

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.

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POSTER

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

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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 $\geq$ 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 $\geq$ 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.

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POSTER

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

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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.

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POSTER

Novel view on Bcr PH domain as a protein binding partner

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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 eluted 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-ionization 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 PLC α 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.

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POSTER

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

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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

with prolonged disease-free survival after curative surgery. The biological function of CLDN-10 in carcinogenesis is lacking. The aim of the current study is to evaluate the biological function of CLDN-10 in HCC by functional assays.

Material and Methods: CLDN-10 was overexpressed in Hep3B, an HCC cell line with low invasive ability and siRNA-mediated knockdown of CLDN-10 was performed in a highly invasive HCC cell line, HLE. The effect on invasion, migration, proliferation and survival was then investigated by in vitro function assays. MMP levels were evaluated by gelatin zymography. Expressions of MT1-MMP and claudin family members were examined by semi-quantitative RT-PCR and Western blotting.

Results: Functional studies demonstrated that increased expression of CLDN-10 enhanced the metastatic potential of HCC by promoting cancer cell survival, motility and invasiveness. More importantly, in the CLDN-10 transfectants, there was increase in mRNA transcription and protein expression of MT1-MMP, a protease shown to promote intrahepatic metastasis in HCC in our earlier study. In addition, CLDN-1, -2 and -4 was up-regulated in CLDN-10 overexpression transfectants, indicating that the expression of claudin family members in cancer cells might affect each other. On the contrary, CLDN-10 siRNA strongly inhibited invasion, MMP2 and MT1-MMP expression. These findings highlighted that CLDN-10 promotes metastatic potential in HCC by enhancing invasion through up-regulation of MT1-MMP and MMP2 expression.

Conclusion: CLDN-10 is important for MMP activation, HCC invasion and migration. It also modifies the claudin family expression profile. These findings underline the contributions of CLDN-10 in HCC progression.

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POSTER

The difference in two types of HSV thymidine kinase's antiviral activity depends on cellular localization – in vitro study

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Background: The administration of Herpes simplex virus thymidine kinase gancyclovir-dependent (HSV-TK-GCV) viral constructions is among the first clinical protocols in cancer gene therapy. In spite the advantages of non-toxicity and safety the clinically certified effectiveness is relatively low compared to methods of standard cancer therapy. The aim of our work was to study the possible attitudes of TK activity enhancement comparing two types of viral thymidine kinases transfected into mammalian cells.

Methods: The cDNA of standard therapeutic constructions HSV-TK1 was amplified from pUT649 plasmid, wild type HSV-TK2 was amplified from Herpes simplex virus type 2 genome with primers: for. aa aga tct ATG GCT TCT CAC GCC GGC CA, rev. aa aag ctt CTA AAC YYC CCC CAY CTC GCG GGC AA. Both HSV-TK genes were ligated into pDsRed2-C1 frame downstream of the red fluorescent reporter gene and into p2FP-RNAi replaced turboGFP gene without any tags. pDsRed2-C1-TK1, pDsRed2-C1-TK2, p2FP-RNAi-TK1 and p2FP-RNAi-TK2 were transfected into Cos-7 and CHO-K1 cell cultures. Protein expression and intracellular distribution were analyzed with scanning confocal microscopy. TK expression was determined with Western blot using antibodies produced in laboratory. The GCV-mediated cell suicide activity was assessed with MTT cytotoxicity assay.

Results: Transfection efficiency for all vectors with TK was 50% and higher. The expression of TK1 and TK2 in CHO-K1 cells was higher than in Cos cell line. Confocal examination after different periods of transfectant's selection showed TK1 tagged with DsRed2 enters the nucleus unlike DsRed2-TK2 that was distributed more evenly throughout the cytoplasm. It occurred to be easy to obtain stably transfected cell lines with TK2 and no one TK1 transfectants survived 6 weeks of G418 selection. We observed interference in p2FP-RNAi transfectants appeared in increasing of TK expression after several days since transfection was made. Comparison of two TK types in short-time MTT assays proved TK1 GCV-mediated cytotoxic activity was 2 times higher than for TK2. However after several days of expression and G418 pre-selection TK2 in both pDsRed2 and p2FP-RNAi vectors provided 2–3-folded higher cytotoxic activity starting with lower doses of GCV versus TK1.

Conclusion: We suppose intracellular localization of the TK1 and TK2 proteins due to sequence alterations and able to affect apoptotic activity of the ferment. TK2 cytoplasmic distribution might be important factor for new gene therapeutic vectors development.

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POSTER

A probiotic strain of enterococcus faecium CRL183 reduces DMH-induced large intestinal tumors in male Wistar rats

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Background: Lactic acid bacteria have been reported to have a positive effect on colon cancer. We investigated the influence of the Enterococcus faecium CRL 183 on 1,2-dimethylhydrazine (DMH)-induced colon cancer, aberrant crypt foci (ACF) and modulation the immune response in male Wistar rats.

Material and Methods: 8-week old rat were given subcutaneous DMH injections at 20 mg/kg once a week during three months. Four groups were used: (1) non-treatment control; (2) DMH control; (3) Enterococcus faecium CRL 183-DMH; (4) Enterococcus faecium CRL 183 control. The all groups were compared histologically and TNF- α , IFN- γ and IL-4 cytokines.

Results: The non-treatment control and Enterococcus faecium CRL 183 control not develop tumor. The E. faecium CRL 183-DMH group showed a 50% inhibition in incidence in average number of tumors compared to DMH-control. ACF formation decreased in Enterococcus faecium CRL 183-DMH group and the results were statistically significant in the DMH control. TNF- α , IFN- γ and IL-4 cytokines increasing in this group.

Conclusions: These results show that Enterococcus faecium CRL 183 reduced tumor progression by modulation the immune response

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POSTER

Involvement of AP-1 in cannabinoid antiproliferative action

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We have previously observed that δ^9 -tetrahydrocannabinol (THC), the most important cannabinoid in terms of potency and abundance, reduces human breast cancer cell proliferation by blocking the progression of the cell cycle and by inducing apoptosis. In order to study in further detail the mechanism of cannabinoid action, we performed a DNA microarray-based study of human breast cancer cell response to THC. After normalization and filtering we obtained a total of 28 genes modulated by THC that might be involved in its action. Amongst these genes, we focused our attention on JunD, a member of the activator protein-1 (AP-1) transcription factor family, which is up-regulated by cannabinoid challenge. We validated the involvement of JunD in THC action by two different approaches:

- By means of siRNA, we observed that breast cancer cells lacking JunD were more resistant to THC than the corresponding controls.
- We compared the effect of THC on wt vs. JunD^{-/-} immortalized fibroblasts. As for siRNA JunD-knocked down breast cancer cells, JunD-deficient fibroblasts were more resistant to THC than their corresponding wt partners.

Taken together, these data support that the AP-1 family, especially JunD, is involved in the antiproliferative effect of cannabinoids in breast cancer cells. These results expand our knowledge on the mechanism of cannabinoid action and might set the bases for a cannabinoid-based therapy for the management of breast cancer.

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POSTER

Spleen tyrosine kinase as a novel candidate metastasis suppressor for human oral squamous cell carcinoma

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Background: Spleen tyrosine kinase (Syk) is a non-receptor type of protein-tyrosine kinase that is widely expressed in several epithelial cells. Firstly, aberrant expression of Syk has been reported in breast cancer. Furthermore, recent finding suggests that loss of Syk is linked to poor prognosis and metastasis. However, expression level of Syk in oral cavity remains unclear. In the current study, we investigated the expression levels of Syk mRNA and protein expression in oral squamous cell carcinoma (OSCC)-derived cell lines and human primary OSCCs to elucidate the potential involvement of Syk in OSCC.