Colorectal cancer detection in rats using fluorescent fingerprint

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Introduction

Animal models of colorectal carcinogenesis - invaluable research tool for investigating colorectal cancer (CRC)

Dimethylhydrazine (DMH) induced tumours in rats - opportunity for studying certain aspects of tumours that cannot be effectively studied in humans, morphological similarity to human CRC (1)

Cancer – alteration of metabolism including metabolism of native fluorophores (2)

Native fluorophores are released into biological fluids - the information of pathologic change caused by cancer (3)

Urine – non invasivity; multi-component mixture including variety of fluorescent metabolites (4)

Metabolome - the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes

Fluorescence spectroscopy – suitable for fingerprint approaches - high sensitivity and simplicity

Concentration matrix (CM) - fluorescent fingerprint created by measuring of synchronous single spectra of selected urine dilutions with ultrapure water (5) (Fig. 1)

Materials and methods

Model of chemically induced carcinogenesis - 18 rats (DMH at a dose of 21 mg / kg subcutaneously, a total of 5 times at weekly intervals, in the 2nd, 3rd, 4th, 5th, and 6th week of experiment), Healthy control group - 18 rats

Colonic tissue samples - histological examination - considered as precancerosis

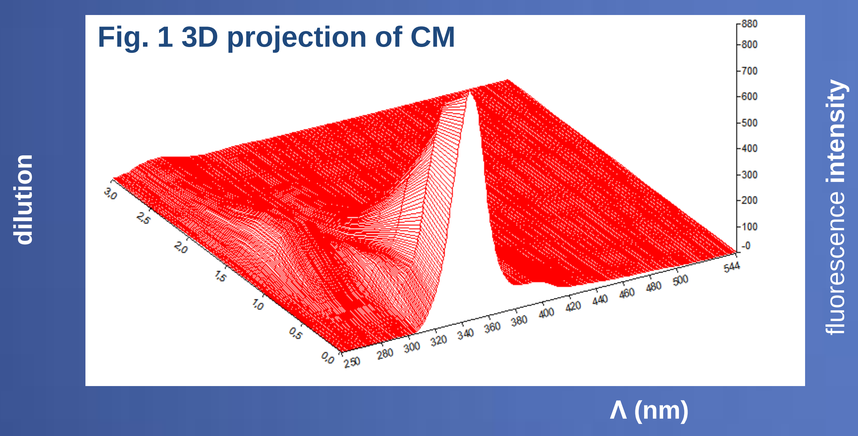
Urine samples - by bladder punction *post mortem,* Centrifugation of urine samples(10 min, 5000rpm), Dilution - (via geometric progression) with ultrapure water - set of 12 dilutions

Fluorescent analysis of urine – luminescence spectrophotometer Perkin Elmer LS 55, quartz microcuvette (3mm), Data analysis - software FL WinLab

Every dilution – single synchronous spektrum (Δλ 30 nm, λ start - end 250-550nm, excitation/emission slit 5/5 nm, speed 1200 nm/min)

Resolution analysis of CM – comparing of maximal values of fluorescence intensity (300 nm) (Fig. 2)

Statistical analysis – Student´s t - test



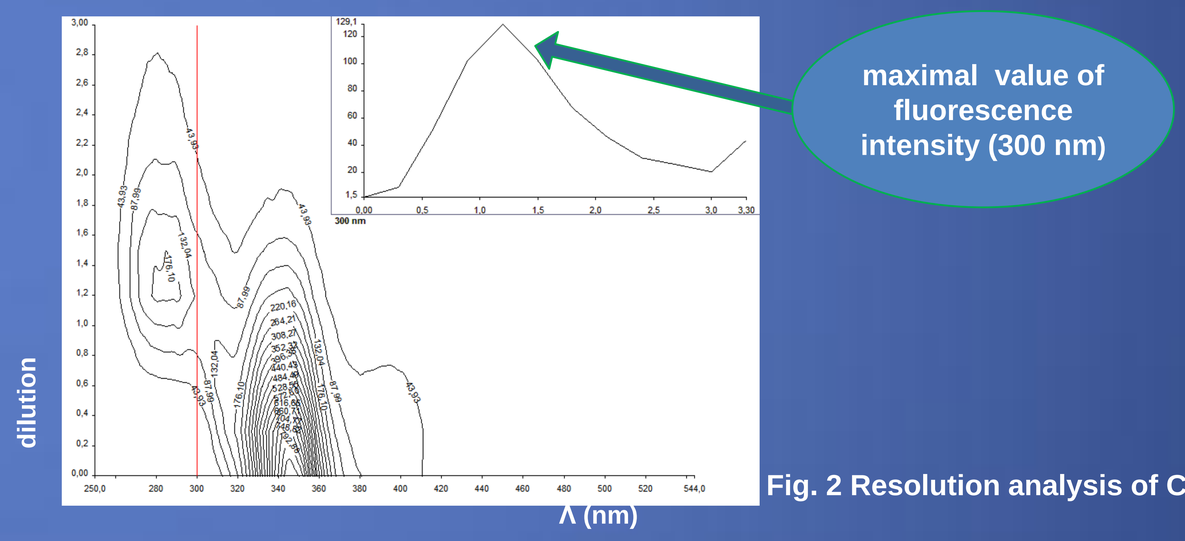
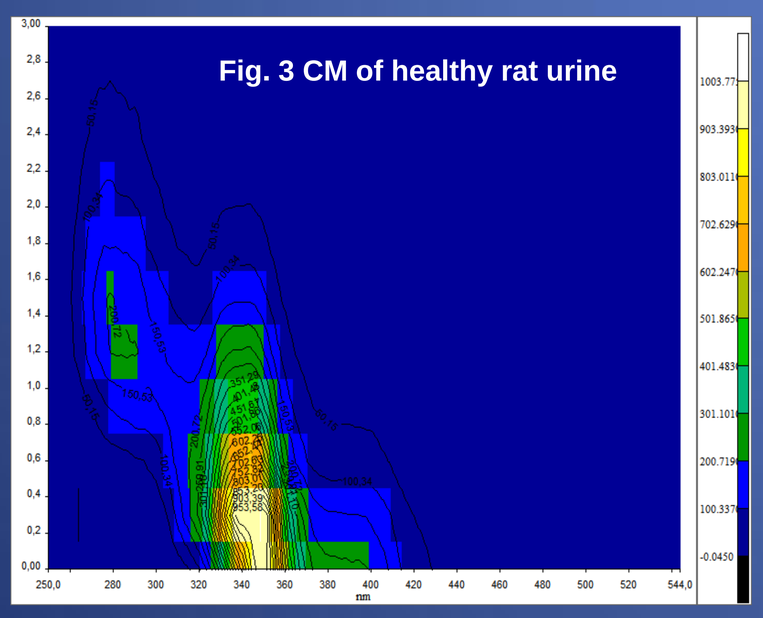
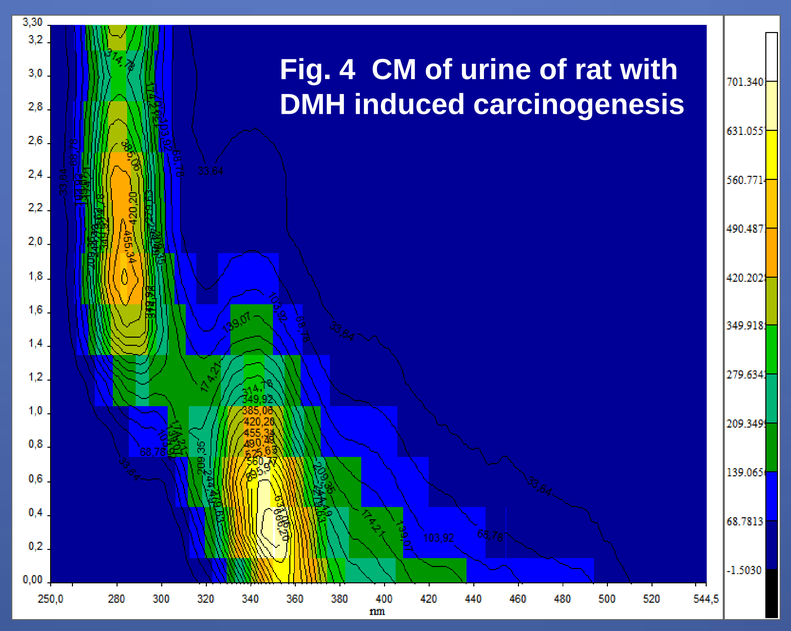


Fig. 2 Resolution analysis of CM

Results





CM of urine

- very sensitive
- differences between healthy rat urine CM and CM of urine of rat with DMH induced carcinogenesis are detectable at the first sight
- able to reveal also small metabolic changes caused by DMH – induced CRC with high efficiency (p= 0,046)

Conclusion

By comparing healthy rat urine fluorescence with the fluorescence of urine obtained from rats with cancer, significant and specific differences were detected. We proved that changes in rat urine fluorescence can be used for cancer detection. The analysis of autofluorescence of urine by fluorescent fingerprint could help in the clinical diagnostics of cancer including precancerosis although further research is required.

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P04-58
Balance of human choline kinase isoform expression is critical for cell cycle regulation: Implications for the development of choline kinase-targeted cancer therapy

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The enzyme choline kinase (CK), which uses ATP for phosphorylation of choline to form phosphocholine (PCho), has an essential role in the biosynthesis of phosphatidylcholine, the major constituent of all mammalian cell membranes. CK is encoded by two separate genes expressing the three isoforms called CKalpha1, CKalpha2, CKbeta that are active as homo- or hetero-dimeric species. Metabolic changes observed in various cancer cell lines and tumors have been associated with differential and marked up-regulation of CKalpha genes, and specific inhibition of CKalpha activity has been proposed as a potential anticancer strategy. As a result, less attention has been given to CKbeta and its interaction with CKalpha. With the aim of profiling the intracellular roles of CKalpha and CKbeta, we used RNA interference as a molecular approach to down-regulate CK expression in HeLa and MCF-7 cells. Individual and simultaneous RNAi-based silencing of the alpha and beta isoforms was achieved using different combinations of knockdown strategies. Efficient knockdown was confirmed by immunodetection using isoform-specific antibodies, and by quantitative real-time PCR. Our analyses of the phenotypic consequences of choline kinase depletion showed the expected lethal effect of CKalpha knockdown. However, CKbeta- and double-silenced cells had no aberrant phenotype. Thus, our results support the hypothesis that the balance of the alpha and beta isoforms is critical for cancer cell survival. The suppression of the cancer cell killing effect of CKalpha silencing by simultaneous knockdown of both isoforms implicates that a more effective choline kinase-based anti-cancer strategy can be achieved by reducing cross-reactivity with CKbeta.

P04-59
Activity of gelatinases in chosen cancer cell lines

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Gelatinases are members of matrix metalloproteinase family – group of zinc/calcium-dependent proteolytic enzymes with capability of degradation of several matrix and non-matrix proteins. Because of intra- and extracellular activity and broad range of substrate specificity, gelatinases are considered to be an important factor in regulation of metastatic process, involved in gaining of cell motility, breakdown of extracellular matrix, and more. The role of gelatinases as putative diagnostic markers relies on the widely described differences in gelatinases' expression patterns in tumor tissues and their normal equivalents, as well as on the imbalance between expression of gelatinases and their native inhibitors, observed in cancer cells.

Present work deals with the comparison of proteolytic activity of gelatinases in eight cell lines, representing different types of cancer disease. Activities of cell sonicates have been determined by fluorometric assays, in the presence or absence of specific and non-specific inhibitors. The clue of the second part of experiment was to estimate changes of proteolytic potencial of cancer cells after treating with mild or strong detergent.

Significant differences between investigated cell lines were obtained, all in relation to overall proteolytic activity and to the susceptibility to inhibitors used. Consecutively, we observed diversified influence of detergent on studied cell lines. Particularly, by contrast to the others, application of mild detergent to black melanoma cell sample caused bigger increase of proteolytic activity than the disruption of cells with strong detergent.

P04-60
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Among instrumental techniques, fluorescence spectroscopy is recognized as one of the most sensitive diagnostic tool with high efficiency compared to many routine medical diagnostic tools for many disorders diseases, especially for diagnosis of early cancer stage. Many metabolic compounds like porphyrins and NADH are produced in excess amounts in cancerous tissues due to rapid metabolism of cancerous tissue. These compounds are responsible for fluorescence spectra, what can be used for diagnosis of cancer along with other parameters. Fluorescence spectroscopy of biomolecules is considered a promising method to discriminate normal tissue from malignant tissue at various sites. However, only few studies have been reported on the feasibility of exploiting fluorescence spectroscopy of biological fluids to characterize pathological changes usable in diagnostic oncology. The fluorophore composition of biological fluids is related to different metabolic pathways. Urine contains a variety of organic and inorganic compounds including a number of natural fluorescent metabolites. The analysis of fluorescence from a sample of urine without any added reagents can provide useful information. Experimentally induced tumors in laboratory rats provide opportunity for studying certain aspects of tumors that cannot be effectively studied in humans. Significant information on human colorectal cancer etiology or factors influencing it has derived from studies using dimethyl hydrazine (DMH) model that is one of the experimental models appreciated for its morphological similarity to human. In our experiment we investigated urine samples from rats with early stages of DMH – induced colorectal cancer using fluorescent spectroscopy. As a model of chemically induced carcinogenesis we used 18 rats in which was induced carcinogenesis using DMH at a dose of 21 mg/kg subcutaneously, a total of five times at weekly intervals, in the 2nd, 3rd, 4th, 5th, and 6th week of experiment. The alterations of colonic tissue which are considered as precancerosis were also confirmed histologically. Eighteen rats served as healthy control group. Urine samples were obtained by bladder punction *post mortem*. After urine centrifugation (10 min, 5000 rpm), the supernatant was relocated into a new test tube and diluted (via geometric progression) with ultrapure water to get set of 12 dilutions. To create fluorescent fingerprint, synchronous fluorescence spectra (deltaλ30 nm) of every dilution were measured. By comparing healthy rat urine fluorescence with the fluorescence of urine obtained from rats with cancer, significant and specific differences were detected ($p = 0.003$). The analysis of autofluorescence of urine by fluorescent

fingerprint could help in the clinical diagnostics of cancer although further research is required. Supported by CEEMP-ITMS: 26220120067 (100%).

P04-61
Effects of radioiodine therapy on extracellular matrix degradation in papillary thyroid carcinomas with/without autoimmune thyroid diseases

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Background: The thyroidectomy followed by radioiodine (I-131) ablation of the residual thyroid tissue, after thyrotropin (TSH) stimulation, is considered the ideal treatment for papillary thyroid carcinomas (PTC) and papillary thyroid carcinomas associated with autoimmune thyroid diseases (PTC + AITD). We aimed to evaluate the effects of therapeutic irradiation with I-131 on imbalance between expression of matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in PTC with/without AITD patients.
Methods: We selected 45 patients with PTC (6M/39F, mean age 44.2 ± 19.1 years) and 38 with PTC + AITD (3M/35F, mean age 38.2 ± 19.6 years), who received the same dose of I-131 (3.7 GBq). All patients had elevated serum levels of TSH (> 30 mU/l). PTC + AITD patients had positive titers of anti-Tg autoantibodies (TgAb). Peripheric blood samples were collected before and at 96 hour after I-131 administration. The serum levels of TSH, TgAb, transforming growth factor-beta1 (TGF-β1), MMP-9 and TIMP-1 were measured by ELISA.
Results: Before I-131 therapy, the MMP-9 and TIMP-1 concentrations of PTC + AITD patients (635.12 ± 370.03 ng/ml, 140.27 ± 33.76 ng/ml) were higher than those of the PTC patients (484.98 ± 377.50 ng/ml, 128.6 ± 43.38 ng/ml). We found that I-131 therapy of PTC + AITD patients was associated with an increase in titers of TgAb (1.18-fold), TGF-β1 (1.27-fold) and a decrease in MMP-9 (1.27-fold) and MMP-9/TIMP-1 ratio (1.24-fold). In PTC patients, the serum levels of TGF-β1, MMP-9 and MMP-9/TIMP-1 ratio decreased 1.03-, 1.72- and 1.79-fold after irradiation.
Conclusions: In PTC patients the blockade of TGF-β1 signaling by I-131 therapy has almost halved the imbalance between MMP-9 and TIMP-1 and this decrease may reduce tumor cell viability and migratory potential. In PTC + AITD patients, increased TgAb titers partially block the beneficial effect of I-131. These titers are associated with increased TGF-β1 concentrations and with a lower decrease of MMP-9/TIMP-1 ratio, after I-131 administration, than in PTC patients.

P04-62
RNase A strongly inhibits metastasis development through the alteration of miRNA profiles in tumor tissue and blood serum

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Recent data on the involvement of miRNA in regulation of tumorigenesis showed a great prospect for these molecules as a novel class of therapeutic targets and gave a new start for the study of ribonucleases as potential antitumor and antimetastatic agents.

Previously we have shown that the administration of ultra-low doses of RNase A to mice bearing Lewis lung carcinoma (LLC) inhibits metastases development by 60–90% and causes the retardation of primary tumor growth by 30%. It was found that the observed antitumor and antimetastatic effects of RNase A are accompanied by reduction of pathologically elevated levels of extracellular RNA and increase in ribonuclease activity of blood plasma of tumor-bearing animals. Here in order to search for possible molecular targets of RNase A miRNA fractions were isolated from the serum and tumor of C57/Bl mice with LLC received treatment with RNase A or without treatment. Libraries of these small RNAs were prepared and analyzed using the SOLiD V3.5 sequencing system. Sequencing data revealed that treatment by RNase A resulted in decrease of the levels of serum miRNAs and increase of levels of timorous miRNAs whose direct and indirect targets are mRNAs encoding proteins of cell adhesion (ITGB1-3, ITGA5, ITGAV, LOX), proteolysis (TIMP3, ADAM17), angiogenesis (VEGF-A, HIF-1A) as well as cell proliferation (PTEN, PHB, RAS). Obtained data give the evidence that antitumor and antimetastatic effects of RNase A is associated with alteration of miRNA profiles in tumor tissue and blood serum which regulate the tissue architectural changes associated with malignancy.

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P04-63
Lipid lowering therapy decreases LDL-S-homocysteinilation levels in chronic kidney desease patients

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Chronic kidney disease (CKD) patients are characterized by diabetes, hypertension, obesity and dyslipidemia with a highly atherogenic profile: increased total and LDL cholesterol, triglyceride, and a decreased HDL-cholesterol. Moreover, elevated levels of LDL-homocysteinilation has been found in these patients and it is reported that this modification increase LDL-atherogenicity. The dyslipidemia control through lipid lowering therapy is one of the targets for the treatment of CKD. By this pilot study we aimed to evaluate the effect of hypolipidemic drugs on the levels of low molecular weight thiols bound to LDL in nephropatic patients. We enrolled thirty CKD randomized to receive three different hypolipidemic regimens: simvastatin alone (40 mg/day) or ezetimibe/simvastatin combined therapy (10/20 or 10/40 mg/day). Considering that proteins are thiolated in response to oxidative stress, evaluation of free malondialdehyde plasma levels, allantoin/uric acid ratio (All/UA) was also performed to monitor OS in patients during drug treatment. LDL thiolation decreased in all treated patients, but a greater efficacy was attained from a combined therapy with a higher simvastatin dose, by which a 31% decrease of all S-bound thiols was reached after one year of therapy. In particular, in this patients group the reduction of apoBHcy was > 40%. The concomitant decrease of the oxidative stress indices during the therapy brings to the hypothesis that decreased levels of protein bound thiols may be a consequence of oxidative stress improvement. Therefore, among the several beneficial effects described for lipid lowering drugs we also propose their ability to reduce the quantity of LDL linked homocysteine thus decreasing not only LDL levels but also LDL atherogenicity.