P3

IR-spectrum of blood and redox: diagnostics of cancer

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Early studies of blood or serum thin layer on glass plate by IR spectrometer as a potential cancer diagnostic tool were initially received with considerable excitement. But many intrinsic specimen characteristics may have no relationship with the disease in question. Therefore a diagnosis was confirmed only in 60% cases. It has been known for a few decades that a relationship exists between cancer and level of –SH group in patient blood. Some insights into the value of SH group in blood have been obtained from studies performed in experimental animals and in vitro. These studies provided some of the first evidence for differential value IR parameters (band Amid I) between exsiccate thin layer of blood or serum of intact animals and tumor animals ($p < 0.005$). IR parameters correlate highly with level of SH group protein of serum or pure albumin ($r = 0.97$). Human serum albumin (HSA) is the most abundant protein in the circulatory system and it has a number of functions. One of a number functional roles of this molecule seems to be the maintenance of the redox potential in the extracellular fluid, because it is a mixture of mercaptalbumin (reduced form; in humans, HMA) and non-mercaptalbumin (oxidized form; in humans, HNA), i.e., a major part of the redox couple in plasma. Our studies indicate that state of SH group of cysteine of albumin can be related to indicator of redox in term of IR parameters. Based on the structure of a molecule of albumin we developed the theoretical norm of redox for IR data. This method offers important advantages for quantitative estimation as common number oxidized/reduced form as individually one. Use selection IR data (1700cm-1–1600cm-1) on level of SH group without proteomic profiling we obtained nevertheless more exact diagnosis of women with breast cancer. This method offers also important advantages for monitoring of treatment. However, additional control experiments are essential, such as using control subjects with other types of inflammatory disease, or even different cancers.

P4

Purification and characterization of a novel monocot lectin with mitogenic and in vitro anti-proliferative activity from Arisaema curvatum

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A lectin with in vitro anti-proliferative activity and potent mitogenic and has been purified from tubers of a wild monocot plant Arisaema curvatum Schott by affinity chromatography on asialofetuin linked amino-activated silica. A single band of pure lectin corresponding to subunit Mr 13.0 kDa was observed in SDS-PAGE at pH 8.3. The native molecular mass as determined by gel filtration chromatography was 52 kDa, suggesting a homotetrameric structure. ACL gave multiple bands in isoelectric focusing and in native PAGE at pH 8.3 like other monocot lectins. ACL was inhibited by N-acetyl-D-lactosamine (LacNAc), a disaccharide and asialofetuin, a complex desialylated serum glycoprotein. When treated with denaturing agents, the lectin was stable in the presence of urea (4 M), thiourea (3 M) and guanidine HCl (4 M). The lectin had no requirement for divalent metal ions. ACL was a glycoprotein with a carbohydrate content of 1.5%. Amino acid analysis revealed high content of aspartic acid, glutamic acid, glycine and threonine and a very low amount of methionine but complete absence of cysteine. Amino acid modification studies of ACL revealed the involvement of tryptophan and tyrosine residues involved in lectin-sugar interaction. The lectin showed potent mitogenic response towards human lymphocytes. The mitogenic activity of ACL was even more than that of Con A, a standard well-known plant mitogen. ACL exhibited a fluorescence emission maximum (λmax) at 341 nm upon excitation at 295 nm. Using Far UV CD spectra the estimated secondary structure was 38% α-helix, 27% β-sheet and 35% random contributions. In vitro anti-proliferative activity of ACL was tested on eleven different human cancer cell lines viz. MCF-7 (Breast), SK-N-SH (CNS), 502713 (Colon), Colo-205 (Colon), HCT-15 (Colon), HT-29 (Colon), SW-620 (Colon), Hep-2 (Liver), IMR-32 (Neuroblastoma), DU-145 (Prostate) and PC-3 (Prostate). The concentrations of ACL which produced 50% inhibition (IC50) of cancer cell lines viz. Colo-205, SK-N-SH, HCT-15, IMR-32, SW-620, Hep-2 and HT-29 was 28, 33, 42, 44, 45, 48 and 81 μg/ml respectively. ACL is specific for LacNAc, which is one of important cancer markers. The inhibitory effect of ACL was not associated with toxicity to the cell lines. Future research is focused find in vivo effect of lectins on appropriate animal models of human diseases and other agents (e.g., synthetic compounds) to explore the synergistic effect of these agents to prevent cancer.

P5

Impression cytology of ocular surface diseases of the bulbar conjunctiva as non-invasive technology for diagnostic and monitoring for cancer prevention

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In recent years, impression cytology has been used in diagnostic various ocular surface diseases.

Aim: To evaluate the accuracy of impression cytology employing a Biopore membrane device in the differentiating bening, premalignant and malignans conjunctival and corneal tumors.

Methods: Impression cytology was used to study of conjunctival and corneal tumors in 45 individuals (19–65 years). From the 45 patients studied 29 had a tumors of melanocytic origin of the conjunctiva, 16 had ocular surface squamous neoplasia (OSSN). Cytological and histological diagnoses were compared in 25 cases. All cytological and histological specimens were examination by two independent investigators. The cytology specimens were obtained using the Biopore membrane device (Biopore Millicell®-CM 0.4mm, PICM01250 MILLIPORE, USA). The specimens were fixed in 95% alcohol, stained with of Schiff's Feulgen and Papanicolaou stains and mounted on the slides for interpretation. The squamous metaplasia cytology was graded according to the classification of Nelson. Cytopatological and histopatological changes were estimated by the differential criteria of atypia cells classified them according to the UICC.

Results: Cytological diagnosis were verificated: from tumors of the melanocytic system, bening nevus, 20 cases, proliferation nevus, 3 cases, melanosis, 2 cases, malignant melanoma, 4 cases; from ocular surface squamous neoplasia (OSSN), squamous cell hyperplasia, 5 cases, squamous cell papilloma, 3 cases, dysplasia (mild-grade), 3 cases, dysplasia (high grade), 2 cases, squamous cell carcinoma, 2 cases. In 80 % (20/25) of cases cytological

diagnosis correct predicted the histological diagnosis. The patients with diagnosis dysplasia were recommended to using of impression cytology monitoring effects of the preventive treatment.
Conclusion: This study shows impression cytology employing a Biopore membrane to be a useful diagnostic tool in differentiation of pigmented tumors and suspected OSSN of the bulbar conjunctiva. Atypical melanocytes, which have migrated to the epithelial surface, can be detected by this technique. This technique is reproducible, economical and high informative. Impression cytology may be successfully used as non-invasive technology for diagnosing all the surface tumors and monitoring for cancer prevention.

P6
Effect of hyperhomocysteinemia on NMU-induced rat mammary tumorigenesis

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Despite the advent of new and aggressive therapeutics, breast cancer remains a leading killer among women; hence there is a need for the prevention of this disease. Hyperhomocysteinemia (HHcy), a consequence of disturbed methionine metabolism, is a well-known factor for several types of carcinoma, including breast cancer. Furthermore accumulating bodies of epidemiological studies have suggested an inverse association of dietary intake and blood concentrations of folate and vitamins B6 and B12, which are key cofactors directly involved in Hcy catabolism, with the risk of breast cancer. However, the major unanswered question whether or not HHcy is associated with cancer pathogenesis and is an indicator of tumorigenesis, remains elusive. Taking into account all above mentioned data, the aim of the work was to study the influence of experimental model of HHcy induced by feeding animals methionine enriched diet and HHcy corrected with vitamins B6, B9, B12 on the development of N-methyl-N-nitrosourea (NMU)-induced mammary tumors in female Wistar rats.

The study was carried out on seven-week-old virgin Wistar rats ($n = 100$). Animals were kept at standard conditions with free access to water and experimental semi-synthetic diets. Diets were: 1) normal semi-synthetic diet; 2) 2% methionine-enriched diet; 3) 2% methionine-enriched diet with 5-fold increased level of vitamins B6, B9, and B12 compared to control and methionine enriched diets. Rats were fed diets supplemented beginning from seven days before initiation with NMU (50 days of age) to termination of latency period (90 days after NMU).

The results of study suggest that the HHcy modulates the susceptibility of mammary gland to NMU carcinogenesis. Despite the decrease of mammary tumor incidence by 26% ($p < 0.05$) as well as of multiplicity of palpable tumors by 33% ($p < 0.05$), the latency period decreased 1.23 times ($p < 0.05$) and portion of mammary adenocarcinomas induced by NMU increased by 27% ($p < 0.05$) compared to the analogous characteristics of animals from control group. Enriched of methionine diet with vitamins B6, B9 and B12 promoted (i) to the decrease of mammary tumour incidence and multiplicity of palpable tumours, (ii) to the decrease of the portion of malignant tumours among all the NMU-induced tumours, and (iii) to the 1.3 times increase of latency period compared to the animals obtaining the methionine diet.

Obtained results have demonstrated that the risk of mammary tumor incidence under HHcy conditions increases. This risk can by substantially reduced by HHcy corrections by vitamins B6, B9 and B12. A negative influence of HHcy on the chemical induced mammary gland carcinogenesis is obviously due to them interaction of NMU mutagenic activity and modulation influence of HHcy on the epigenetic mechanisms of the regulation of the expression of genes associated with the malignant cell transformation.

P7
Flavonoid-positive plant fractions yield a potent inducer of promyelocytic leukemia cell differentiation with no enzyme inhibitory action within the XO system

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Bioactivity-guided fractionation was carried out on flavonoid-positive fractions of A. squamosa, a popular fruit tree with known medicinal uses. The chemical purification process yielded an isolate characterized via physical and spectral means, and dereplicated using high-resolution mass spectrometry. When assayed for xanthine oxidase, an enzyme correlated with brain tumors, the compound showed non-inhibition of uric acid product, suggesting inability to act directly as enzyme inhibitor while suppressing the superoxide free radicals of the metabolic pathway. Verification of the compound's identity may find more analogs to positive controls and elucidate the ambivalent role assigned to uric acid as either cause or protector of oxidative burst. Tests conducted on HL60 promyelocytic leukemia cells in culture revealed a strong inducer of differentiation, presenting an alternative approach with less adverse effects usually accredited to chemotherapeutic agents.

P8
Antitumor effect of experimental soybean curd produced from thermally treated soy

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Aim: to find optimal technological modifications of soybean foods for use in nutrition of cancer patients and in preventive nutrition of patients of cancer risk groups.
Results: Two modifications of soybean curd – produced from fresh or thermally treated soy (FS or TS) – were elaborated. Both variants were tested on healthy and Walker W-256 carcinosarcoma or Guerin's carcinoma (GC) bearing rats. The TS curd, if moderately consumed during the term from tumor transplantation to the end of the experiment or the same term + 7–14 days before transplantation, caused growth retardation of W-256 (36–48%) and GC (26%). The same product consumed excessively (ad libitum) accelerated W-256 growth (100%). If the same product consumption started 2 days after W-256 transplantation, tumors growth rate was permanent. FS curd consumption even if it was moderate accelerated W-256 growth (26%). Anticancer effect of TS curd and negative effect of FS curd was found both in perfect in vivo experiments and in express-tests. In all experiments (both positive and negative in anticancer effect) antioxidant and anti-inflammation action of both products were found. So, the results obtained show TS