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.

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

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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.

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

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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.

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

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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.

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

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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

mitochondrial kinases responsive to oxidative stress communicate to the rest of the cell.

Using pharmacological and immunochemical methods we tested the role of mitochondrial permeability transition (MPT) and the Bcl-2 proteins in oxygen-dependent radiosensitivity. Treated or untreated cervical cancer HeLa, breast cancer MCF-7 and melanoma MeWo cell lines were irradiated at 6.2 Gy under normoxic and hypoxic conditions (<0.2% O2 x 1h) then allowed to proliferate for seven days. Reduction of resazurin to resorufin was used as an index of cell growth.

MPT blocker cyclosporin A (2μM) strongly protected HeLa but not the other two lines against oxygen-dependent radiosensitivity. By contrast, bongkrekic acid (50μM) had only marginal effect and calcineurin inhibitor FK-506 (0.1 μM) had none. Nor was evidence found for MPT modulation by Bax/Bcl-2 signaling, mitoKATP channels or mitochondrial Ca2+ uptake.

Calcineurin-independent protection by cyclosporin A suggests that MPT but not mitoKATP or the mitochondrial apoptosis pathway plays a causal role in oxygen-dependent radiosensitivity of HeLa cells. Targeting MPT may therefore improve the effectiveness of radiotherapy in some solid tumours.

We gratefully acknowledge Aid Cancer Treatment, Cork, for financial support.

07 July 2008 08:00 - 08:50

EDUCATIONAL LECTURE

Angiogenesis / Hypoxia

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The von Hippel-Lindau protein: insights into hypoxic signaling and cancer

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Inactivation of von Hippel-Lindau (VHL) tumor suppressor gene plays an important role in clear cell renal carcinoma, hemangioblastoma, pheochromocytoma, as well as some other tumors. Individuals with germline VHL mutations (VHL disease) are at increased risk for these tumors in an allele-specific manner (genotype-phenotype correlation). The VHL gene product (pVHL) has multiple functions including serving as the substrate recognition subunit of an E3 ubiquitin ligase that targets the alpha subunits of the heterodimeric transcription factor HIF (Hypoxia-inducible Factor) for destruction. HIFα must be hydroxylated on one (or both) of two conserved prolyl residues by members of the EglN family (also called PHD or HPH family), which are oxygen-dependent enzymes that also require reduced iron, 2-oxoglutarate, and ascorbic acid, in order to bind to pVHL. Under low oxygen conditions, or in cells lacking wild-type pVHL, HIFα accumulates and activates 100-200 genes involved in adaptation to hypoxia. Deregulation of HIFα (especially HIF2α) appears to play a causal role in clear cell renal carcinoma and almost certainly contributes to the development of hemangioblastomas, which are blood vessel tumors. Loss of pVHL might explain why clear cell renal carcinomas are high angiogenic, overproduce the HIF-responsive gene product VEGF, and are particularly sensitive (among solid tumors) to VEGF inhibitors. We are also conducting "synthetic lethal" screens in search of kinases that are particularly important for the survival of VHL-/- tumor cells compared to pVHL-proficient cells. In theory inhibitors of such kinases would kill VHL-/- tumor cells while sparing normal cells. In addition, we are using high density SNP arrays, gene expression profiling, and siRNA functional screens to identify mutations that cooperate with VHL loss in renal carcinogenesis. It is hoped that these studies will identify additional 'drugable' targets in renal carcinoma.

Higher metazoans, including people, have three EglN family members (EglN1, EglN2, and EglN3). We generated a conditional EglN1 mouse (EglN1-/- embryos are not viable) and confirmed cell culture experiments that suggested EglN1 is the primary HIF prolyl hydroxylase. Our recent studies suggest that EglN2 and EglN3 play roles in control of cell proliferation and apoptosis, respectively. We found, for example, that the genes that, when mutated, cause familial paraganglioma and pheochromocytoma define a pathway that is activated in sympathetic neuroblasts during embryological development by growth factor withdrawal. Interestingly, this pathway impinges upon EglN3, which is both necessary and sufficient for apoptosis in this setting. In an unbiased screen for shRNAs that confer protection against EglN3-induced apoptosis, we identified an shRNA directed against KIF1Bβ, which maps to 1p36.2. This region of the genome is frequently deleted in a variety of tumors, including neuroblastoma. Notably, this gene is also one of only 6 annotated genes located within a 500 kB homozygous deletion in a neuroblastoma line. Restoration of KIF1Bβ function in this line induces apoptosis and we have identified germline loss of function KIF1Bβ mutations in some neuroblastoma and pheochromocytoma patients, arguing that KIF1Bβ is a potential tumor suppressor gene. Preliminary data suggest that KIF1Bβ haploinsuffiency is sufficient to protect from apoptosis, which might account for the observation that many 1p deleted tumors retain a wild-type KIF1Bβ allele.

07 July 2008 08:00 - 08:50

EDUCATIONAL LECTURE

Drug targets screening

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An in vitro systems approach to predicting and understanding clinical responses to molecularly targeted therapeutics

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Background: We are developing methods that allow targeted treatment of individual cancer patients by using in vitro models of response to identify molecular signatures that predict clinical utility. Materials and Methods: We use a well-characterized panel of more than 50 breast cancer cell lines to model the clinical responses of breast cancers to molecularly targeted and traditional anti-cancer agents. The panel of cell lines reflects the substantial heterogeneity of breast cancers at the genomic and transcriptional levels. Traditional growth assays are used to assess cell line responses to individual agents and are then compared to the mutational spectra, copy number aberrations, and transcriptional profiles to identify predictors of response. These predictors can then be deployed clinically to determine which patients are likely to benefit from a given agent. Results: We have created molecular signatures that predict cell line response for more than 20 therapeutic agents including traditional chemotherapeutics (i.e. carboplatin) and targeted agents. Several of our predictors from the cell line system make implicit biological sense (i.e. ErbB2 expression level is an excellent predictor of response for ErbB2 targeting agents, or mutations in the Akt pathway are excellent predictors of response for at least one anti-Akt agent). We have successfully validated some of these signature in both tissue culture and clinical materials using the Quantigene gene expression platform that allows multiplex mRNA level measurements for ~100 genes from a single 10 micron tumor section and with no purification of RNA. Conclusion: The process of identifying patients who might benefit from particular therapeutic regimens is unlikely to be solved by clinical trials. Additionally, as molecularly targeted therapeutics target ever-smaller subsets of the patient population it is necessary for clinical trials to enrich for patients that are likely to respond. In vitro systems can be effectively utilized to identify predictive signatures that can either help clinical trials achieve success or identify therapeutic regimens of approved drugs for patients.

07 July 2008 08:00 - 08:50

EDUCATIONAL LECTURE

Antibody engineering

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Vascular targeting antibodies: from the bench to the clinic

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BACKGROUND: One avenue towards the development of more selective anti-cancer drugs consists in the targeted delivery of bioactive molecules (drugs, cytokines, procoagulant factors, photosensitizers, radionuclides, etc.) to the tumor environment by means of binding molecules (e.g., human antibodies) specific to tumor-associated markers. In this context, the targeted delivery of therapeutic agents to newly-formed blood vessels ("vascular targeting") is particularly attractive, because of the dependence of tumors on new blood vessels to sustain growth and invasion, and because of the accessibility of neo-vascular structures for therapeutic agents injected intravenously. MATERIALS AND METHODS: Human