regardless their molecular mechanisms. Furthermore, we found the positive linear relationship between relative activity of AKT and ERK1/2 combined and cell viability as r = 0.948 in the same manner. The results obtained suggest quantitative cross-talk between the main two pathways regardless molecular mechanisms, which may aid cancer drug selection to a patient.

396 POSTER

Human papillomavirus (HPV) infection, p53 overexpression and histopathologic factors in colorectal cancer

A. Motlagh[1], P. Azadeh[2], M. Hashemi[3], M. Molaei[4], A. Fazlalizadeh[2], A. Alidoosti[2], F. Ghaderi[4], B. Noorinayaer[4], M. Davaei[1]. *[1]Shahid Beheshti Medical University, Cancer Research Center (CRC), Tehran, Iran; [2]Imam Hossein Hospital, Radiation Oncology, Tehran, Iran; [3]Imam Hossein Hospital, Pathology, Tehran, Iran; [4]Shahid Beheshti Medical University, Research Center for Gastrointestinal and Liver Disease (RCGLD), Tehran, Iran*

Background: There is evidence of a possible etiological role of human papillomaviruses (HPVs) in the development of colorectal cancer. Loss of p53 tumor suppressor gene function has been found in many malignancies and it can occur in a variety of ways, including gene mutation and interaction with the E6 protein of oncogenic human papillomaviruses (HPVs). The aim of this study was to verify the prevalence of HPV infection and p53 overexprssion in colorectal cancer tissue samples and its association with histopathologic factor.
Materials and Methods: Sixty tissue sections from CRC patients were investigated immunocytochemically for aberrant expression of p53 using the streptavidin-biotin-peroxidase method with monoclonal antibodies. HPVstatus was also analyzed using type-specific primers for HPV16/18 by polymerase chain reaction (PCR).
Results: Overall, 21of 60 patients (35%) presented HPV DNA; HPV 18 was detected in 19of 60 samples (31.7%) and HPV16 in 11 of 60 (18.3%). An abnormal expression of tumor-suppressor protein p53 were observed in 29 0f 60 (48.3%) samples. P53 overexpression was observed in 15/21 (71.4%) of HPV positive and 14/39 (35.8%) of HPV negative patients (P = 0.009). Same significant difference were found between HPV18 and p53 (P = 0.007) but not in HPV16 (P = 0.261). HPV DNA presentation was not significantly associated with histopathologic factor including tumor stage (P = 0.428), grade (P = 0.668), PNI (P = 0.265) and LVI (P = 0.275).
Conclusion: Our results suggest that p53 inactivation caused by HPV infection may play a role in the pathogenesis of colorectal cancer but there is not any association between HPV infection with histopathologic factor.

397 POSTER

Effects of cisplatin exposure on the expression of Bcl-2-family proteins: differences between cisplatin-sensitive and -resistant malignant pleural mesothelioma cells

V. Janson, K. Grankvist. *Umeå University, Dept. of Medical Biosciences Clinical Chemistry, Umeå, Sweden*

Background: Resistance toward apoptosis is one of the hallmarks of cancer, and cancer therapy failure is often attributed to apoptosis resistance. Malignant mesotheliomas (MM) are aggressive tumors that frequently acquire drug resistance during treatment. The chemotherapy regimens available often include the chemotherapeutic drug cis-diamminedichloroplatinum(II) (cisplatin, CDDP). In MM, there is evidence that the apoptosis-resistant phenotype is a consequence of suppressed mitochondrial membrane permeabilisation (MMP). The Bcl-2 family of proteins, which includes both pro-apoptotic proteins (e.g. Bim, Puma, Bid, Bad, Bmf) and pro-survival proteins (e.g. Bcl-2, Bcl-XL), is essential for the regulation of the MMP. We compared a malignant pleural mesothelioma cell line (P31wt) with its CDDP-resistant sub-line (P31res) regarding CDDP effects on the expression of Bcl-2-family proteins.
Materials and Methods: After 0.5, 2 or 6 h CDDP exposure, protein expression in cell lysates was determined with Western blotting. Equitoxic concentrations, 10 mg/L (P31wt) and 40 mg/L (P31res), of CDDP were used: 72 h after a 6-h exposure to CDDP, 50% of the cells had died of apoptosis, as determined by TUNEL staining.
Results: Under control conditions, some proteins were differently expressed: (1) the P31res cells did not express the most potent isoform of Bim; (2) P31res cells had a reduced expression of Puma; and (3) the P31res cells had a higher expression of P-Bcl-2 and P-Bad. In P31wt cells, which have a primary resistance toward CDDP compared to many other cancer cell lines, CDDP exposure (1) increased the expression of Bim and Puma, (2) increased the expression of Bad, and (3) decreased the expression of Bcl-2. In P31res cells, which have an acquired resistance toward CDDP, CDDP exposure (1) increased the expression of Puma, (2) decreased the expression of P-Bad, and (3) decreased the expression of pro-survival Bcl-2 and Bcl-XL.
Conclusions: Compared to P31wt, the P31res cells had lower expression of potent pro-apoptotic proteins and higher expression of P-Bad and P-Bcl-2. Cisplatin exposure reduced the expression of pro-survival proteins in both cell lines, but the effect on pro-apoptotic proteins differed: in P31wt most of the pro-apoptotic proteins increased, in P31res cells only Puma expression increased. These results suggest that the regulation of pro-apoptotic proteins can have an important role in CDDP resistance.

398 POSTER

Clinical requirements of "In Silico Oncology" as part of the integrated project ACGT (Advancing Clinico-Genomic Trials on Cancer)

N. Graf[1], C. Desmedt[2], A. Hoppe[1], M. Tsiknakis[3], D. Dionysiou[4], G. Stamatakos[4]. *[1]Universitätsklinikum des Saarlandes, Paediatric Oncology and Haematology, Homburg/Saar, Germany; [2]Free University of Brussels, Institut Jules Bordet, Bruxelles, Belgium; [3]Foundation for Research & Technology-Hellas, Forth, Heraklion, Greece; [4]National Technical University of Athens, Institute of Communication and Computer Systems, Zografos, Greece*

Background: New methods and technologies in molecular biology will result in an exponential increase of information that can be handled by the advances of high-computing and informatics. It is of paramount importance to gather this information with clinical data to gain new knowledge for developing better and more individualized treatments for cancer patients. This approach results in clinico-genomic trials, as ACGT (Advancing Clinico-Genomic Trials on Cancer) is running.
Materials and Methods: Substantial efforts have been made in mathematically simulating tumour growth and response to treatment resulting in a discipline called In Silico Oncology. Such in silico experiments might help clinicians to find the best way of treating an individual patient by simulating different treatments in the computer before starting the treatment in reality.
Results: From a clinical point of view two preconditions are of utmost importance, before a physician can rely on predictions of in silico simulations:
1. every in silico experiment has to be part of a clinico-genomic trial
2. every prediction of an in silico experiment has to be compared with the reality.

In the process of developing in silico experiments it is necessary to define the necessary and available data in a first step, including data from the tumor (molecular biology, pathology, imaging), from the patient (clinical data) and from possible treatments (pharmacokinetics of drugs, the treatment schema). Because the amount of data is restricted by the availability of tumour material, imaging data and clinical data, In Silico Oncology has to be part of clinico-genomic trials based on GCP criteria.
The simulation prediction of each in silico experiment has always to be compared with the reality. Only if there are no or minimal deviations between the prediction and the reality the in silico experiment is allowed to be used in a clinical setting. Before an in silico experiment can be accepted as a routine method for treatment stratification, a prospective and randomised trial has to show that patients treated according to the result of the in silico simulation experiment do better, than those treated regardless of the result.
Conclusions: In ACGT in silico models of breast cancer and nephroblastoma are tested regarding tumour growth and response to treatment.
This work was supported in part by the European Commission under the project "ACGT: Advancing Clinico-genomic Trials on Cancer" (FP6–2005-IST-026996)

399 POSTER

Analysis of coding and non-coding regions of thymidylate synthase gene in colorectal cancer patients and its possible relationship with 5-fluorouracil drug response

A. Calascibetta[1], G. Gulotta[2], M. Cajozzo[3], S. Feo[4], A. Agrusa[2], G. Sanguedolce[1], R. Sanguedolce[1]. *[1]University of Palermo, Dipartimento di Scienze Farmacologiche, Palermo, Italy; [2]University of Palermo, Dipartimento di Chirurgia generale e d'urgenza, Palermo, Italy; [3]University of Palermo, Dipartimento di Discipline Chirurgiche e Oncologiche, Palermo, Italy; [4]University of Palermo, Dipartimento di Oncologia Sperimentale e Applicazioni Cliniche, Palermo, Italy*

Background: Thymidylate synthase (TS) catalyzes methylation of dUMP to dTMP and is the target of 5-fluorouracil (5-Fu). TS gene has regulatory tandemly repeated sequences in its 5′ and 3′ untraslated regions (5′-3′ UTR). TS levels vary considerably among tumors and the response to 5-FU is influenced by the intratumoral activity of the enzyme, with high levels generally being associated with a poor response. A recently detected 6 bp deletion polymorphism in the 3′UTR of the TS gene might also

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