239 POSTER

Single nucleotide polymorphisms of cancer related genes involved in signal-transduction pathways in Korea lung cancer patients

Y.H. Kim[1,2], H. Choi[1,2], J. Sung[1,2], K. Park[1,2], I. Choi[1,2], J. Seo[1,2], S. Shin[1,2], J. Kim[1,2]. [1]*Korea University Anam Hospital, Medical Oncology, Seoul, Korea;* [2]*Lung, Breast/Ovary Cancer Genome Research Center, Seoul, Korea*

Background: Lung cancer is a leading cause of cancer death in Korea. Single nucleotide polymorphisms (SNPs) represent an important class of genetic variation and susceptibility of individual. To search Korean specific SNPs for lung cancer, we explored SNPs of genes involved in diverse cancer related signal transduction pathways.
Methods: We analyzed 11 SNP points from 3 genes (ERBB2, ATM, CDK5) by SNaPshot assay and Restriction Fragment Length Polymorphism (RFLP) using genomic serum DNA from 291 Korean lung cancer patients and 281 healthy controls. Written informed consent was obtained from all patient study subjects, and the study protocol was approved by the institutional review board. The allele frequencies of each SNP between cases and control were estimated by the chi square test and odds ratios (OR) with 95% confidence interval (95% CI).
Result:

			p-value
CDK5 (rs1549760, −904)	G:A = 0.654:0.346 (LP)	0.711:0.289 (N)	**0.041**
CDK5 (rs2069442, −270)	C:G = 0.657:0.343 (LP)	0.659:0.341 (N)	0.947
CDK5 (rs2069443, −238)	A:C = 0.659:0.341 (LP)	0.665:0.335 (N)	0.849
ATM (rs228589, −5144)	A:T = 0.531:0.469 (LP)	0.518:0.482 (N)	0.709
ATM (rs189037, −4519)	G:A = 0.570:0.430 (LP)	0.542:0.458 (N)	0.417
ATM (rs664677, IVS22)	C:T = 0.603:0.397 (LP)	0.604:0.396 (N)	0.976
ATM (rs609429, IVS48)	G:C = 0.558:0.442 (LP)	0.540:0.460 (N)	0.543
ERBB2 (rs2643194, −3444)	C:T = 0.603:0.397 (LP)	0.631:0.369 (N)	0.392
ERBB2 (rs2934971, −1985)	G:T = 0.603:0.397 (LP)	0.619:0.381 (N)	0.567
ERBB2 (rs189200, I655V)	A:G = 0.875:0.125 (LP)	0.888:0.112 (N)	0.534
ERBB2 (rs1058808, P1170A)	C:G = 0.610:0.390 (LP)	0.625:0.375 (N)	0.638

Conclusion: We identified differences in frequencies of 11 SNP points in Korean lung cancer. In result, A polymorphism in the promoter region of CDK5 (−904) gene was statistically significant but others not. Further study needed to explore the association between SNP points and histopathological factors of the lung cancer patients and to evaluate the role of each polymorphism in lung cancer.

240 POSTER

Comparison of gene expression profile between RER+ and RER− sporadic colorectal tumours

F. Rodríguez Jiménez[1], P. Iniesta[1], A. Diaz-López[1], C. García-Aranda[1], C. Frías[1], C. de Juan[1], A. Sánchez-Pernaute[2], A. Torres[2], E. Díaz-Rubio[2], M. Benito[1]. [1]*Facultad de Farmacia, Bioquímica y Biología Molecular, Madrid, Spain;* [2]*Hospital Clínico San Carlos, Servicios de Cirugía y de Oncología Médica, Madrid, Spain*

Background: Replication error positive (RER+) and negative (RER−) sporadic colorectal tumors display distinctive pathological features and prognosis. To elucidate their gene expression profile may contribute to understand their different molecular pathogenesis and their corresponding clinical evolution.
Material and methods: Seventy sporadic colorectal tumours were classified according to their RER status. Total RNA extracted from normal tissues as well as from RER+ and RER− tumours was reverse transcribed. The labelled cDNA was used to hybridize a commercially available cDNA array corresponding to 96 human extracellular matrix and adhesion molecule genes. The candidate genes were selected by computational analysis (using Gearray analyzer software). Verification of the cDNA array results was performed by Real Time Quantitative PCR using TaqMan probes. The resulting expression of tumor tissues was normalized using GAPDH as housekeeping gene and referred to the expression of normal tissues.
Results: We observed common events occurring in RER+ and RER− tumours. Thus, the matricellular glycoprotein osteopontin (SPP1) was expressed at high levels in both kinds of tumors in comparison to normal tissue. However, the expression profile of this gene defined two subgroups of RER− tumours. On the other hand, significant overexpression of MMP11 in RER− in comparison to RER+ tumors was observed as a differential event. Interestingly, RER− tumors subgroup which showed higher expression level for SPP1 also expressed more MMP11 mRNA transcripts, both gene profile contributing to better define both RER− subgroups. The first RER− subgroup expressed MMP11 mRNA at significant higher level (P = 0.021) in comparison with RER+ tumours. In contrast, the second RER− subgroup did not display this tendency (P = 0.252)
Conclusion: RER− tumours show a more heterogeneous expression profile than RER+ tumours. Our knowledge of the differential molecular events between RER+ and RER− sporadic colorectal tumours may allow us to a better understanding of their different clinicopathological outcome.

241 POSTER

Detection of CEA mRNA expressing cells in peripheral blood 7 days after surgery influences relapse in colorectal cancer

Y. Maeda[1], S. Sadahiro[1], T. Suzuki[1], A. Kamijo[1], H. Makuuchi[1], C. Murayama[2]. [1]*Tokai University, Surgery, Isehara, Kanagawa, Japan;* [2]*Tokai University, Radiology, Isehara, Kanagawa, Japan*

Background: No consensus has been reached on whether cancer cells detected in blood during surgery in patients (pts) with colorectal cancer(CRC) may correlate with prognosis. Cancer cells could not be detected when blood was taken 7days after intravenous injection in experiment (Fidler, 1970). The aim of this study was to examine the detection of cancer cells in blood at more than 7 days after curative resection may serve as a prognostic indicator.
Methods: Two hundred and one pts with CRC who underwent curative surgery were the subjects. Peripheral blood was collected between 7 and 10 days after resection. Cancer cells were detected using RT-PCT targeting CEAmRNA. The median follow-up period was 42 months (range 22−59 months).
Results: Recurrence has been confirmed in 51 pts (25%). Recurrence was noted in liver in 16 pts, locoregional in 13 pts, lung in 10 pts, peritoneum in 6 pts, lymph node in 3 pts. Cancer cells were detected in blood in 45 pts (22%). Recurrence was observed in 18 out of 45 pts positive for CEAmRNA (40%) and in 33 out of 156 pts negative for CEAmRNA (21%) (p = 0.02). There were statistical differences in disease-free survival and recurrence-free survival between pts with positive CEAmRNA and pts with negative CEAmRNA, respectively (p = 0.04, P = 0.03). However, difference did not reach the significance in overall survival (P = 0.28). There was no correlation between recurrence site and positivity of CEAmRNA. There was no significant correlation between pathological stage and PCR status.
Conclusion: Detection of cancer cells in blood taken 7 days after curative resection was an independent prognostic indicator in CRC.

242 POSTER

Interaction of biological variables in tumour cells and stroma, and their clinicopathological significance in colorectal cancers

X. Sun. *Colorectal cancer research Group, Institute of Biomedicine and Surgery, Department of Oncology, Linköping, Sweden*

Background: Disruption of stromal-epithelial interactions and cell adhesion alters cellular signalling, which influences proliferation, angiogenesis, differentiation, motility, death, genomic integrity and other phenotype in the tissues. We examined the relationships of the factors expressed in tumour cells or/and stroma including legumain, Nup88, MAC30, PINCH (particularly interesting new cysteine-histidine rich protein) and ST3 (stromelysin-3) as well as their clinicopathological significance in colorectal cancers.
Material and methods: The study included the matched distant normal mucosa, adjacent normal mucosa, primary tumour and metastasis in the lymph nods from 212 patients with colorectal cancer. Methods included immunohistochemistry, Western blotting and immunofluorescence.
Results: Legumain, Nup88 and MAC30 mainly in tumour cells, while PINCH and ST3 were expressed in stroma mainly in the fibroblasts, myofibroblasts and a proportion of endothelial cells of the tumor vasculature. The expression of the five proteins had positive relationships. Legumain expression was increased from normal mucosa to primary tumour, MAC30 was increased from primary tumour to metastasis. Nup88 and PINCH expression was increased from normal mucosa to primary tumour and to metastasis. Legumain, Nup88 and PINCH expression were more intense at the invasive margin of tumour than their expression in intratumour/intrastroma. Legumain expression was positively related to poorer differentiation/mucinous carcinoma and higher degree of necrosis. Nup88 expression was positively related to distal tumour location and infiltrative growth pattern. ST3 expression was positively related to infiltrative growth pattern. Strong expression of legumain, Nup88 and PINCH predicted unfavourable survival, independent of clinicopathological factors.
Conclusions: Legumain (as an early event), MAC30 (as a later event), Nup88, PINCH and ST3 had a positive interaction in the development and

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