is based upon DNA-heteroduplex chemical cleavage of mismatches formed at the mutation point. To analyse specificity and sensitivity of chemical cleavage we elaborated the model system that may be used also to control the possibility of false-positive and false-negative result appearance, when different protocols for detection of DNA mutations are applied. The series of heteroduplexes with all types of mismatches and extrahelical nucleotide residues surrounded by either A●T or G●C pairs were formed via pair wise hybridization of 50-mer synthetic oligonucleotides differing in only one nucleotide at the central position. Heteroduplexes immobilized on magnetic beads by means of biotin-streptavidin interaction were modified at mismatched T and C with chemicals, which able to attack only nucleobases flipped out from the helix: potassium permanganate and hydroxylamine, respectively, and cleaved further by piperidine treatment. The fragments formed were visualized by denaturing polyacrylamide gel-electrophoresis and silver staining. The chemical reactivity of different mismatches was shown to correlate clearly with the target local structure in a particular sequence context. The intensity of heteroduplex cleavage increased in dependence on duration and temperature both of $KMnO_4$ or NH_2OH modification treatment. Heteroduplexes were revealed when their ratio in mixture with homoduplexes comprised 5−10% or 2% after primer extension. The data obtained demonstrate that modification of heteroduplex mixtures by potassium permanganate and hydroxylamine allows to reveal any non-canonical base pair and suggest its type and neighboring nucleotides from the nature of chemical as well as its localization from the length of cleavage products. High sensitivity and wide specificity of the method demonstrated in our model system are important for mutation detection in clinical oncology when the sample analyzed contains small amounts of mutant DNA in the mixture with normal one.

This research was supported by RFFI grants 06−04–49204a; 07−04–01194a.

404 POSTER

Relationship between non-enzymic antioxidant profile and mean prostate specific antigen (mPSA) levels of known prostate cancer patients

O. Adaramoye[1], O.I. Kareem[2], O. Akinloye[3]. *[1]University of Ibadan, Department of Biochemistry, Ibadan, Nigeria; [2]University College Hospital (UCH), Cancer Screening Unit (CSU), Ibadan, Nigeria; [3]Ladoke Akintola University of Technology, Department of Chemical Pathology, Osogbo, Nigeria*

Background: Oxidative stress has been implicated in the etiology of several pathologies, prostate enlargement inclusive. This study was designed to relate the non-enzymic antioxidant levels in prostate cancer patients with their mean prostate-specific antigen (mPSA) values.
Materials and Methods: Participants were recruited (with informed consent) from the Cancer Screening Unit (CSU), University College Hospital (UCH), Ibadan, Nigeria. 120 prostate cancer patients were assigned into 3 groups on the basis of mPSA values; group 1 with mPSA of 6.5 mg/L, group 2 with mPSA of 15.9 mg/L and group 3 with mPSA of 73.8 mg/L. Patients had no recent hormone therapy and/or radiation therapy. Likewise, 120 apparently normal subjects were recruited as control and had mPSA value of 2.8 mg/L. The study was approved by Ethical Committees of the UCH.
Results: Patients with mPSA ? 6.5 mg/L to 73.8 mg/L had significantly lower serum uric acid and vitamin E levels ($p < 0.001$) than the control. Significant reduction ($p < 0.001$) in patients with mPSA ? 6.5 mg/L to 73.8 mg/L when compared to the control. Specifically, LPO was elevated by 28%, 35% and 46% in patients with mPSA of 6.5, 15.9 and 73.8 mg/L, respectively. Furthermore, serum selenium levels were decreased by 35%, 34% and 38% in patients with mPSA of 6.5, 15.9 and 73.8 mg/L, respectively.
Conclusions: These results indicate an inverse relationship between the non-enzymic antioxidant profile of prostate cancer patients and their respective mPSA values.

405 POSTER

Expression of cellular apoptotic markers in Kaposi's sarcoma (KS) tumor biopsies

H. Kadyrova. *N.N. Blokhin Russian Cancer Research Center, laboratory of Viral carcinogenesis, Moscow, Russian Federation*

Intensive experimental investigations help and greatly improve of therapy of the vascular tumor virus-associated disease. Our aim was to analyze of several cellular apoptotic protein expressions in KS tumor biopsies at different clinic stages.
Materials and Method: There were analyzed 25 tumor tissues samples from 17 HIV-negative KS patients; HHV-8-associated in 64% cases: group I included 13 KS initial stage samples (patches and plaques); group II – 12 samples of KS patients at the advanced stage (nodules and tumors). As control we have investigated 9 cases of different skin endothelial benign pathologies (group III). The analysis of expression and protein localization was performed using immunohistochemical (IHC) method at 4−5 ?? formalin-fixed paraffin-embedded tissue sample sections with mono- and polyclonal antibodies to: CD95/FASR (ICO160; Russian); Bcl-2, FasL, p53, Ki67/KiS5, Bax (DAKO Corp., USA).
Results: Received IHC results showed that KS progress leads to: (1) the expression of all tested by us cellular apoptotic markers, greatly varying in tumor samples, perhaps, reflecting first of all immune status of patient, his genetic peculiarities and stage/sub stage of tumor development; (2) the decrease (around twice) of all tested apoptotic proteins expression in endothelial and spindle cells. At the same time proliferate index Ki67 in group II was increased in 1.5 times. Analyzing all tissue structures, not only endothelial and spindle cells, we have observed the change of expression level only for two apoptotic markers of different signal ways (bcl2, FASR) and proliferation index (Ki67) also.
Conclusion: Our data permit to think about the crucial role of two cellular markers expressions during aggressive KS development – anti-apoptotic bcl-2 and pro-apoptotic antigen FASR; perhaps diagnostic and prognostic value of nuclear proliferation index Ki67 analysis also. Last marker as other investigators have showed earlier for a row of tumors, determines common survival of patients. Cellular apoptotic protein expressions, especially bcl-2 and FASR, in such tissue type structures as, for example, epidermis, stroma, keratinocytes, hair papilla, glandular structures and more, perhaps reflect their paracrine mechanism of influence at the spindle cells.

406 POSTER

Novel view on Bcr PH domain as a protein binding partner

D. Miroshnychenko, A. Dubrovska, S. Maliuta, G. Telegeev. *Institute of Molecular Biology and Genetics, Molecular Genetics, Kyiv, Ukraine*

Reciprocal translocation t(9;22) leads to the Philadelphia (Ph) chromosome formation and allows tree types of hybrid Bcr-Abl protein to be formed. Since the Abl part remains the same size in all chimeric Bcr-Abl proteins, the difference between the tree forms of Bcr-Abl depends on the Bcr fragment. It's suggested that transforming potential of Bcr-Abl is determined by Abl tyrosine kinase, which is deregulated. However, the Bcr moiety contribution to the hybrid protein functions needs to be clarified.
In the focus of our research is PH domain that is absent in the Bcr-Abl shortest variant p190 but is found in two other types – p210 and p230. p190 Bcr-Abl corresponds to acute lymphoblastic leukaemia and p210 is found in chronic myelogenous leukaemia cases. PH domain is known to bind to lipids but its protein-protein interactions are not investigated well. To determine Bcr PH domain binding partners recombinant his-tagged PH protein was used. K562 cells were labeled with [35S]-methionine and cell lysates were loaded on PH-bound column. Column with empty his-tag was used as a control. After incubation with K562 lysates columns were washed several times. Bound proteins were eluated and resolved by two-dimensional gel electrophoresis. Spots corresponded to K562 proteins bound to Bcr PH domain were selected. Interacting proteins were identified by peptide mass fingerprinting by matrix-assisted laser desorption-dimensional time-of-flight mass spectrometry (MALDI TOF MS). We identified 20 proteins that formed complexes with PH domain. To verify some of the interacting proteins immunoprecipitation assay and pull down experiments were performed. Binding to SMC1 (structure maintenance of chromosome protein) and β-tubulin was observed in vitro in pull down assay. Interaction with PLCå and zizimin1 was confirmed in vivo.
It's established that the cellular compartment in which Bcr-Abl is localized is important in determining whether the outcome of its deregulated kinase activity is pro- or antiapoptotic. PH domain is a possible regulator of Bcr-Abl localization since it's able to bind lipids of cellular membranes or form complexes with various proteins. Moreover, detecting the roles and relative importance of Bcr-Abl domains in leukaemogenesis in vivo should help to understand the molecular mechanisms underlying the phenotypes of leukaemia and thus to identify targets for developing therapeutic interventions.

407 POSTER

Claudin-10 enhanced metastatic potential in hepatocellular carcinoma with MMP activation and modified expression profile of other claudin family members

Y.C. Ip, S.T. Cheung, Y.T. Lee, J.C.Y. Ho, K.K. Lo, S.T. Fan. *The University of Hong Kong, Surgery, Hong Kong, Hong Kong*

Background: Claudins, a group of integral membrane proteins, are important components of tight junctions. Increasing evidence shows that claudins are differentially regulated in a variety of malignancies and involved in cancer progression. Previously, we demonstrated that down-regulation of CLDN-10 in hepatocellular carcinoma (HCC) is associated