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

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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

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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

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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

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

particles in tissue has so far precluded their use against solid tumors. We have developed a new form of brachytherapy that enables the treatment of solid tumors with alpha radiation, which was termed- Diffusing Alpha-emitters Radiation Therapy (DART). The basic idea of DART is to insert into the tumor sources loaded with ^{224}Ra atoms, which release from their surface short-lived alpha-emitting atoms. These disperse inside the tumor and deliver a lethal dose through their alpha decays. The present study examines the anti-tumoral effects resulting from the release of alpha emitting radioisotopes into solid lung carcinoma (LL/2 and A427) tumors. We assessed the efficacy of the short-lived daughters of ^{224}Ra, which are released into the malignant tissue, to produce tumor growth retardation and prolong life.

Methods: Radioactive wires (0.3 mm diameter and 5 mm long) with ^{224}Ra activities in the range 12-33 kBq were inserted into LL/2 tumors in C57BL/6 mice and into human derived A427 tumors in athymic mice. Tumor development was recorded during 21 days (LL2) or 28 days (A427) and survival was monitored for 45 days (LL2) or 120 days (A427). An in-vitro set-up tested the dose dependent killing of tumors cells exposed to alpha particles.

Results: The insertion of a single DART wire into the center of 6-7 mm (130 mm^3 average volume) tumors had a pronounced retardation effect on tumor growth in the murine model, leading to a significant inhibition of 49% (LL2) and 93% (A427) in tumor development, and prolongations of 48% (LL2) in life expectancy. These observed effects were strengthened when tumors were treated with two DART wires. In the human model more than 80% of the treated tumors disappeared or shrunk. Autoradiographic analysis of the treated sectioned tissue revealed intratumoral distribution of radioactive atoms around the wires, and histological analysis revealed corresponding areas of necrosis. In-vitro experiments demonstrated a dose-dependent killing of tumors cells exposed to alpha particles.

Conclusions: The results indicate that DART causes significant damage to lung carcinoma, and prolongs survival. DART holds great potential for the treatment of human lung cancer, and might be augmented by chemotherapy and other modalities like immunotherapy or anti growth factors.

214 Poster

Ionizing radiation fosters cancer invasion through transcriptional up-regulation of the MET oncogene

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It is debated whether ionizing radiation (IR) employed in radiotherapy could unleash tumor invasion and metastasis, as suggested by animal models. We have shown that, in epithelial and melanoma cell lines, IR induces overexpression of MET, the tyrosine kinase receptor for hepatocyte growth factor (HGF). MET is an unconventional oncogene that drives "invasive growth", a complex genetic program, which controls tissue development and regeneration in physiological conditions, but can promote metastasis in cancer cells. We have found that MET induction by IR is supported by transcription factors activated in response to DNA damage and extracellular stress stimuli. We have then shown that MET upregulation is crucial for the biological effects elicited by IR. In fact, in vitro IR induces spontaneous cell invasion that requires MET expression and kinase activity. Moreover, IR exposure sensitizes cancer cell lines to HGF, and turns the physiological response to this signal (branching morphogenesis) into disorganized cell spread and migration in tri-dimensional matrices. These findings support the notion that MET upregulation is part of a transcriptional program elicited in response to DNA damage, and likely aimed to promote cell recovery and repair. However, MET expression/activation in irradiated cancer cells can convert a healing response into an invasive program leading to cancer dissemination, thus providing a molecular explanation for the adverse effects of radiotherapy.

215 Poster

Suppression of DNA-PK effected the radiosensitivity and cell cycle of HeLa

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In response to DNA damage, cell survival can be enhanced by activation of DNA repair mechanisms and of check-points that delay cell cycle progression to allow more time for DNA repair. DNA Double-strand break (DSB) is the most lethal of all DNA lesions and is repaired by two major repair pathways: homologous recombination (HR) and nonhomologous end-joining (NHEJ). It is generally believed that NHEJ plays a more important role than HR in mitotically replicating cells especially in human cells. DNA-dependent protein kinase (DNA-PK), including Ku80, Ku70 and DNA-PK catalytic subunit (DNA-PKcs), is the most important kinase plays in NHEJ. We produced deficiencies of Ku80 by small hairpin interfering RNA (shRNA) in human cervical carcinoma cell line HeLa, before exposure to 6MV X-ray. The shRNA led to a ~96% reduction of Ku80 expression, and made the dose-reduction factor (DRF) of 10% cell survival got to 1.68; Transfection DNA-PKcs-targeted shRNA into HeLa/Ku80-shRNA gave rise to a DRF of 2.47 compared to control cells; while after pretreated with 50μM LY294002, a chemically specific phosphatidylinositol (PI) 3-kinase inhibitor (DNA-PKcs and the major HR protein ATM are both members of PI 3-kinase gene family), HeLa/Ku80-shRNA's DRF of 10% cell survival comparison to control cells even got to 3.36. As for cell cycle distribution after 6Gy X-ray exposure, just like control cells, HeLa deficiency of Ku80 singly or in combination with DNA-PKcs showed the cell accumulation in G2/M phase, and the G2/M accumulation got to vertex at 48h post-irradiation. However, HeLa cells transfected only with DNA-PKcs-targeted shRNA or pretreated with LY294002 had a prolong G2/M delay, the percentage of cells in G2/M phase even didn't get to highest at 72h post-irradiation, it suggests that the accumulation of significant unrepaired DNA damage following inhibition of DSB repair proteins. These data indicates that the role of Ku80 in DSB repair could be compensated by other DSB repair protein after its inhibition, co-inhibition of both NHEJ and HR proteins would be a suitable stratagem to enhance the radiosensitivity of cancer cells; Based of the cell cycle consequences, we presumed that DNA-PKcs and Ku80 had different contributions in cell cycle distribution, Ku80 firstly acted in cell cycle response, and then induced the participation of the DNA-PKcs, when Ku80 protein was inhibited, the HR protein would compensate completely, when DNA-PKcs was suppressed, for the "occupying" of Ku protein, neither HR nor NHEJ could act normally, resulted in the delay of cell cycle.

216 Poster

Gefitinib enhances radiosensitivity of glioma neurospheres by inhibition of Akt signalling and DNA repair mechanisms

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Background: Brain tumor stem cells (BTSCs) have recently been identified as critical for maintenance of tumor mass, contributing to chemo-radioresistance and tumor relapses. We investigated the effect of irradiation on stem-like glioma neurospheres and determined whether the tyrosine kinase inhibitor gefitinib could enhance radiosensitivity by inhibition of Akt activation and DNA repair mechanisms. Methods: Malignant glioma tissues were collected with informed consents from patients in accordance with protocols approved by the institutional review boards at National Cancer Centre, Singapore. Tissues were minced, trypsinised and cultured for growth of neurospheres and differentiated glioma cells. Neurospheres that exhibited characteristics of BTSCs (self-renewal and multipotent) were used in this study. Dose-dependent effects of irradiation (0-10 Gy) on neurospheres and differentiated glioma cells were analysed for clonogenic formation (14 days), gamma-H_2AX (marker for double-strand DNA breaks) immunostaining and CD133+ (marker for neural stem cells) cell population. Neurospheres and differentiated gliomas treated with gefitinib (5 μM), irradiation (5 Gy) or both were assayed for clonogenic formation, Akt phosphorylation (Ser 473) and DNA-PK complex formation. Results: Irradiation did not affect clonogenic survival and gamma-H_2AX immunostaining of glioma neurospheres, but significantly reduced clonogenic survival and increased gamma-H_2AX immunostaining of differentiated glioma cells in a dose-dependent manner. Irradiation significantly increased CD133+ cell population in neurospheres, but not in differentiated glioma cells. Gefitinib significantly inhibited clonogenic survival of irradiated neurospheres, but not differentiated glioma cells. Akt phosphorylation was inhibited by gefitinib alone and when combined with irradiation in neurospheres, but not in differentiated glioma cells. Irradiation slightly inhibited DNA-PK complex formation in neurospheres, with further inhibition by combined gefitinib and irradiation. In differentiated glioma cells, irradiation enhanced DNA-PK complex formation with no further effect by combined gefitinib and irradiation. Conclusions: Stem-like glioma neurospheres are resistant to irradiation-induced DNA damage, with greater clonogenic survival than irradiated differentiated glioma cells. Gefitinib enhanced radiosensitivity of stem-like glioma neurospheres by inhibition of Akt activation and DNA repair mechanisms.