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

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

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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)

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

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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.

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Thymidylate synthase gene polymorphisms in Croatian population

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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

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

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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