influence transcriptional and/or translational efficiency or mRNA stability. Response to 5-FU also depends on TS structure and some researchers showed that variant structural forms of TS in tumour cell lines confer resistance to fluoropyrimidines. We intend to find any relationship between the polymorphisms in the 5′ and 3′ UTR with the TS levels and to analyze the whole coding regions of the TS gene.
Materials and Methods: We performed the TS-DNA gene sequence in 68 colorectal cancer (CRC) samples from patients of different Dukes' stages (A, B, C). The 5′ UTR polvmorphism was evaluated amplifying DNA by PCR, amplification products were electrophoresed in 3% agarose gel. Products of 116 bp (2R/2R), and 144 bp (3R/3R) or both (2R/3R), depending on the TS genotype were obtained. The 3′ UTR analysis was carried out by RFLP. The quantification of TS expression, was obtained by Light Cycler- TS mRNA quantification Kit (Roche). The sequence of the exons was performed amplified every exon by PCR and sequencing them by the SequiTherm EXCEL II DNA sequencing kit on a LI-COR sequencer.
Results: Significantly higher values of TS mRNA were found in the 3R/3R group and 2R/3R group compared with 2R/2R respectively. No significant association was found for the polymorphism of the 3′UTR and the TS mRNA levels. The sequencing of the 7 exons of the gene did not show any mutation.
Conclusions: 5-FU improves survival in a subgroup of CRC patients but predictive markers are required to identify patients that benefit from such treatment. Low intratumoral TS levels are associated with a good response to chemotherapy, so patients with the genotype 2R/2R, associated with lower TS levels, could have a better prognosis and a better response to 5-Fu. Because of missing mutation in the TS exons we intend to widen our study to D Dukes' metastatic CRCs that in spite of their high genomic instability could present mutations explaining their frequent 5-Fu drug resistance, and a worse prognosis.

400 POSTER

Measurements of residual radiation spectra from neutron activation in a medical linear accelerator

D. Schifter[1], T. Djamil[2], T. Schory[3]. *[1]Tel aviv Sackler Medical Center, Radiation Oncology, Tel-Aviv, Israel; [2]Cleveland Clinic Foundation, Radiation Oncology, Cleveland, USA; [3]Navotek Ltd., R&D, Yokneam, Israel*

Background: Linear accelerators are known to produce neutrons by the atomic reaction (g,n). It is of an interest to measure the residual spectra of activated materials in the treatment rooms.
Method and Materials: Measurements were done in two treatment rooms in two clinical facilities. The accelerators used were both Elekta Synergy S units with beam modulators. The measurements were done with 6 MV and 18 MV photon energies on one accelerator and with 6 MV, 10 MV, and 15 MV on the other. A collimated scintillation BGO detector (Bismuth Germinate or Bi4Ge3O12) was used. The detector is connected to a Multi-Channel Analyzer (MCA), which is in turn connected to a laptop equipped with an QtmMCA software. The detector was calibrated using Co57, Ba133, Cs137 sources. An Ir192 was placed in the detection area for reference.
Results: For both accelerators, there was no observable change in the spectra before and after the 6 MV irradiation. However, following the 10 MV, 15 MV, and 18 MV, residual radiation was detected. For 10 MV, there was an activation peak with half life of about 134 seconds. For 15 MV, the peak half life was about 114 seconds. For 18 MV, there was a significant peak at about 150 KeV with half life of about 145 sec. The suspected isotope is one of the following: Sr79, Fr87, Cd48, Pm61, Pr59, Os66. Another peak was about 1.5 MeV, which probably belongs to Al28.
Conclusion: There is no significant activation from 6 MV photon beam. There is a significant activation following 10 MV, 15 MV, and 18 MV beams with relatively long half live, 134 sec, 114 sec, and 145 sec, respectively. It should be taken into consideration when department policies are written. When using mixed beams set up, it should be preferred to treat the high energy beams first.

401 POSTER

Methylation and chromosomal losses in saquamous cell carcinoma of the head and neck

Y.H. Joo, D.I. Sun, J.H. Cho, Y.H. Park, K.J. Cho, S.H. Cho. *The Catholic university of Korea, Deaprtment of Otolaryngology-HNS, Seoul, South Korea*

Background: DNA methylation is essential for normal differentiation and development and aberrant DNA promoter methylation is a common feature of many human cancers. Another thing is unilateral chromosomal losses important role in Head and Neck cancer. We investigate the extent of chromosomal losses and the status of CpG methylation in Head and Neck cancer in relation with the clinicopathologic factors.
Material and Methods: Normal mucosa and tumor tissue 17 cases were examined with a methylation-specific PCR on 15 cancer-linked genes and a total of 29 cases were examined with a PCR-based loss of heterozygosity (LOH) analysis using a panel of 41 microsatellite markers on 8 chromosomes.
Results: The pattern of methylation changes between the paired normal mucosa and tumor site was variable the total of 206 cases examined for the methylation status of non-CpG island showed 34 cases hypomethylation changes, 26 cases hypermethylation changes, 31 cases no methylation changes and CpG island showed 8 cases hypomethylation changes, 17 cases hypermethylation changes, 31 cases no methylation changes. The degree of methylation changes showed a tendency to cluster in a range of U1 and M1 low-grade changes. As a result of the relation between methylation changes and clinicopathologic factors, non-CpG island in several genes mainly showed hypermethylation but CpG island in several genes rarely showed hypermethylation. Furthermore, relation between methylation and lymph node invasion, in the event of lymph node invasion, p16 stream 0.7 kbp, p16 upstream 1.0 kbp, hMLH1 upstream 1.0kbp showed hypomethylation and BGLAP upstream 4.5 kbp, Runx3 upstream 1.7 kbp, KIAA downstream 0.4kbp showed hypermethylation but the rest of the genes were not changed.
In 29 tumor foci, a LOH was found most frequently on chromosome 3p, 8p, 9p, 13q. Chromosomal loss and yielded an overall mean value of 4.79±2.2 per tumor focus.
Conclusions: The head and neck cancer and its progression generally need the proper level of chromosomal losses were accomplished cancer progression or development but over the level of changed could not affect cancer progression. This study showed that methylation pattern and LOH might be important rules and target event in head and neck cancer. From now on there will be a experiment about finding a point of the genetic modification and find the way to prevent the cancer.

402 POSTER

Bioengineering reconstruction of the upper respiratory tracts in rabbits

I. Reshetov[1], V.I. Chissov[1], E.V. Batuchtina[1], A.V. Vasiljev[2], O.S. Rogovaya[2]. *[1]P.A. Hertzen Moscow Research Oncological Institute, Microsurgery, Moscow, Russian Federation; [2]Koltzov Institute of Developmental Biology, Tissue Biology, Moscow, Russian Federation*

Background: The Objective of this study is: To perform the reconstruction of the upper respiratory tract using tissue-equivalent. The tissue-equivalent includes cellular culture of fibroblasts and of epidermic keratinocytes attached to the polymer biocompatible net.
Materials and Methods: For the reconstruction of the upper respiratory tract was used the bioengineering transplant. Transposed muscular flaps are used as the basis for implantation of stem-cells. Mucous membrane is restored by means of a tissue-equivalent. The tissue-equivalent includes allogen cellular culture of fibroblasts and of epidermic keratinocytes attached to the polymer biocompatible net. 10 rabbits were treated by this method.
Results were compared with a control group that for the reconstruction of the defect upper respiratory tract using a only sternocleidomastoid muscle. Wound healing and the epithelization of muscular flap were compared between groups.
Results: All reconstructed animals survived the postoperative period. After 2 weeks of the muscular flap was epithelized to 50% in the experimental group of the animals, to 20% in the control group. After 4 weeks muscular flap was completely epithelized in both groups of the animals. A morphological study showed the presence allogen tissue-equivalent during 30 days on the muscle after the implantation.
Conclusion: The research done covers the fundamental mechanisms of tissue reparation and practical aspects of application of tissue cellular transplants. Allogenic transplant contributes to epithelization and is capable to remain after implantation in the course of 30 days.
Protocol of experiment approved by Ethics committee.

403 POSTER

Specificity and sensitivity of point mutation detection in tumor suppressor genes via chemical cleavage of mismatches

A.A. Belyakova[1], M.G. Yakubovskaya[1], V.K. Gasanova[1], G.A. Belitsky[1], N.G. Dolinnaya[2]. *[1]Blokhin Cancer Research Center, Institute of Carcinogenesis, Moscow, Russian Federation; [2]Lomonosov Moscow State University, Department of Chemistry, Moscow, Russian Federation*

Tumor suppressor genes are inactivated by random point mutations the appropriate identification of which is applied for cancer diagnosis, prognosis, monitoring, target chemotherapy. Elaboration of the methods which detect random point mutations of all types and are sensitive enough to reveal mutant DNA in the presence of wild-type DNA represents an actual goal for modern molecular oncology. The most promising approach

is based upon DNA-heteroduplex chemical cleavage of mismatches formed at the mutation point. To analyse specificity and sensitivity of chemical cleavage we elaborated the model system that may be used also to control the possibility of false-positive and false-negative result appearance, when different protocols for detection of DNA mutations are applied. The series of heteroduplexes with all types of mismatches and extrahelical nucleotide residues surrounded by either A●T or G●C pairs were formed via pair wise hybridization of 50-mer synthetic oligonucleotides differing in only one nucleotide at the central position. Heteroduplexes immobilized on magnetic beads by means of biotin-streptavidin interaction were modified at mismatched T and C with chemicals, which able to attack only nucleobases flipped out from the helix: potassium permanganate and hydroxylamine, respectively, and cleaved further by piperidine treatment. The fragments formed were visualized by denaturing polyacrylamide gel-electrophoresis and silver staining. The chemical reactivity of different mismatches was shown to correlate clearly with the target local structure in a particular sequence context. The intensity of heteroduplex cleavage increased in dependence on duration and temperature both of $KMnO_4$ or NH_2OH modification treatment. Heteroduplexes were revealed when their ratio in mixture with homoduplexes comprised 5−10% or 2% after primer extension. The data obtained demonstrate that modification of heteroduplex mixtures by potassium permanganate and hydroxylamine allows to reveal any non-canonical base pair and suggest its type and neighboring nucleotides from the nature of chemical as well as its localization from the length of cleavage products. High sensitivity and wide specificity of the method demonstrated in our model system are important for mutation detection in clinical oncology when the sample analyzed contains small amounts of mutant DNA in the mixture with normal one.

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404 POSTER

Relationship between non-enzymic antioxidant profile and mean prostate specific antigen (mPSA) levels of known prostate cancer patients

O. Adaramoye[1], O.I. Kareem[2], O. Akinloye[3]. *[1]University of Ibadan, Department of Biochemistry, Ibadan, Nigeria; [2]University College Hospital (UCH), Cancer Screening Unit (CSU), Ibadan, Nigeria; [3]Ladoke Akintola University of Technology, Department of Chemical Pathology, Osogbo, Nigeria*

Background: Oxidative stress has been implicated in the etiology of several pathologies, prostate enlargement inclusive. This study was designed to relate the non-enzymic antioxidant levels in prostate cancer patients with their mean prostate-specific antigen (mPSA) values.
Materials and Methods: Participants were recruited (with informed consent) from the Cancer Screening Unit (CSU), University College Hospital (UCH), Ibadan, Nigeria. 120 prostate cancer patients were assigned into 3 groups on the basis of mPSA values; group 1 with mPSA of 6.5 mg/L, group 2 with mPSA of 15.9 mg/L and group 3 with mPSA of 73.8 mg/L. Patients had no recent hormone therapy and/or radiation therapy. Likewise, 120 apparently normal subjects were recruited as control and had mPSA value of 2.8 mg/L. The study was approved by Ethical Committees of the UCH.
Results: Patients with mPSA ? 6.5 mg/L to 73.8 mg/L had significantly lower serum uric acid and vitamin E levels ($p < 0.001$) than the control. Significant reduction ($p < 0.001$) in patients with mPSA ? 6.5 mg/L to 73.8 mg/L when compared to the control. Specifically, LPO was elevated by 28%, 35% and 46% in patients with mPSA of 6.5, 15.9 and 73.8 mg/L, respectively. Furthermore, serum selenium levels were decreased by 35%, 34% and 38% in patients with mPSA of 6.5, 15.9 and 73.8 mg/L, respectively.
Conclusions: These results indicate an inverse relationship between the non-enzymic antioxidant profile of prostate cancer patients and their respective mPSA values.

405 POSTER

Expression of cellular apoptotic markers in Kaposi's sarcoma (KS) tumor biopsies

H. Kadyrova. *N.N. Blokhin Russian Cancer Research Center, laboratory of Viral carcinogenesis, Moscow, Russian Federation*

Intensive experimental investigations help and greatly improve of therapy of the vascular tumor virus-associated disease. Our aim was to analyze of several cellular apoptotic protein expressions in KS tumor biopsies at different clinic stages.
Materials and Method: There were analyzed 25 tumor tissues samples from 17 HIV-negative KS patients; HHV-8-associated in 64% cases: group I included 13 KS initial stage samples (patches and plaques); group II – 12 samples of KS patients at the advanced stage (nodules and tumors). As control we have investigated 9 cases of different skin endothelial benign pathologies (group III). The analysis of expression and protein localization was performed using immunohistochemical (IHC) method at 4−5 ?? formalin-fixed paraffin-embedded tissue sample sections with mono- and polyclonal antibodies to: CD95/FASR (ICO160; Russian); Bcl-2, FasL, p53, Ki67/KiS5, Bax (DAKO Corp., USA).
Results: Received IHC results showed that KS progress leads to: (1) the expression of all tested by us cellular apoptotic markers, greatly varying in tumor samples, perhaps, reflecting first of all immune status of patient, his genetic peculiarities and stage/sub stage of tumor development; (2) the decrease (around twice) of all tested apoptotic proteins expression in endothelial and spindle cells. At the same time proliferate index Ki67 in group II was increased in 1.5 times. Analyzing all tissue structures, not only endothelial and spindle cells, we have observed the change of expression level only for two apoptotic markers of different signal ways (bcl2, FASR) and proliferation index (Ki67) also.
Conclusion: Our data permit to think about the crucial role of two cellular markers expressions during aggressive KS development – anti-apoptotic bcl-2 and pro-apoptotic antigen FASR; perhaps diagnostic and prognostic value of nuclear proliferation index Ki67 analysis also. Last marker as other investigators have showed earlier for a row of tumors, determines common survival of patients. Cellular apoptotic protein expressions, especially bcl-2 and FASR, in such tissue type structures as, for example, epidermis, stroma, keratinocytes, hair papilla, glandular structures and more, perhaps reflect their paracrine mechanism of influence at the spindle cells.

406 POSTER

Novel view on Bcr PH domain as a protein binding partner

D. Miroshnychenko, A. Dubrovska, S. Maliuta, G. Telegeev. *Institute of Molecular Biology and Genetics, Molecular Genetics, Kyiv, Ukraine*

Reciprocal translocation t(9;22) leads to the Philadelphia (Ph) chromosome formation and allows tree types of hybrid Bcr-Abl protein to be formed. Since the Abl part remains the same size in all chimeric Bcr-Abl proteins, the difference between the tree forms of Bcr-Abl depends on the Bcr fragment. It's suggested that transforming potential of Bcr-Abl is determined by Abl tyrosine kinase, which is deregulated. However, the Bcr moiety contribution to the hybrid protein functions needs to be clarified.
In the focus of our research is PH domain that is absent in the Bcr-Abl shortest variant p190 but is found in two other types – p210 and p230. p190 Bcr-Abl corresponds to acute lymphoblastic leukaemia and p210 is found in chronic myelogenous leukaemia cases. PH domain is known to bind to lipids but its protein-protein interactions are not investigated well. To determine Bcr PH domain binding partners recombinant his-tagged PH protein was used. K562 cells were labeled with [35S]-methionine and cell lysates were loaded on PH-bound column. Column with empty his-tag was used as a control. After incubation with K562 lysates columns were washed several times. Bound proteins were eluated and resolved by two-dimensional gel electrophoresis. Spots corresponded to K562 proteins bound to Bcr PH domain were selected. Interacting proteins were identified by peptide mass fingerprinting by matrix-assisted laser desorption-dimensional time-of-flight mass spectrometry (MALDI TOF MS). We identified 20 proteins that formed complexes with PH domain. To verify some of the interacting proteins immunoprecipitation assay and pull down experiments were performed. Binding to SMC1 (structure maintenance of chromosome protein) and β-tubulin was observed in vitro in pull down assay. Interaction with PLCå and zizimin1 was confirmed in vivo.
It's established that the cellular compartment in which Bcr-Abl is localized is important in determining whether the outcome of its deregulated kinase activity is pro- or antiapoptotic. PH domain is a possible regulator of Bcr-Abl localization since it's able to bind lipids of cellular membranes or form complexes with various proteins. Moreover, detecting the roles and relative importance of Bcr-Abl domains in leukaemogenesis in vivo should help to understand the molecular mechanisms underlying the phenotypes of leukaemia and thus to identify targets for developing therapeutic interventions.

407 POSTER

Claudin-10 enhanced metastatic potential in hepatocellular carcinoma with MMP activation and modified expression profile of other claudin family members

Y.C. Ip, S.T. Cheung, Y.T. Lee, J.C.Y. Ho, K.K. Lo, S.T. Fan. *The University of Hong Kong, Surgery, Hong Kong, Hong Kong*

Background: Claudins, a group of integral membrane proteins, are important components of tight junctions. Increasing evidence shows that claudins are differentially regulated in a variety of malignancies and involved in cancer progression. Previously, we demonstrated that down-regulation of CLDN-10 in hepatocellular carcinoma (HCC) is associated