224 Poster

Hypoxia strongly upregulate the expression of EGFRvIII in glioma cells

Y. Li[1], N. Lieuwes[1], B. Jutten[1], R. Chiu[1], J. Theys[1], M. Koritzinsky[1], P. Lambin[1], B.G. Wouters[1], G. Lammering[2]
[1]Maastricht University, Radiation Oncology, Maastricht, The Netherlands; [2] Maastro Clinic, Radiation Oncology, Maastricht, The Netherlands

Introduction: Lots of scientific literatures suggest frequent existence and clinical importance of an extra cellular deletion variant of EGFR (EGFRvIII) in a variety of human tumor. The recent data also show using hypoxia inducible factor1 alpha as indicator of tumor responses to EGFR-targeted therapy in preclinical studies and in the clinical setting is expected. Previous work has identified the detection of EGFRvIII in vivo and not in the corresponding cells under in vitro conditions. Then we have therefore hypothesized that its expression might be regulated by the microenvironment.

Materials and Methods: The human glioma cell line (U373 MG) was used and genetically modified to stably express EGFRvIII or a control vector. Cells were grown in a hypoxia chamber under acute anoxic conditions following a prolonged time course of in total 24 hours. Every 6 hours, EGFR and EGFRvIII protein and mRNA expression levels were monitored on Western blots and qRT-PCR, respectively. Phospho- immunoblots quantified the phosphorylation levels of EGFR and its downstream effectors Akt and Erk. Finally, clonogenic survival assays were performed at different time points daily starting after 24 hours anoxia to a maximum of 4 days. Survival fractions were calculated after correction for plating efficiency under normoxic conditions.

Results: The protein but not the mRNA expression levels of EGFRvIII significantly increased under hypoxic conditions compared to EGFR by at least 4 fold. Interestingly, basal phosphorylation levels of EGFRvIII behave differently according to different phosphorylated sites. In line with controls, p-ERK levels continuously decreased during the different time points of hypoxia treatment. In contrast, however, p- Akt levels increased, suggesting a survival benefit for cells with an induced EGFRvIII expression under hypoxia.

Conclusion: This work indicates that EGFRvIII is an important modulator of radiation responses in vivo. In addition, the results suggest an important role for hypoxia in the regulation of EGFRvIII expression in vivo. If this proves to be correct, it would dramatically improve our understanding of the importance of EGFR in radiation responses, since hypoxia is known to negatively influence radiosensitivity in tumor cells.

225 Poster

Radiosensitization and cell cycle phases distribution induced by paclitaxel in human fibrosarcoma HT1080 cell line in vitro

R. Moleron[1], J. Romero[1], I. Zapata[1], A. De la Torre[1], M.J. Citores[2], J.A. Vargas[2], R. Sanchez-Prieto[3]
[1]Hospital Universitario Puerta de Hierro, Oncologia Radioterapica, Madrid, Spain; [2] Hospital Universitario Puerta de Hierro, Medicina Interna, Madrid, Spain; [3] Univ Castilla-La Mancha, Centro Regional de Investigacion Biomedica, Albacete, Spain

BACKGROUND: Recent studies suggest that taxanes cuold be useful in sarcoma treatment. As taxanes are well known radiosensitizers, this study evaluates in vitro radiosensitization by paclitaxel-cremophor in human fibrosarcoma cell line HT1080.

MATERIALS AND METHODS: HT1080 cells were grown in 10% FBS supplemented DMEM. After plating, paclitaxel-cremophor at different concentrations was added to treated wells of a 24-wells plate. After 24 hours, cell cultures were irradiated at 0, 2, 4 and 6Gy and left to grow for 10 days. Citotoxicity was evaluated by the crystal violet method. Distribution in cell cycle and apoptosis was evaluated by DNA stain with propidium iodide and flow citometry analisys.

RESULTS: HT1080 cells were sensitive to paclitaxel with an IC50 range of 4 to 10nM. Dose enhancement ratio (DER) at 0,25 survival level was 1,38; 1,91 and 4,1 for 1, 5 and 7nM drug concentrations, respectively. A marked arrest in G2-M cell cycle phase was observed in treated cells (24% vs. 90,84% for control and treated cells after 12 hours). A moderate amount of apoptosis (23,7%) was observed after 36 hours exposure.

CONCLUSIONS: Paclitaxel radiosensitizes human fibrosarcoma HT1080 cells in vitro by a G2-M cell cycle arrest.

226 Poster

A clinical study for the adjuvant brachytherapy in the treatment of soft tissue sarcomas

K. Takeda[1], F. Nakatani[1], M. Miyagi[1], E. Kobayashi[1], Y. Suehara[1], A. Kawai[1], H. Chuman[1], Y. Beppu[1], M. Sumi[2], H. Mayahara[2]
[1]National Cancer Center, Orthopaedic Surgery, Tokyo, Japan; [2] National Cancer Center, Radiation Oncology, Tokyo, Japan

Background: Soft tissue sarcomas (STSs) bear a risk for local recurrences, especially in high-grade sarcomas. Brachytherapy (BRT) has been used as a tool to obtain better local control in the treatment of STSs. We examined the treatment outcome for STSs using adjuvant interstitial BRT in our single institution retrospectively.

Materials and methods: Twenty-five patients were treated from1994 to 2007 with adjuvant BRT (16 male, 9 female; median age, 51 years). Median follow-up was 37 months (5-146 months). Fifteen patients were treated as primary disease and ten as recurrent. Eleven patients had tumors in lower extremities, 8 patients in upper extremities, and 6 patients in trunk. Histologically, 19 patients suffered high-grade sarcomas, and 6 patients had low-grade sarcomas. Fifteen patients were operated with musculocutaneous flap reconstructions including 11 free flaps after excisions of the tumors. Eight patients were treated with various types of neo-adjuvant chemotherapies. Four patients were pre-treated with neo-adjuvant radiotherapy before the operations. We placed applicators for BRTs in the tumor bed with the cooperation of radiotherapists. The dose of BRT was 6 Gy per fraction, and the frequency was two times a day. The total dose of BRTs ranged from 30 to 42 Gy.

The overall survival rate was 76.3% and 55.5% at 3 and 5 years. Local relapses were observed in 5 patients. The overall local control rate was 87.5% and 75.3% at 1 and 5 year. Wide surgical margins were achieved in 6 patients, marginal margins in 11 patients, intralesional margins in 8 patients. Eight patients experienced complications after BRTs, including infection of surgical site, seroma, delayed wound healing, hematoma, bleeding from pedicle vessels of free musculocutaneus flap and fracture of radiated site. We performed additional surgeries for two cases for the complication.

Conclusions: In our series, we have performed limb sparing surgery in the relatively challenging cases. However, the local control rate after BRTs was almost similar to the previous reports. All of the musculocutaneous flaps were successfully adopted after BRTs, except for one case, which suffered rupture of sutured pedicle vessel. This ruptured case might be associated with fibrosis of surrounding tissues after the BRT, and suggested the need to consider the location of applicators for BRTs.

227 Poster

Neoadjuvant chemotherapy followed by concurrent chemo-radiation therapy in patients with locally advanced nasopharyngeal carcinoma preliminary results of 21 patients

R. Djekkoun[1], N. Ferdi[1], K. Boudaoud[1], B. Boudraa[1], W. Boughrara[1], S. Taleb[1]
[1]Centre Hospitalier Universitaire Benbadis, Oncology-Radiotherapy, Constantine, Algeria

Purpose: To evaluate the feasibility and toxicity of neoadjuvant Cisplatinum and Doxorubicin Chemotherapy (CT) followed by a concurrent Cisplatinum Chemo-Radiation Therapy (CRT)

In patients with locally advanced Nasopharyngeal Carcinoma (NPC).

Methods and Materials: Twenty one patients with locally advanced NPC, and without distant metastasis, were treated at the Benbadis University Hospital of Constantine, between January 2006 and June 2007, by neoadjuvant Cisplatinum and Doxorubicin CT followed by Cisplatinum CRT. The median age of patients was 39.8 years range (16-55 years), the sex-ratio was 2.0 (14 male and 7 female). 17 pts (81.0%) had stage III and IV according to the 1997 International Union Against Cancer/American Joint Committee on Cancer classification system. 13 pts (61.9%) had cervical lymph node involvement (N2/N3). Histopathologically, all patients had undifferentiated NPC type. Patients underwent three cycles of induction neoadjuvant CT with Cisplatinum 100 mg/m2 and Doxorubicin 60 mg/m2 on days 1, 21 and 42, followed by a locoregional hyper-fractionated radiotherapy (1.6 Gy twice a day with an interval of 6 hours), with 4 cycles of concurrent weekly Cisplatinum 40mg/m2 on days 1, 8, 29, and 36 of radiotherapy. The total dose to the primary tumor and involved nodes was 70.4 Gy and to the supraclavicular nodes was 45 Gy.

Results: 19 pts completed the scheduled treatment and 2 pts had voluntary stopped the treatment. The response to treatment was evaluated 2 months after the completion of therapy. Objective response (OR) rate was 90.5% (19pts), with 52.4% (11pts) of complete response (CR) and 38.1% (8pts) of partial response (PR). At a median follow-up of 13 months (5-18), 5 pts (23.8%) had failed treatment: one patient (with T4 primary) had a locally relapse after 12 months and 4 pts (all with N3 nodes) developed distant metastases. The median time for first relapse was 11 months (8-16). All patients completed the four concurrent cycles of Cisplatinum. However, 3 of them required the delay of the cycles due to toxicity. Acute Grade 3 and 4 reactions were observed during CRT: leukopenia 42.8% and 4.7%, infection in 14.3% and 0%, mucositis in 66.7% and 4.7%, and skin reaction in 47.6% and 4.7%, respectively. Weight loss was observed in all patients with a median loss of 6.8 kg (4-10 kg) after the completion of the treatment.

Conclusions: Preliminary results show that neoadjuvant CT followed by concurrent CRT is a safe and effective regimen of treatment for locally

advanced NPC. However, the higher acute toxicity needs the use of conformal radiotherapy technique to reduce this toxicity. Longer follow-up and further investigations are required to evaluate this regimen.

228 Poster

Nasopharyngeal carcinoma in children and adolescents - 15 years experience at the benbadis hospital of constantine - long-term results of 284 patients

R. Djekkoun[1], K. Boudaoud[1], M.S. Bali[1], N. Ferdi[1], W. Boughrara[1], B. Boudraa[1], S. Taleb[1], A. Djemaa[1], T. Filali[1]
[1]Centre Hospitalier Universitaire Benbadis, Oncology-Radiotherapy, Constantine, Algeria

Purpose: To report a retrospective analysis of epidemiologic, clinical, and therapeutic aspects of 284 children and adolescents with newly diagnosed nasopharyngeal carcinoma (NPC), and to evaluate the efficacy and toxicity of a sequential neoadjuvant Chemotherapy (CT) and bifractionated Radiotherapy (RT) regimen.

Methods and Materials: Between January 1990 and December 2004, 284 consecutive, previously untreated, children and adolescents less or equal to 20 years old were diagnosed with NPC and treated at the Benbadis University Hospital of Constantine. The median age was 14.6 years (6-20). The sex-ratio was 2.02 (190 males and 94 females). After excluding patients presenting with distant metastases (27 pts), 257 pts were identified as having only primary locoregional disease and they serve as the study population. 221 pts (86%) had locally advanced primaries (T3/T4) and 178 pts (69%) with cervical lymph node involvement (N2/N3). Histopathologically, undifferentiated type predominates, representing 96% of cases. All patients received neoadjuvant CT, 234 of them (91%) were treated combining Cisplatin and Epirubicin, 3 or 4 cycles every 21 days. The CT was followed by a loco-regional bifractionated external beam RT (1.6 Gy twice a day). The total radiation dose for the primary tumor was 70.4 Gy and for uninvolved regions was 45 Gy. All patients completed the scheduled treatment. Evaluation was repeated at completion of CT and of therapy.

Results: The objective response rate (OR) after neoadjuvant CT at the primary site was 95.7% (246 pts), with a 44.7% (115 pts) of complete response (CR) rate, 51% (131 pts) of partial response (PR) and 11 pts (4.3%) did not respond or progressed during CT. At completion of therapy, CR was recorded in 218 pts (84.8%), PR in 31 pts (12.1%) and progression in 8 pts (3.1%). The median follow-up time was 74 months (11 – 192). Patients failed rarely locally (16.3%), but generally distantly (28.4%) as first event. The median time for first relapse was 13 months. The actuarial 5-year and 10-year overall survivals (OS) were respectively 65.3% and 54.2%, and disease-free survivals (DFS) rates were respectively 51.7% and 43.8%. OS and DFS were plotted using Kaplan Meier method. Chemotherapy-related toxicity was mycosis Grade II-III in all patients and nephrotoxicity Grade I-II occurred in 19 pts (7.4%). Different late toxicity affecting quality of life was found, especially among patients less than 13 years of age (xerostomia, skin fibrosis, trismus, dental caries, hearing loss, hypothyroidism, and growth retardation).

Conclusions: NPC in children and adolescents is diagnosed late usually in an advanced stage of disease. However, this combined modality management is quite effective and results in good outcome with a satisfactory locoregional control and overall survival. It seems to give similar results to those in adults, but perhaps with more late induced toxicity.

229 Poster

Membrane-remodeling controls the death of microvascular cells engaged by radiotherapy

C. Niaudet[1], S. Bonnaud[1], S. Gouard[1], M.H. Gaugler[1], I. Corre[1], F. Paris[1]
[1]INSERM U601, Radiobiology, Nantes, France

This study aims at correlating the biophysical and molecular pathways through which a single high-dose irradiation can engage endothelium destruction.

p38-phosphorylation, ceramide generation, membrane remodeling and apoptosis quantification were assessed on the Human Microvascular Endothelial Cell line (HMEC-1), irradiated at 15 Gy.

We show here that generation of ceramide in the membrane of HMEC-1 appears in the five minutes following 15 Gy irradiation. The apoptosis wave detected within 24 hours is decreased by use of the pharmacological inhibitors of aSMase, desipramine and monensin (respectively from 52% and 32%). This is consistent with the in-vivo protection of microvasculature offered by knocking-out aSMase.

Ceramide is well known to induce the coalescence of rafts microdomains. We detected a deep relocalisation of the raft-marker ganglioside GM1, from a scattered, discret pattern, to large areas on the cell membrane, following irradiation, and this membrane reorganisation could be mimicked by addition of exogenous ceramide or bacterial SMase, or conversely be totally prevented by addition of desipramine.

High-dose irradiation is also known to induce the death-pathway p38 in microvascular endothelial cells. A 15 Gy irradiation is indeed able to activate rapidly a durable phosphorylation of p38 in HMEC-1, detected by immunofluorescence and phospho-blot. p38 blockade by MAPK inhibitor III or sh RNA decreased radiation induced death of HMEC-1 by respectively 30% and 43%. The p38-activator anisomycin leads to a durable activation of the pathway, and a wave of apoptosis 24 hours post-exposition, confirming the critical role exerted by this pathway in microvascular apoptosis.

We finally investigated whether these two concomitant phenomena, i.e. ceramide-induced raft coalescence and p38 death-pathway activation, could be connected. Disorganization of rafts by drugs as nystatin, hindered the radiation-induced rafts coalescence, the activation of p38 and the subsequent death-induction of microvascular cells (death-decrease by 35%).

In the present work, a cell-culture model allows us to arrange the cascade of until-then disconnected events leading to microvascular cells destruction: the 15 Gy-irradiation activates ceramide generation by aSMase, which leads to a thorough reorganization at the membrane surface, through rafts coalescence. These large platforms in turn strongly activate the p38 pathway, and hence the apoptosis of the endothelial cells.

230 Poster

Carotid vessel changes and risk factors in patients receiving radiotherapy for head and neck cancers

Z. Kadehci[1], N. Dagoglu[1], A. Ucar[2], H. Oflaz[3], Y. Dizdar[1], M. Altun[1]
[1]Istanbul Medical Faculty, Radiation Oncology, Istanbul, Turkey; [2] Istanbul Medical Faculty, Radiology, Istanbul, Turkey; [3] Istanbul Medical Faculty, Cardiology, Istanbul, Turkey

AIM:Late effects of radiation on medium and large sized vessels including the carotid arteries have not been thoroughly studied.The role of interaction between radiation and known risk factors for atherosclerosis is unclear either.This study aims to evaluate the changes in carotid vessels,the interaction of these changes with risk factors and to determine the value of carotid vessel screening.MATERIAL AND METHOD:81 patients suffering from head and carcinoma and Hodgkin lymphoma and had received external radiotherapy to the cervical region between 1981-2000, were consecutively recruited for the study. The study group included patients who had received high doses (50-70Gy) and relatively low doses (30-36Gy).Results were compared with a control group of 59 volunteers. All patients and controls were questioned for risk factors of an arterial disease. Changes in bilateral carotid arteries were evaluated by doppler ultrasonography. RESULTS:68 patients received high dose (50-70Gy), and 13 low dose (30-36Gy) radiotherapy. 55 patients received concomitant chemotherapy and 19 (%23) undergone surgery. Smoking, hypertension and hypercholesterolemia were identified as risk factors. 35 patients (45%) had a history of smoking,5(7%) had hypertension and 3(5%) had hypercholesterolemia. Median arterial thickness was higher in the radiotherapy group ($p<0.0001$).Smoking was a significant risk factor($p<0.0001$),but sample sizes for hypertension and hypercholesterolemia were too small to reach a significant result. Chemotherapy was found to increase the thickness only for common carotid artery. Sample size for surgery may not be enough to reach significant difference. Radiation dose did not have a significant effect, however low dose group had only 13 patients. According to NASCET criterias, atherosclerotic plaques causing stenosis over 50% were detected in 10 patients.Three patients had advanced stenosis;one had endarterectomy prior to ultrasonographic evaluation and 2 had stent application.DISCUSSION:External radiotherapy to the cervical region caused thickening of carotid artery intima-media ($p<0.0001$). Considering,10 patients (12.3%) had stenosis and 3 necessiated theraupetic intervention,these results suggest screening with doppler ultrasonography after neck radiotherapy, especially for patients with known risk factors. This would be beneficial for early interventions or preventing possible complications. The frequency and timing of screening should be further evaluated.

231 Poster

Mitochondrial modulation of oxygen-dependent radiosensitivity in some human tumor cell lines

S. Anoopkumar-Dukie[1], G.D. Sisk[1], T. Conere[2], A. Allshire[1]
[1]University College Cork, Pharmacology & Therapeutics, Cork, Ireland; [2] Cork University Hospital, Medical Physics, Cork, Ireland

Oxygen-dependent radiosensitivity of tumour cells reflects direct oxidative damage to DNA but non-nuclear mechanisms including signaling pathways may also contribute. Mitochondria are likely candidates because not only do they integrate signals from each of the main kinase pathways but