Camargo, Department of Pathology, Sao Paulo, Brazil; [2] University of São Paulo, Department of General Pathology, Sao Paulo, Brazil; [3] Santa Casa de São Paulo, Morphology Department of the School of Medical Sciences, Sao Paulo, Brazil; [4] Hospital do Câncer de Barretos, Department of Head and Neck Surgery, Sao Paulo, Brazil

Background: Squamous cell carcinoma of the oral cavity (OSCC) is a common malignancy characterised by a high degree of local aggression and metastasis to cervical lymph nodes. Behaviour of this type of neoplasm is related to disturbance in several molecular cascades and cell cycle molecules play a central role. Cell cycle control is complex and involves numerous molecules. Phases of cell cycle progress aided by promoter molecules termed cyclins (A, B, E, C, D and H) and cyclin-dependent kinase (CDK). Inhibition of the cycle involves molecules called ckd-inhibitor (as p16, p21, p27, p57) and the classical proteins RB e p53. Other molecules also play essential role in critical processes of cell cycle and cell proliferation such as Ki-67 and Topoisomerase II. Alterations in these molecules are associated with poor prognosis in many human neoplasms and may be important molecular predictors of biological behaviour of OSCC. Methods: This study analysed cell-cycle related proteins - Cyclin D1, Cyclin B1, Cyclin A, p16, p21, p27, p53, Rb, Ki-67 and Topoisomerase using immunohistochemistry in tissue microarray of 136 cases of OSCC, and associated their expression with clinico-pathological features and survival rate, for predicting tumour prognosis. The results were evaluated quantitatively by the automated cellular imaging systems (ACIS III DAKO), which detects, counts, and classifies cells based on colour, shape, and size. Results: The results were compared to clinical-pathological features. Kaplan-Meier method and χ^2 tests were used for statistical analysis. Expression of Cyclin B1, Cyclin A, p16, p21, p27, RB, Ki-67 and Topoisomerase proteins had no significant association with clinical pathological parameters tested (age, sex, race, clinical stage, tobacco and alcohol consumption, histological grade, perineural invasion, vascular embolization, lymph nodes status and capsular rupture). Cyclin D1 overexpression was significantly correlated with advanced clinical stage (T3/T4) (p=0,003). Expression of p53 protein was correlated with poorly differentiated tumours (p=0,028). Significance between Cyclin D1 and p53 and other clinicopathological features was not statistically established. Tumours with p16 downregulation were related to patients with a smaller survival rate (analysis of a 10-year overall survival) (p=0,025). Conclusion: Our results suggest that overexpression of Cyclin D1 and p53 proteins and downregulation of p16 protein might be indicators of poor outcome in patients with OSCC. Supported by FAPESP.

POSTER SESSION

Radiobiology / Radiation oncology

212 Poster

PARP inhibition vs. PARP-1 silencing: different outcomes in terms of single-strand break repair

C. Godon[1], F.P. Cordelieres[2], D.S.F. Biard[3], F. Megnin-Chanet[1], J. Hall[1], V. Favaudon[1]
[1]Curie, U612 Inserm, Orsay, France; [2] Curie, Imagerie, Orsay, France; [3] CEA, IRCM-LGR DSV, Fontenay-aux-Roses, France

Poly(ADP-ribose) polymerase-1 (PARP-1) and XRCC1 are crucial effectors in the short patch repair (SPR) branch of the base excision repair pathway (BER), an essential mechanism for the repair of DNA single-strand breaks (SSBs). We have determined the consequences of PARP-1 disruption on SSB repair (SSBR) during the S phase of the cell cycle using isogenic human HeLa cells exposed to a PARP inhibitor or stably silenced for PARP-1 (PARP-1KD) or XRCC1 (XRCC1KD) gene expression. We found that both PARP-1 inhibition or silencing prevented the recruitment of XRCC1 to DNA damage sites using laser microirradiation and live cell microscopy. Strikingly, alkaline elution analysis of DNA showed that PARP-1KD or XRCC1KD cells were able to rejoin radio-induced SSBs as rapidly as control cells. These data suggest that a PARP-1- and XRCC1-independent pathway operates to repair SSBs when SPR is deficient. The long patch repair (LPR) branch of BER appears to be the likely mechanism, as PCNA recruitment at sites of DNA damage was not affected by the absence of PARP-1. In contrast, inhibition of PARP-1 in HeLa cells exposed to γ-rays in S phase, dramatically slowed down SSBR as measured by alkaline elution. In addition, PARP-1 inhibition also triggered the accumulation of a large amount of PARP-1 and PCNA at sites of microirradiation which persisted for over 20 min. It is proposed that this accumulation results in steric hindrance and slows down the recruitment of other intermediates of the BER process. Thus we demonstrated that inhibiting or silencing the PARP-1 protein has different outcomes in terms of SSBR in the S phase of the cell cycle.

213 Poster

Growth retardation and survival prolongation of experimental lung carcinoma by interstitial Ra-224 loaded wires releasing diffusing alpha-emitting atoms

T. Cooks[1], M. Efrati[1], H. Bittan[2], M. Schmidt[2], L. Arazi[3], I. Kelson[2], Y. Keisari[1]
[1]Tel Aviv University, Human Microbiology Faculty of Medicine, Tel Aviv, Israel; [2] Tel Aviv University, School of Physics and Astronomy Faculty of Exact Sciences, Tel Aviv, Israel; [3] Tel Aviv University, School of Physics and Astronomy Faculty of Exact Sciences and Althera Medical, Tel Aviv, Israel

Background & Objectives: Alpha particles are substantially more effective in cell killing than photons and electrons. However, the short range of alpha