aggressiveness of colorectal cancer. Legumian, Nup88 and PINCH were independently prognostic factors in the patients.

243 POSTER

Increasing detection efficiency of microsatellite instabilities in colon carcinoma by applying a label-free method

O. Mueller[1], N. Barta[2], R. Salowsky[1], S. Baldus[2], M. Odenthal[2]. [1]*Agilent Technologies, Waldbronn, Germany;* [2]*University Clinic Cologne, Institute of Pathology, Cologne, Germany*

Microsatellite instability (MSI) is caused by a failure of the DNA mismatch repair system and occurs frequently in various types of cancer. Since MSI, associated with approximately 10 to 15% of colorectal, gastric or endometrial carcinoma, impact clinical prognosis, MSI analysis is an important tool of molecular pathology. This study aimed to develop a simple and efficient procedure of MSI detection. 40 cases with no (27), low (l) or high (h) MSI (13), pre-identified by conventional fluorochrome-associated PAGE technology, were selected out of a panel of 150 patients with colon carcinoma.
Microdissected non-tumor (N) and tumor (T) tissue areas of one or two 4 μm-sections were de-paraffinized and DNA was extracted. Primer sequences recognizing the five microsatellite loci BAT25, BAT26, D5S346, D17S250, D2S123, were selected according to the recommendation of the 1997 National Cancer Institute-sponsored conference on MSI. Primer sets were applied in label-free duplex or single PCR assays for DNA amplification and amplicons were analysed by microfluidics based on-chip electrophoresis.
In all 40 cases, chip linked microcapillary electrophoresis of the amplicons, arisen from tumor and non-tumor DNA, resulted in highly resolved, distinct patterns of each of the microsatellite loci. Label-free detection of MSI could be demonstrated by microsatellite loci-associated deviations in the electropherogram profiles of tumor and non-tumor material, and confirmed the prediagnosis of the MSI cases by conventional technology.
Here, we present a simple and robust approach for MSI detection, which allows a label-free microsatellite analysis of uncharacterized microdissected tissue areas within 30 minutes.

Publication
Molecular predictive assays (including: genetics, genomics, molecular diagnostics, prognostic factors, proteomics)

244 PUBLICATION

Fundamental aspects of mutation detection analyses via chemical cleavage of DNA mismatches

A.A. Neschastnova[1], M.G. Yakubovskaya[1], V.K. Gasanova[1], G.A. Belitsky[1], M.G. Ivanovskaya[2], N.G. Dolinnaya[2]. [1]*Inst. of Carcinogenesis, Cancer Reseach Center RAM, Lab. of Carcinogen Screening Methods, Moscow, Russian Federation;* [2]*Lomonosov Moscow State University, Chemical Department, Moscow, Russian Federation*

Carcinogenesis is considered as accumulation of genetic alterations, in particular, point mutations: substitutions, deletions or insertions of one or several base pairs. Detection of point mutations is applied for cancer diagnosis, disease prognosis, monitoring, choice of treatment strategy and therapeutic effect determining. Chemical Cleavage of Mismatches (CCM) is the most sensitive method, which reveals unknown point mutations of random location and determines their positions and type. CCM consists of a heteroduplex formation, accomplished by consecutive denaturing and annealing the amplified normal and analyzed DNA mixture and their chemical cleavage at mismatches formed at the mutation points. Heteroduplexes modified at mismatched T and C by potassium permanganate and hydroxylamine, correspondingly, are cleaved further by piperidine treatment. Then fragments obtained are visualized by denaturing polyacrylamide gel-electrophoresis. Random probes with several known mutations are used usually as positive controls. However, according to physicochemical investigations, mismatch influence on duplex conformation depends on its type and the neighbouring residues. Chemical reactivity of heterocyclic bases of different mismatches should vary significantly affecting the sensitivity and specificity of the method. We estimated the influence of mismatch type, orientation and its flanking nucleotides on the CCM rate and efficacy. The set of heteroduplexes with all types of mismatches and extrahelical nucleotide residues was obtained via pair wise hybridization of five sense and five antisense 50-base oligonucleotides differing in only one nucleotide at the central position. The point of structural abnormality in constructed heteroduplexes was surrounded by A/T pairs.
We demonstrated that hydroxylamine induced cleavage of heteroduplexes containing only mismatched C, and cleavage intensity was independent on mismatch type. Potassium permanganate modification resulted in cleavage of all heteroduplex at the point of mismatched T and neighbouring T as well. The most intensive cleavage was observed for extrahelical T and C/T mismatch. The intensity of cleavage increased in dependence on treatment duration (from 1 min to 1.5 h). Heteroduplexes were revealed when their ratio in mixture with homoduplexes comprised 5–10%. It is important for mutation detection in clinical oncology when the analyzing sample contains small amounts of mutant DNA in the mixture with normal one.

245 PUBLICATION

Thymidylate synthase gene polymorphisms in Croatian population

M. Jokic. *Rudjer Boskovic Institute, Division of Molecular Medicine, Zagreb, Croatia*

Thymidylate synthase (TS) is crucial enzyme in the nucleotide biosynthetic pathway because it calalyzes the reductive methylation of dUMP by 5, 10-methylentetrahydrofolate to form dTMP which is very important reaction for cell proliferation. Thus, TS gene has been an important target for a variety of chemotherapeutic drugs auch as 5-FU. Inhibition of TS by such an agent causes cytotoxicity leading to thymineless death or sometimes chronic uracil misincorporation into DNA. Resistance to fluoropyrimidines which is not rare arises from many different mechanisms including TS protein expression. The human TS promoter region includes a *cis*-acting enhancer which is polymorphic containing two or three 28-bp tandem repeats and has been implicated in affecting on TS mRNA expression as well as TS mRNA translational efficiency. The majority of individual human TS alleles harbor either a double repeat (2R) or a triple repeat (3R) for this polymorphism, creating genotypes of 2R/2R, 2R/3R i 3R/3R. Individuals who are homozygous for the 3R were found to have elevated intratumoral TS mRNA and protein level compared with 2R homozygous.
A novel G→C SNP in the second repeat of the 3R alleles identified recently has shown that the 3R sequence with G has three to four times greater efficiency of translation than the 3R with C and the 2R sequence. Genotypes 2R/3G, 3C/3G, 3G/3G are associated with high expression of TS and genotypes 2R/2R, 2R/3C and 3C/3C with low expression. Due to associations of the TS polymorphisms with the prognosis of several tumor types, we performed a study to determine the distribution of TS polymorphisms in Croatian population.
A total of 125 healthy unrelated individuals were genotyped for the TS 5′ UTR polymorphisms using PCR-RFLP method with HaeIII restriction enzyme. Genotype frequencies for 5′ UTR TS polymorphisms were 26.4%, 16%, 2.4%, 42.4%, 8.8% and 4% for 2R/3G, 3G/3C, 3G/3G, 2R/2R, 2R/3C, 3C/3C genotype respectively.
Our results showed that in Croatian population low TS expression genotypes were more frequent (55.2%) than high TS expression genotypes (44.8%) but not significant.
Key words: Thymidylate synthase, 5′ UTR polymorphism

246 PUBLICATION

Response of prostacyclin to low dose irradiation in the development of radiation myelopathy

A. Shirazi[1], S.R. Mahdavi[1], K.R. Trott[2]. [1]*Tehran University of Medical Science, Department of Biophysics, Faculty of Medicine, Tehran, Iran;* [2]*Gray Cancer Institute, MSc in Radiobiology, Northwood, UK*

Purpose: The priority of vascular and its secretory profile changes in pathogenesis of radiation myelopathy have recently been discussed. In this study the model of prostacyclin concentration changes after low doses of X-irradiation within a short period of time was studied.
Method and Materials: Wistar rats were irradiated with doses of 2.4 and 6 Gy's of X-rays. After 24 hours, 2 and 13 weeks post-irradiation, samples of spinal cord were prepared for evaluation of prostacyclin and histopathologic changes. Prostacyclin content was determined by quantification of 6-keto-prostaglandin-F1α (prostacyclin stabilized metabolite). Irradiated segments of spinal cord were stained routinely for histological studies.
Results: Twenty four hours post-irradiation, finding shows decrease in the content of prostacyclin after doses of 0.5 and 1 Gy with 91.67±1.47% 96.80±2.17% of age-matched control group. After 2 weeks concentration of prostacyclin shows significant decreases after 6 Gy. After 13 weeks irradiation shows marked differences even after a small dose of 2 Gy ($p < 0.001$) and after doses of the low dose group. The differences between concentration values at doses of 4 Gy and 6Gy in compare to control are significant ($p < 0.001$ and $p < 0.002$, respectively).
Discussion and conclusion: In the vascular theory, circulation disturbance following vascular injury secondarily induces white matter lesions. The interpretation of this finding can be that radiation affects the synthesis of prostacyclin at both vascular and parenchymal sources responsible

to produce prostacyclin in the spinal cord. Our study also supports the hypothesis that the asymptomatic interval after radiation is characterized by sequential physiological changes that are imperfectly reflected in routine histological study and that even in the histologically unaffected spinal cord, severe impairment in substructures and biochemistry of irradiated spinal cord are present.

Key words: Prostacyclin, radiation myelopathy, low dose irradiation, spinal cord.

247 PUBLICATION

Germline mutations in BRCA1 and 2 genes in Polish population, modulation of cancer risk by tri- and dinucleotide repeats in AR (androgen receptor) and ESR1.2 (estrogen receptor) genes

E. Grzybowska[1], J. Pamula[1], W. Pekala[1], H. Zientek[1], J. Rogozinska-Szczepka[2], E. Nowicka[2], B. Utracka-Hutka[3]. *[1]Centre of Oncology – Maria Sklodowska-Curie Memorial Institute, Department of Tumor Biology, Gliwice, Poland; [2]Centre of Oncology – Maria Sklodowska-Curie Memorial Institute, Radiotherapy Clinics, Gliwice, Poland; [3]Centre of Oncology – Maria Sklodowska-Curie Memorial Institute, Oncology Clinics, Gliwice, Poland*

Introduction: Germline mutations in BRCA1/2 genes predispose to breast and ovary cancers. However, the penetrance of mutations is incomplete (70–90% for breast cancer and 20–50% for ovary cancer). The aim of this work was to identify possible other genetic factors: AR (androgen receptor) gene and ESR1, 2 (estrogen receptor) gene with much lower penetrance which can modulate the penetrance of BRCA mutations.
Methods: Patients with breast and ovary cancer and healthy persons with family history of these cancers were recruited through Chemotherapy Clinics and Genetic Counseling. Controls were identified among the town clerks. Each person filled out detailed questionnaire including family history and signed the consent. Germline mutations in BRCA1 and 2 genes were detected using direct sequencing and when the spectrum of mutations was determined, ASA and RFLP PCR method was used for screening of mutations.
CAG and GGN repeats in AR gene, TA repeats in ESR1 gene and CA repeats lengths in ESR2 gene were measured also in ABI PRIZM DNA sequencer. The primers for PCR reactions were labeled with FAM and NED fluorescence dyes and run with the length marker GS-500.
Results: Since 1997, 252 carriers of germline mutations in BRCA1/2 genes belonging to 178 families were identified. Mutation 5382insC in BRCA1 was prevalent in Polish population and it was found in 63% of all carriers. When Amsterdam criteria were applied for pedigree analysis and direct sequencing was used to detect mutations, we found germline mutations in 45% families. For further analysis ASA and RFLP PCR detecting 6 most prevalent mutations in both genes were used. Germline mutations were found in 8.2% of consecutive breast cancer cases below 50 years of age, 13.5% of consecutive ovary cases regardless of age, 12.5% of synchronous bilateral breast cancer and 34.2% of metachronous bilateral breast cancer. CAG repeats length polymorphisms in exon1 of AR gene did not modify significantly the risk of cancer in HBOC families. GGN longer repeats in the same gene were associated with increased risk of developing malignancy in the group of carriers of BRCA1/2 mutations diagnosed with breast and/or ovary cancer (OR = 3.44, p = 0.00005). Short TA repeats in ESR1 gene also increased the risk of cancer in the same group under study (OR = 3.14, p = 0.00007). By contrast short CA repeats in ESR2 gene showed slight protective effect against malignancy (OR=0.68, p = 0.08430) at the border of significance.
Discussion: In our study we proved that germline mutations in BRCA1 and 2 genes can be also detected in the patients without family history of cancers and strict Amsterdam criteria allow only to identify the part of HBOC families in the population. Analysis of tri- and dinucleotide repeats in AR and ESR1, 2 genes respectively indicates that other genetic factors are able to modify very high penetrance of mutations in BRCA genes.

248 PUBLICATION

Pyruvate kinase activity in colon, stomach and breast tissue as a marker of malignancy

K. Bajin-Katic[1], Z. Kovacevic[2], K. Stankov[1]. *[1]Clinical center Novi Sad, Department of Biochemistry, Novi Sad, Serbia; [2]Serbian Academy of Sciences and Arts, Branch in Novi Sad, Novi Sad, Serbia*

Pyruvate kinase (PK; E.C. 2.7.1.40) is one of the key enzymes in the synthesis of pyruvate, which is the most important metabolite in aerobic and anaerobic metabolism of carbohydrates. Since the pioneering work of Wartburg (1956), the differences in metabolic profile between normal and malignant tissues are the subject of many studies directed towards advancement in diagnostics and therapy of cancer.

The family of PK enzymes comprises 3 different classes of isoenzymes, which are designated according to the tissue where they are predominant: L isoenzyme – the main isoenzyme of the liver and erythrocytes, M – predominant in muscles and brain, K – in kidneys. These enzymes and their dimeric hybrids are designated as: M_4, KM_3, K_2M_2, K_3M and K_4. Many studies showed the aberrations in isoenzymic profile of PK in malignant hepatomas, gliomas, meningeomas, carcinomas of the lung and in experimental tumors, in comparison with isoenzymatic profiles in corresponding normal tissues.
In our study, we determined the total activity of PK and PK-K_4 isoenzyme activity as well as the effects of the inhibition of PK induced by L-alanine in samples of 20 colon carcinomas and corresponding surrounding tissue (2 and 10 cm distant from tumor), 14 gastric carcinomas and corresponding surrounding tissue (2 and 10 cm distant from tumor) and 15 samples of breast carcinoma and 14 samples of benign dysplasia of the breast.
The results obtained in our study showed significantly increased total activity of PK in malignant tissue samples in comparison with normal tissue of colon (2×) and stomach (3.5×) or with benign breast hyperplasia (10×). We observed significantly higher proportion of PK-K_4 isoenzyme in total PK activity in malignant tissues, showing that the increase in total PK activity is due to PK-K_4 isoenzyme activity increase. The activity of PK-K_4 isoenzyme was 4x higher in colon carcinoma samples, 6x higher in gastric carcinoma samples and 50× higher in breast carcinoma samples in comparison with non-malignant tissue samples. The percentage of the L-alanine induced PK inhibition was significantly increased in malignant tissue samples. Our results showed that the increase in PK-K_4 isoenzyme activity is a significant, highly specific and sensitive parameter, which may enable the differentiation between malignant and normal tissue.

249 PUBLICATION

Interactions of genetic polymorphisms of folate metabolizing enzymes in modifying colorectal cancer risk in a Hungarian population-based case-control study

V. Adleff[1], V. Komlosi[1], B. Budai[1], A. Reti[1], E. Hitre[1], A. Hajnal[2], J. Kralovanszky[1]. *[1]National Institute of Oncology, Clinical Research, Budapest, Hungary; [2]National Blood Transfusion Service, Serologic Laboratory, Budapest, Hungary*

Complex interactions of candidate polymorphic genes in CRC risk assessment make individual association estimates difficult. Pathway guided risk evaluation is possibly more adequate for gene-gene interaction studies. TS enhancer region (TSER), TSER G>C SNP, and TS1494del6, MTHFR C677T, cSHMT C1420T genetic variants were analyzed in 325 Hungarian primary CRC patients, and compared with the genotype data of a 200 individual control group of same population origin.
Population analysis showed a significant TSER heterozygote defficiency overall, and also in the rectum cancer (RC) subgroup, and a slight heterozygote excess in RC at cSHMT locus. No disequilibria were found at the TS1494del6 and MTHFR loci, or for any of the polymorphisms in the control population. The OR between TSER homo-/heterozygotes was higher among RC vs. CC (1.63 CI 1.08–2.4, vs. 1.42 CI 0.92–2.18), and rooted mainly in a 3R3R frequency increase for all sites, compared to the control. An important gender difference was apparent: we found 3R3R women most susceptible for RC and men for distal CC (OR 2.06 CI: 1.09–3.8; and 2.7, 1.28 to 5.8, respectively). Variant homozygote cSHMT genotypes (TT) were less affected by CRC (OR 0.50, CI 0.27–0.93), with closely the same effect in both RC and CC, which was significant for the women subgroup alone (p = 0.05), as well. No associations with TS SNP and TS1494del6 occurred, nor the reported protective effect of the MTHFR TT variant was seen on our study group, conversely, we found an increased risk for TT genotype, especially for proximal cancers in men. In a multivariate logistic regression analysis TSER 3R3R and cSHMT CC were found independent positive risk factors for CRC, with a cumulative risk of the compound genotype 3R3R&CC OR = 1.78 CI: 1.0 to 3.0; and for the least affected 2R3R&TT OR = 0.42 CI: 0.18 to 0.98, while MTHFR and sex were not influencing alone. Instead, modeling the overall and also the site-specific susceptibilities, important two-way interaction terms (e.g. between MTHFR and cSHMT genotypes) modifying risk emerged, raising the hypothesis that hierarchical models could be useful in folate-related molecular epidemiology studies of CRC.
Our findings support the proposals of pathway-driven multi-locus assessments of genetic susceptibility for CRC, and were able to identify independent risk factors with additive properties at TSER and cSHMT loci, and also MTHFR-cSHMT gene-gene interactions in Hungarian CRC population.