

STUDY OF THE STRUCTURE AND BIOLOGICAL ACTIVITY OF TRIORGANOTIN AMINO BENZOATES

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Trimethyl, tributyl and triphenyltins- ters of 3- and 4- aminobenzoic acids have been synthesized and charac- terized by a variety of spectroscopic methods including X-ray diffraction and tested for their in vitro antibacterial and antitumor activity. Within this study their interaction with DNA, and various lines of bacteria (*E. coli* TOP-10 and TOP- 10F) and human tumor cells (K562, 293T/17 and HeLa) have been studied in order to verify the mechanism of their action.

Solution NMR studies for ¹H and ¹³C reveal that in chloroform the tin envi- ronment may be considered to be tet- rahedral whereas in DMSO the solvent co- ordinates to the metal centre enhanc- ing its coordination number. The active organotin compounds studied are the DMSO adducts since they were dis- solved in this medium prior to their use in the biological assays in order to facili- tate their solubility.

MTT assays verify, in accordance with literature, that the tributyltin derivatives possess the highest cytostatic activity against all human tumor cell lines and the trimethyl the lowest, whereas the activity towards the studied bacteria is generally considerably lower. No direct interaction was observed with plasmid DNA. The above observations account for an activity which is well established for tri- organotin compounds and is re- lated to their exotoxicant behavior, i.e. their po- tential interaction with cell mem- branes and the subsequent inhibition of various signaling processes.

MECHANISM OF ACTION OF A FRACTION ISOLATED FROM GALEGA OFFICINALIS L., STUDIED BY FLOW CYTOMETRIC ASSAYS WITH MONOCLONAL ANTIBODIES

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Galega officinalis L. is a plant used for the treatment of diabetes mellitus. A fraction from crude extract of the plant has been purified by chromatography on Sephadex G-25. The preliminary analyses of the fraction show that it consists of about 23% protein and 74% polysaccharides. The fraction is a strong inhibitor of platelet aggregation induced by adenosine 5'-diphosphate (ADP). The mechanism of action of a fraction was studied using flow cytometric assays with monoclonal antibodies CD62P-FITS, specific for P-selectin expression in activated platelet. The experimental results clearly documented action of fraction like inhibitor of the P-selectin expression -a support of platelet adhesion. Possibly the fraction is antagonist of GPIIb/IIIa receptors for fibrinogen, since has a strong disaggregating effect on aggregated by ADP platelet-rich plasma.

NOVEL NANOSTRUCTURED MATERIALS FOR ACCELERATION OF OSTEOINTEGRATION

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The aim of this study was to improve the integration of implants with weak osteoporotic bone by developing of nanostructured implant materials based on ultrananocrystalline diamond (UNCD) coatings in autologous and cell-seeded forms for enhancing osteointegration. To investigate how the nanostructured UNCD films can influence the cell response and osteointegration, the interaction between Si, Ti, as-grown UNCD, UNCD modified with O₂ plasma (UNCD-O) or NH₃/N₂ plasma (UNCD-N) and different cells (MG-63, EPCs, EA.hy926) were studied by proteome analysis. The results showed that UNCD coatings applied on different implant materials can enhance the cells attachment and growth. The proteome analysis revealed the identification of about 19 000 proteins (extracellular and cytosolic). According to their gene ontology annotation database (www.uniprot.org) 178 extracellular proteins were detected from the extracellular matrix (ECM, of total 278) for EPCs. In addition, differences in the abundance of ECM components were found, when comparing the hydrophilic surface modifications and hydrophobic as-grown UNCD. The interaction network analysis revealed differences in the connectivity of inferred protein networks between the ECM niches, which suggest the presence of specific cell microenvironments on UNCD-O2 and UNCD-NH₂ surfaces. Our results provide initial evidence that synthetic nanomaterials may exhibit specific architecture and environment which may serve as superior scaffolding for promoting the cell attachment, differentiation and biomineralization.

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EVALUATION OF THE MITOTIC SPINDLE PROTEIN TPX2 AS TARGET FOR ONCOLOGIC PHARMACOTHERAPY

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Microtubuli-associated proteins could be potent novel targets for anti-cancer therapy. To test this hypothesis, a HeLa cell line was generated, in which the reverse tetracycline-dependent transactivator rtTA2S-M2 drives the expression of eGFP and a miRNA, inducing RNAi-dependent inhibition of TPX2 expression, from the bidirectional tet-regulated promoter (HeLa EM2-11-TPX2). As a negative control for respective experiments a similar cell line was generated (HeLa EM2-11), in which the presence of the inducer doxycycline (dox) activates expression of the genes mCherry and firefly luciferase. First, the effect of TPX2 knockdown on in vitro proliferation was determined by MTT assay. Treatment with vincristine (VC) or docetaxel (DCX) caused a concentration-dependent decrease in proliferation in vitro, whereas knockdown of TPX2 reduced cell growth by 35-45%. The proliferation of HeLa EM2-11-TPX2 was synergistically reduced by the combination treatment dox and VC and additively reduced by dox and DCX when compared to the single agent exposures. Next, the efficiency of tumor growth inhibition following knockdown of TPX2 alone or in combination with VC or DCX was tested in a nude mouse xenograft model. Dox was given via drinking water from day 14 after cell injection until the end of the observation period. VC or DCX were administered twice on days 14 and 21 after tumor implantation. In nude mice, TPX2-knockdown alone caused a 71% reduction in tumor growth. VC or DCX reduced tumor growth by 59% or 76%, respectively. The combination of dox and VC or DCX inhibited tumor growth by 93% or 97%, respectively. Both combination treatments were significantly superior to the respective single treatment arms ($p < 0.05$). We could demonstrate that persistent inactivation of TPX2 expression was highly effective in inhibiting tumor growth, both in vitro and in vivo. It is concluded that TPX2 is a valuable treatment target, causing effective tumor growth inhibition, which can be successfully combined with other anti-mitotic agents.

NEW THERAPEUTIC APPROACHES TO STUDY BLADDER CANCER

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Photodynamic therapy (PDT) is a noninvasive treatment for solid malignant and infiltrative tumors. Light activated sensitizers catalyze photochemical reactions that produce reactive oxygen species which can cause cancer cell death. In this work we investigated the photophysical properties of the photosensitizer ruthenium (II) (RuP), along with its PDT efficiency onto rat bladder cancer cells (AY27). Optical spectroscopy verified that RuP is an excellent photosensitizer, capable to activate singlet oxygen via blue and red absorption bands and inter system crossing (ISC) to the triplet state. In vitro experiments on AY27 indicated increased photo-toxicity of RuP after cell illumination (at 435 nm, 13 mW/cm²), as a function of light exposure, compared to the low 'dark' cytotoxic effect. The surviving fraction was reduced significantly to 15% after illumination (20 min) with RuP (20 μM), compared to 90% in the case of "dark" toxicity. Structural and morphological changes of cells were observed, due to RuP accumulation and light-dependent cell death, using confocal microscopy. Flow cytometry verified that PDT-RuP (50 μM) triggered significant photo-induced cellular damage (79-87 % ±7 %) via late apoptotic/ necrotic mode action. RuP compared to the conventional chemotherapy drug cisplatin, showed higher toxicity, both evaluated by MTT assay. We registered an enhanced effect of the photochemical treatment by RuP in combination with cisplatin, resulting in efficient decrease of cell viability. In conclusion, the present investigation of synergistic interaction of RuP and cisplatin may enable the development of a new combined approach of chemophotodynamic therapy for bladder cancers.

NUCLEOLIN IS A KEY PROTEIN REQUIRED FOR THE TRANSCRIPTION OF GENES INVOLVED IN CELL DIVISION AND PROLIFERATION

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Growth and proliferation of eukaryotic cells require coordination of many cellular processes. Ribosome biogenesis, which allows protein synthesis, involves the simultaneous synthesis of many proteins necessary for the production of ribosomal RNA and ribosome assembly. It must itself be coupled to the output of the cellular factors required for cellular growth and proliferation. The understanding of these mechanisms, and the identification of factors involved in these processes are major challenges in biology. In eukaryotes, ribosome biogenesis takes place in a dedicated space the nucleus, the nucleolus. Nucleolin (NCL) is a highly conserved protein in eukaryotes enriched in the nucleolar compartment. Cell depletion in NCL induces a rapid stop of transcription by RNAPI and an abrupt cessation of proliferation and cell division. NCL interacts with rDNA chromatin and is required for the maintenance of the euchromatin state and transcription elongation of rDNA.

Data from transcriptomics and genome-wide ChIP-seq have identified specific interaction sites (10 bp motif) of NCL in promoters of genes whose transcripts accumulation is affected in the absence of NCL. This motif was previously identified in the promoters of genes involved in cell cycle regulation and protein synthesis. We have also developed a transgenic mouse model that allows the constitutive or inducible inactivation of the Ncl gene. Using these models we show that Ncl is an essential gene required for early embryonic development and in adult. Altogether, our data indicates that NCL is a key protein to coordinate RNPAI and RNAPII transcription of genes required for cell division and proliferation.

3D METAL COMPLEXES OF MELOXICAM DECREASE VIABILITY AND PROLIFERATION OF HUMAN GLIOBLASTOMA MULTIFORME AND CERVICAL CARCINOMA CELLS

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Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications in the world due to their remarkable ability to reduce pain and inflammation and anti-pyretic effect. Epidemiological and clinical studies suggest that some NSAIDs also express anticancer properties. Involving the COX 2 in tumorigenesis and its overexpression in many cancers makes this enzyme an attractive therapeutic target for the prevention and treatment of a number of malignancies. The aim of the study was to evaluate the influence of selective non-steroidal anti-inflammatory drug meloxicam and its metal (Zn(II), Cu(II), Co(II), Ni(II)) complexes on viability and proliferation of cultured human HeLa (carcinoma of the uterine cervix) and 8MGBA (glioblastoma multiforme) cells. The investigations were performed by assays with different cellular/molecular targets and mechanisms of action – MTT test, neutral red uptake cytotoxicity assay, double staining with acridine orange and propidium iodide, alkaline variant of single cell gel electrophoresis (Comet assay) and colony-forming method. The results obtained reveal that applied at a concentration range of 10-500 µg/ml for 24h – 96h the compounds examined decrease significantly viability and proliferation of the treated HeLa and 8MGBA cells and lead to cytopathological changes including DNA damages. Metal complexes (especially Cu(II) and Zn(II)) were found to be more pronounced cytotoxic/cytostatic agents as compared to their ligand meloxicam. 8MGBA cells were found to be more sensitive to influence of the compounds examined than HeLa cells.

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CISPLATIN INTRA- AND INTER-STRAND CROSS-LINKED DNA CONFORMATION AND INTERACTIONS WITH HYDRATED ELECTRONS

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Cis-diamminedichloroplatinum (II) (cisplatin, CPT) is a classical chemotherapeutic drug used in the treatment of various cancers. CPT binds to N7 purine sites in DNA, preferentially on guanine creating cytotoxic intra- and inter-strand crosslinks (CL) in between other damages. In clinic, concomitant CPT-chemo-radiotherapy enhances tumor control and patient survival. The synergistic effects have been manifested by enhanced cell kill and correlate with increased DNA base damage and DNA fragmentation (formation of single and double strand breaks, etc.). It has been shown that among different radiation-induced species the hydrated electrons, e_{aq}^- play important role in the radiosensitization of CPT-modified DNA, i.e. Pt-atom acting as an electron-affinity center; Pt(II)→Pt(I) reduction, eventually followed by cisplatin detachment with the generation of DNA-based free radicals via electron-transfer processes.

To elucidate the fundamentals of e_{aq}^- interactions with CPT-modified DNA (intra and inter-strand cross-linked CPT-DNA) we applied molecular modeling and molecular dynamics, MD (including meta-MD). The e_{aq}^- was modeled as a distance-constrained $(H_2O)_6^-$ anionic water cluster, i.e. Kevan's octahedral cavity with a single shell water structure of -1 total charge, distributed as Mulliken partial charges along the six water molecules. The dynamic conformational properties of both intra- and inter-G*G* cross-linked DNAs show common structural deviations from native DNA, including pronounced DNA-axis bending, unwinding and minor groove widening at the CPT site, with an important difference: the intra-CL-DNA bends towards the major groove, while the inter-CL-DNA axis is bent toward the minor groove with the G*- pairing cytosines flipped out from the DNA helix. In both cases, the bending direction follows the particular groove location of the CPT-unit. Consecutively, the results from meta-MD in the presence of hydrated electrons show frequent e_{aq}^- contacts (electrostatic and close VDW atomic interaction) with the CPT-unit located in the major groove (intra-CL-DNA) and with the CPT-unit located in the minor groove (inter-CL-DNA). Structural DNA deviations which control long- and short-range interactions between CPT-DNAs and e_{aq}^- are thoroughly examined.

EPIGENETIC SIGNIFICANCE OF LINKER HISTONES IN AGEING AND STRESS RESILIENCE

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Certainly, everyone is tempted to slow the process of growing old and to live longer, however, only few probably understand that ageing is a very intricate and quite individual process. Apparently, ageing is governed by many interconnected factors, genetic and environmental and therefore is a podium for extensive research. Among the numerous model organisms which are used in ageing - related studies the yeast *Saccharomyces cerevisiae* is famous for its many advantages. It is a unicellular eukaryote with very well-known genetics and biology. Moreover, it is an organism which permits separate studying of chronological and replicative ageing. We will present our recent results on the ageing of yeast double mutants lacking the gene for the linker histone in combination with a point mutation in Arp4p (actin-related protein 4), an important subunit of several chromatin modifying complexes. By compromising higher-order chromatin structures we have followed the chronological ageing in these mutants. A complex interplay between H1 and Arp4p emerged which in turn was imperative for the ageing of the cells. Moreover, the observed interaction between these proteins resulted in altered ability of the cells to withstand different stress conditions.

Keywords: ageing, *Saccharomyces cerevisiae*, higher order chromatin structure, chromatin remodeling complex, linker histone – H1, actin-related protein 4- Arp4p

MAMMALIAN INO80 IN REPLICATION AND THE MAINTENANCE OF GENOME STABILITY

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INO80 is a multi-subunit ATP-dependent chromatin remodeling complex that has been shown to participate in various nuclear processes such as transcriptional regulation, double strand break repair and nucleotide excision repair, chromatid cohesion and chromosome segregation. We wanted to investigate the role of mammalian INO80 complex in replication and replication-related processes of genome integrity maintenance. The general experimental strategy was to apply RNA interference to knockdown expression of genes encoding unique subunits of the mammalian INO80 chromatin remodeler and to evaluate the functional outcomes on DNA replication, recovery from replication stress and repair of DNA lesions. We found that human INO80 was dispensable for initiation of DNA replication but was needed for replication elongation and we investigated the underlying mechanism. Cells deficient for INO80 subunits had impaired replication restart and accumulated collapsed forks under conditions of replication stress. These observations indicate that the complex is required to stabilize stalled replication forks to allow their subsequent restart. We have also found that INO80 deficiency led to impaired repair of DNA interstrand crosslinks, likely due to reduced speed of replication forks.

IVAN IVANOV

Ivan Ivanov

DOES IT MATTER HOW YOU ENTER? LESSONS FROM A BACTERIA.

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Key words: Salmonella Typhimurium, lipid rafts, Golgi apparatus, intracellular pathogen replication.

Infection with Salmonella Typhimurium is one of the most common causes of gastroenteritis in humans. Entry of *S. Typhimurium* into the human body involves invasion of epithelial cells in the intestinal tract. In the host cell bacteria resides in an membrane bound compartment called SCV. For efficient intracellular replication these SCVs migrate to the perinuclear region in close proximity to the Golgi complex. Intracellular localization in the Golgi region is driven by the bacterial effector SseG and was proposed to be essential for efficient Salmonella pathogenesis.

Using a quantitative proteomics approach (SILAC) we recently identified a number of proteins significantly affected in their abundance in the Golgi fraction upon Salmonella infection. Here we show that the intracellular replication pathway of Salmonella in epithelial cells is predetermined during the entry process and that stomatin-positive cholesterol-enriched microdomains (lipid-rafts) at the plasma membrane direct Golgi-dependent bacterial replication. In the absence of stomatin or lipid-rafts, Salmonella replicates in a dispersed and Golgi-independent manner.

Based on our data we suggest that lipid-enriched, stomatin-positive microdomains at the plasma membrane are essential platforms used during Salmonella invasion of epithelial cells to direct bacterial replication to a Golgi-dependent intracellular niche. These platforms then facilitate the signaling cascade driven by the effector molecules SopE/E2 and the host GTPases, Cdc42 and Rac 1, that results in the Golgi-dependent bacterial replication.

A NEW APPROACH TO DISEASES AND SYNDROMES WITH DNA AND CHROMOSOME ETIOLOGY

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The necessity of the new approach is crucial because of serious issues related to the most common and medically important diseases and syndromes associated with DNA and chromosomal etiology. Among them are: cancer of blood cells and solid tissue tumors, intellectual disability, physical deformity, infertility, spontaneous abortions, Down syndrome and others. After many years of study and billions in spending, these diseases and syndromes are not fully understood and are difficult to predict, treat or prevent. Based on this difficulty, our interdisciplinary international team of scientists has spent 17 years searching for a new approach to understanding the above listed diseases and syndromes. The results obtained reveal an assemblage of 10 previously unknown issues. Four of these issues are related to the main stream theories concerning: the electronic properties of DNA and chromosomes; the importance of heterochromatin; the primary mission of spiralization and packaging of DNA into chromosomes; and the mechanism and mission of chromosomal crossing over. Three issues are related to the breaks, deletions, duplications, amplifications, inversions, translocations, associations, fusions and other DNA and chromosomal abnormalities found in cancer and other diseases and syndromes. Three issues are related to the mechanism responsible for the development of important diseases and syndromes with DNA and chromosomal etiology. All issues have been examined in detail in experimental and clinical studies. The reasons responsible for their development were outlined. New ideas and theories are proposed for solving the issues that have remained unsolved after many years of serious studies of DNA and chromosomes.

ESTABLISHING GENE THERAPY IN THE CLINIC: GLYBERA AND BEYOND

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The presentation will provide a review of uniQure's approach to develop AAV-vector based gene therapy medicinal products. A state-of-the art summary for Glybera will be provided. The clinical development program started 10 years ago with the dosing of the first patient in 2005 (Stroes et al., *Arterioscler Thromb Vasc Biol.* 2008). In total, 3 interventional open label, prospective clinical studies and 2 retrospective analyses on the incidence of disease-related clinical events in the same subjects have been completed in The Netherlands and Canada. EMA approval was achieved in 2012 (Glybera®). Based on this experience we have started clinical development for indications like Haemophilia B and Sanfilippo B. A second focus will be on New Technologies including the AAV-delivery of miRNA and the therapeutic potential of this approach. The lead program in uniQure is for the neurodegenerative Huntington's disease (HD) where the disease-causing huntingtin (HTT) gene is silenced with AAV-delivered miHTT. Proof of concept studies in HD rodent models have shown improvement of neuropathology associated with HTT protein decrease in the brain. Direct intrastriatal delivery of AAV5-GFP by slow or convection-enhanced diffusion (CED) injection or cerebrospinal fluid (CSF) delivery were evaluated in non-human primates (NHP) and minipigs to identify the best biodistribution profile for HD therapy. The AAV-miHTT vector is currently being optimized for future clinical applications. A short summary of this program will be shown including our collaboration with the Institute of Molecular Biology, Sofia. An outline on how we will get from where we are in the pre-clinical program to start a phase I clinical trial will be presented.

EPIGENETIC EFFECT OF AMINO-MODIFIED POLYSTYRENE NANOPARTICLES ON HUMAN CELLS

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PURPOSE: The aim of our investigation was to examine the epigenetic response to nanoparticles in human cells in comparison with their cytotoxic effect. Our standpoint was that nanoparticles affect exposed cells in a complex way.

METHODS: We used 50 nm amino-modified polystyrene nanoparticles in this study. We examined the effect of these nanoparticles on human alveolar epithelial (A549) cells. The cellular adenosine triphosphate (ATP) levels were evaluated by CellTiter-Glo® luminescent assay. The intracellular reactive oxygen species (ROS) were determined by 2',7'-dichlorofluorescein (CM-H2DCFDA) fluorescence assay. The histone H3 lysine 9 acetylation (H3K9) in the chromatin of the treated cells was evaluated in kinetics in a dose-and-time-dependent manner using High Content Analysis (HCA).

RESULTS: The treatment of the cells with high doses (over 50µg/ml) 50 nm amino-modified polystyrene nanoparticles caused ROS formation, strongly reduced the global levels of histone H3K9 acetylation in the chromatin, induced reorganizations in the nuclei of treated cells and triggered apoptosis. A low dose of nanoparticles (25µg/ml) induced cell cycle arrest, which in a time dependent manner was accompanied with modest reduction in H3K9 acetylation in chromatin of treated cells.

CONCLUSION: The results received suggest that the 50 nm amino-modified polystyrene nanoparticles cause epigenetic changes in the chromatin of human alveolar epithelial (A549) cells.

INFLUENCE OF HEPARIN-LIKE OLIGOSACCHARIDES ON HIFN-G – HIFNGR BINDING

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Human interferon gamma (hIFN-g) is an important immunomodulating signaling molecule, also related to a number of autoimmune diseases. The cytokine binds to a specific extracellular receptor, the Interferon gamma receptor (hIFN-gR). Understanding the process of hIFN-g–hIFNgR complex formation is crucial for finding a mechanism for suppressing IFN-g biological activity. We performed extensive molecular-dynamics (MD) simulations for studying the formation of the complex between IFN-g and its receptor. We observed that the positively charged flexible C-termini of the cytokine interact with certain negatively charged domains in the receptor molecules. This interaction impedes the proper binding of hIFN-g molecule with its receptor. The above observation justifies the assumption for involvement of a third participant, facilitating the complex formation. Heparin-derived oligosaccharides are well-known IFN-g ligands. We probed their influence on hIFN-g–hIFNgR binding by means of molecular modeling. For this purpose, we first developed CHARMM compatible force field parameters for the monosaccharide N-sulfated glucosamine. By a series of MD simulations of the interaction between hIFN-g, hIFNgR and heparin-derived oligosaccharides with a different degree of polymerisation, we found that hexa- and octasaccharides bind the C-termini of the cytokine thus favouring the complex formation.

HEPARAN SULFATE: A COMPLEX GLYCOSAMINOGLYCAN THAT “ORCHESTRATES” CYTOKINE’S ACTIVITIES AT THE CELL SURFACE

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Heparan sulfate (HS) is a highly sulfated glycosaminoglycan ubiquitously present in the extracellular matrix and associated to most cell surfaces. This extraordinary complex polysaccharide binds hundreds of proteins, thereby modulating their conformation, stability, local concentration or biological activities. In particular, HS interacts with many signaling proteins at a step prior to receptor recognition, thus regulating a plethora of phenomena such as cell proliferation, cell adhesion, chemoattraction, inflammation, immune response, development, lipid metabolism or angiogenesis.

Chemokines are small cytokines that promote the oriented migration of cells by signaling through G protein-coupled receptors and which binding to HS maintains haptotactic gradients within tissues along which cell can migrate directionally. CXCL12, for example, is a key regulator of leukocyte trafficking and a major chemoattractant for haematopoietic progenitor cells through the receptor CXCR4. Functionally, we observed that HS-binding CXCL12 mutants which *in vitro* remain full CXCR4 agonists had a marginal capacity to attract leukocytes or endothelial progenitor cells *in vivo*, demonstrating the importance of HS for this biological system. Surface Plasmon Resonance based binding assays and mutagenesis as well as engineered ¹³C-labeled HS-like oligosaccharides and ¹⁵N-labeled CXCL12, along with multidimensional NMR spectroscopy, enable us to identify the structural attributes of this HS-chemokine complex that could be used to design specific HS-derived compounds for potential therapeutic intervention. Such tools also permit to investigate how cells regulate the structure of their HS (the synthesis of which is a non-template driven process) and in turn modulate their ability to respond to a given chemokine.

REPLICATION COMPLEX INTEGRITY – MECHANISMS FOR CONTROL THE DYNAMICS OF DNA REPLICATION

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The *S. cerevisiae* complex Tof1/Csm3/Mrc1 is physically associated with the replicative MCM helicase and acts to restrain DNA unwinding when synthesis is prohibited. Mrc1 has a more specific role in replication arrest recovery. Keeping the replication machinery intact allows restart of the replication fork when the block is relieved. Ctf4 is necessary for the stable Pol α association to chromatin. It links GINS and Pol α , that is important to connect DNA helicase and Pol α . That is why Mrc1 and Ctf4 can be indicated as key replisomal proteins, involved in helicase and polymerase harmonization during replication.

The aim of this work is to study the behavior of Mrc1 and Ctf4 during normal and perturbed DNA replication. Our results indicate that after prolonged hydroxyurea (HU) incubation, cells bypass the S-phase checkpoint and proceed throughout the cell cycle. This process is accompanied by Mrc1, but not Ctf4 chromatin detachment. During that process Rad53 checkpoint activity is diminished in order to allow S-phase checkpoint escape and completion of the cell cycle, indicating the specific role of Mrc1 in the process of adaptation to HU.

As both Mrc1 and Ctf4 are involved in SCF^{Dia2} E3 ubiquitin ligase association to the replication complex and both are described to be destabilized in a Dia2-dependent manner, our study also aimed to characterize the role of Dia2 in the control of studied replisomal proteins. Our results demonstrate that Ctf4 is destabilized in a Dia2-dependent manner after the completion of S-phase. We show that Dia2 is responsible to keep the levels of Ctf4 low during G2/M phase of the cell cycle. As Dia2 deficient strains seem to have difficulties when entering G2/M phase and also exhibit prolonged G2/M phase, it can be speculated that decreasing Ctf4 levels in a Dia2 dependent manner, after completion of DNA synthesis is an important event, related to replication complex disassembly and G2/M phase entry.

Our results so far indicate that one of the important mechanisms for control the dynamics of replication is the regulation of the levels of key replisomal proteins.

THE HOX GENES FAMILY: DEVELOPMENTAL CONTROL AND EVOLUTIONARY CONSERVATION

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Members of the Hox class homeobox genes encode transcription factors and are found in almost all eukaryotes. They define positional cellular identities along the major and secondary body axes. The Hox genes expression is controlled by a combination of local and long-range cis-regulatory elements. The regulation in both space and time is critical for embryonic regionalisation. The talk will illustrate molecular mechanisms of developmental control in Mammalian systems: hind-brain, axial and appendages skeleton, hair follicle and skin. Then, evolutionary changes in Hox gene expression patterns will be discussed with emphasis on the neural crest cells patterning and its implication in Vertebrate development. The relevance of epigenetic mechanisms in the control of body plans and segmental diversification will be finally reviewed.

ULTRANANOCRYSTALLINE DIAMOND FILMS FOR BIOAPPLICATIONS

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Diamond is a material with quite a number of excellent properties, like extreme hardness, high elastic modulus, high wear resistance, optical transparency in a broad spectral range, controllable resistivity by the level of dopants, etc. which make it a promising candidate for different applications. Due to the outstanding electrochemical properties, superior chemical inertness and biocompatibility, artificially grown diamond has been recognised as an extremely attractive material for (bio-)chemical sensing and as an interface to biological systems. The ultrananocrystalline diamond (UNCD) films are composed of diamond nanocrystallites of up to 10 nm diameter embedded in an amorphous carbon matrix. They are deposited by microwave plasma chemical vapour deposition from methane/nitrogen gas mixtures. The first part of the talk will describe the growth mechanism and the bulk properties of UNCD, comparing them with other types of diamond films. The second part will address the surface modifications of the UNCD films, which is the first step towards preparation of a biosensor or implant coating, including different plasma and chemical processes, the thorough characterization of the resulting surfaces by a variety of techniques (AFM, XPS, ToF-SIMS, contact angle measurements, etc.) and the possibility to pattern the surface properties. The third part will summarize the bio-properties of UNCD films, like cytotoxicity, bioinertness, non-specific interactions with biomolecules, etc., and will demonstrate examples of bio-applications, like platforms for long-term growth and investigation of neurons, coatings of bio-implants, immobilization of biomolecules (proteins, DNA) and others.

HUMANIZED YEAST: A TOOL TO STUDY PARKINSON'S DISEASE

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Parkinson's disease (PD) is associated with the progressive loss of dopaminergic neurons from the substantia nigra. The pathological hallmark of the disease is the accumulation of intracytoplasmic inclusions known as Lewy bodies (LBs) that consist mainly of post-translationally modified forms of α -synuclein. The budding yeast *Saccharomyces cerevisiae* represents an established model system to study the molecular mechanisms associated to neurodegenerative disorders. Expression of α -synuclein in yeast leads to inclusion formation similar to neurons and significant growth reduction, resembling the pathology of the disease. Various modifications change α -synuclein posttranslationally and alter its inclusion formation, cytotoxicity and the distribution to different clearance pathways. Several of these modification sites are conserved from yeast to human. Whereas phosphorylation is one of the major modifications of α -synuclein in LBs, sumoylation has recently been described. The interplay between α -synuclein phosphorylation and sumoylation is poorly understood. Here, we examined the interplay between these modifications as well as their impact on cell growth and inclusion formation in yeast. We found that α -synuclein is sumoylated *in vivo* at the same sites in yeast as in human cells. Impaired sumoylation resulted in reduced yeast growth combined with an increased number of cells with inclusions suggesting that this modification plays a protective role. A defect in α -synuclein sumoylation could be suppressed by serine-129 phosphorylation by the human G protein-coupled receptor kinase 5 (GRK5) in yeast. These findings suggest a complex interplay between sumoylation and phosphorylation of α -synuclein, which may open new horizons for the development of therapeutic strategies for PD.

KINETICS OF THE RECRUITMENT OF DNA REPAIR PROTEINS TO THE SITES OF DNA LESIONS

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Each cell receives tens of thousands of lesions each day. Cells have evolved an impressive array of repair, signaling and by-pass mechanisms to restore the structure of DNA and to coordinate repair with the cell cycle progression so that the adverse effects of the lesions are minimized. Defects of these mechanisms can lead to devastating diseases in particular premature aging and cancer. Although dozens of proteins were found to participate in several specific pathways of DNA repair, the kinetics of these pathways is still vague. The reason for this is the complex interplay between multiple players, whose complex underlies the maintenance of the genome integrity. Therefore, we apply a state of art laser microirradiation and advanced live cell microscopy to systematically study the kinetic of the recruitment and removal of proteins to the sites of DNA lesions.

EXPRESSION, PURIFICATION AND CHARACTERISATION OF HUMAN INTERFERON-GAMMA ANTAGONISTS

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Human Interferon-gamma (hIFN γ) is a key proinflammatory cytokine in the network that regulates the immune system. Due to this its abnormal expression is associated with many inflammatory and autoimmune diseases. Recently we have been exploring the idea to counteract the over-expression of the endogenous hIFN γ by competitive inhibition with inactive hIFN γ mutants. They are designed to have preserved affinity to the hIFN γ receptor, but to be deprived from their capability to trigger the intracellular signal transduction. To this end a library of mutants was created and they were screened for biological activity and ability to compete with the wild type for the hIFN γ receptor. Based on these criteria two proteins were selected for further investigations: K88Q (Q substitution for K in position 88) and K88Q_T27Y (Y substitution for T in position 27 additional to K88Q). The genes encoding both mutants and the wild type hIFN γ were expressed in *E. coli* employing a developed by us successful methodology for a large scale production of aggregation-prone proteins in soluble native form. The biological activity of the pure and stable in solution mutants showed to be between 100 (K88Q) and 1000 times (K88Q_T27Y) lower than that of the wild type hIFN γ . The binding between the purified proteins and the soluble part of the hIFN γ receptor was studied by isothermal titration calorimetry. The estimated thermodynamic parameters showed that both mutants have fully preserved binding affinity. The obtained results identified these mutants as potentially good candidates for future research and development as autoimmune therapeutics. Acknowledgements to Proteros Biostructures GmbH, TIGO GmbH, Grants DMU 03/23/2011 and DRG 02/05/2010 from the National Science FUND of Bulgaria.

STRUCTURE AND FUNCTION OF RUVBL1 AND RUVBL2 PROTEINS IN BIOMOLECULAR MACHINES

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RUVBL1 and RUVBL2 are related proteins that belong to the AAA+ family. They play essential roles in key cellular processes including chromatin remodeling, DNA damage response and repair, transcription, cell division, snoRNP biogenesis among others. Overexpression of RUVBL1 and RUVBL2 have been found in various cancer tumors but their role in preventing cancer is not unknown. The two proteins participate in different ATP-dependent multiprotein complexes, or “biomolecular machines” which could explain the apparently unrelated cellular pathways. The data on the structure and function of RUVBL1, RUVBL2 and RUVBL1/2 complex and are still controversial and their mechanism in different complexes remain largely unknown.

Our previous studies showed the ATP hydrolysis and hexameric/dodecameric organisation and mutations of the human RUVBL1, RUVBL2 and their complex. More recently we analyzed the large-scale conformational flexibility of these complexes using molecular modeling and molecular dynamics simulations. We show the key principles of domain mobility that affect the protein conformation and biochemical properties, including the downregulation of ATPase activity upon protein hexamerization. The analysis was complicated due to the homo- and hetero-oligomeric assemblies of RUVBL1 and RUVBL2. To circumvent these, we analyzed the single ancient RUVBL paralog found in the archaee *Methanopyrus kandleri* (mkTIP49). Biochemical assays, molecular modeling and dynamics simulations reveal the highly conserved organizational principles of mkTIP49 and identify key residues for ATP hydrolysis. The unexpected crosstalk between Walker B and Sensor I motifs depends on the dynamics of water molecules highlighting, within mkTIP49 oligomeric assemblies, the critical role in the mechanism of ATP hydrolysis.

EFFECT OF MILTEFOSINE ON CYTOSKELETON ORGANIZATION AND LIPID METABOLISM IN EPITHELIAL CANCER CELLS

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Background: Miltefosine belongs to a group of a relatively new class of synthetic alkylphospholipids exhibiting broad anticancer activity. These compounds do not interfere with the DNA or the mitotic spindle apparatus of the cell, instead, they incorporate into cell membranes, where they accumulate and interfere with lipid metabolism and lipid-dependent signaling pathways leading to apoptosis. The aim of this study was to investigate the effect of miltefosine on cytoskeleton organization and lipid metabolism in A549 epithelial cancer cells.

Material and methods: A549 epithelial cancer cell line was used. Actin staining and immunostaining for ZO-1 was performed. The lipid fractions were quantified in isolated cellular membranes by thin layer chromatography. Cholesterol was determined by gas chromatography.

Results: The data show that miltefosine induced reorganization and disruption of actin cytoskeleton as well as increased cell permeability due to the interruption of the tight cell-cell contacts. In addition, treatment of cells with miltefosine induced a decrease of the choline-containing membrane phospholipids, phosphatidylcholine and sphingomyelin, as well as a slight increase of cholesterol level.

Conclusion: The changes in actin cytoskeleton and cell monolayer permeability caused by the miltefosine treatment can contribute to the decreased migration rate of tumor cells. The observed alterations in the content of membrane lipids imply that miltefosine treatment induced changes in the ratio between the major components of raft domains which in turn affects transmembrane signal transduction. On the other hand, reduction of the phosphatidylcholine content due to inhibition of its synthesis is basically related to activation of pathways leading to apoptosis.

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DIAMOND-BASED MULTIELECTRODE ARRAYS FOR THE INVESTIGATION OF NEURONS

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Due to its biocompatibility, large electrochemical potential window and high chemical stability, combined with a number of superior physical properties, all types of diamond films are ideal candidates for platforms for biotechnological applications, such as multielectrode arrays (MEAs) for neuronal signal derivation. In our work we investigated the suitability of the integration of diamond into the fabrication process of MEA structures. For this purpose the deposition of our ultrananocrystalline diamond (UNCD) films was investigated on glass substrates as well as metal layers to achieve a closed and uniform coating. Better optical quality was achieved by decreasing the UNCD layer thickness. For this purpose the primary nucleation density was increased by optimized pre-treatment leading to shorter deposition times. In earlier experiments the improved cell adhesion of pacemaker neurons of *R. maderae* was confirmed on UNCD layers with different surface termination. This fact was used to improve the cell culture preparation technique. Additionally it improves the handling of samples for immunocytochemical tests as there is less cell loss during washing steps. A positioning grid embedded below the UNCD layer enables the allocation of measurements done in different setups to certain cells.

ONCOPHARMACOLOGICAL STUDY OF CURCUMIN LOADED PH-SENSITIVE HYBRID LIPID/BLOCK COPOLYMER NANO-PLATFORMS IN CHEMOSENSITIVE AND CISPLATIN-RESISTANT HL-60 CELLS

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The yellow natural pigment curcumin is a promising drug candidate with pleiotropic antineoplastic activity, whose exceptionally low aqueous solubility and poor pharmacokinetic properties have hampered its development beyond the preclinical level. A possible approach to overcome these limitations is the encapsulation of curcumin into nano-carriers, incl. liposomes. The present contribution is focused on feasibility of using hybrid pH-sensitive liposomes, whereby curcumin is entrapped as a free drug and as a water soluble inclusion complex with PEGylated tert-butylcalix[4]arene, which allows the drug to occupy both the phospholipid membranes and the aqueous core of liposomes. The inclusion complexes were further encapsulated in dipalmitoylphosphatidyl choline:

cholesterol liposomes, whose membranes were grafted with a poly(isoprene-b-acrylic acid) diblock copolymer to confer pH-sensitivity. The liposomes were characterized by DLS, ζ -potential measurements, cryo-TEM, curcumin encapsulation efficacy, loading capacity, and in vitro release as a function of pH. Free and formulated curcumin were further investigated for cytotoxicity, apoptosis-induction and caspase activation in chemosensitive HL-60 and its resistant subline and HL-60/CDDP. The latter is characterized by MRP-1 expression and augmented free radical-scavenging and antioxidant defense machinery *vs*. the parent chemosensitive cell line. Nano-formulated curcumin was superior cytotoxic and apoptogenic agent vs. the free drug. The mechanistic assay demonstrated that the potent proapoptotic effects of pH-sensitive liposomal curcumin presumably mediated via recruitment of both extrinsic and intrinsic apoptotic pathways in both HL-60 and HL-60/CDDP cells.

POSTERS

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RECRUITMENT OF TRANSLESION POLYMERASES POL ETA AND POL KAPPA TO SITES OF UV INDUCED DNA DAMAGE

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The DNA of every nucleated cell is under constant attack from endogenous and exogenous factors which cause hereditary changes that can lead to cell cycle arrest, transformation into cancer cell or cell death. The cellular mechanism that prevent these events involve the activation of numerous DNA repair and checkpoint pathways as a result of the lesion in DNA. These pathways aim to halt the cell cycle progression and trigger the DNA repair machinery in order to deal with the damage and restore the dynamics of the cell division cycle. An important pathway of DNA repair named translesion synthesis (TLS) has attracted much scientific interest. Although the set of proteins involved in translesion synthesis has been discovered and relatively well characterized, its intimate regulation mechanisms are not completely understood. In general TLS involves the exchange of the processive replicative DNA polymerases with specific TLS DNA polymerases that facilitate DNA replication by means of bypassing the DNA lesion. Binding of Pol eta and Pol kappa TLS polymerases to DNA requires monoubiquitination of PCNA by Rad18 E3 monoubiquitin ligase at Lys164 but there may be other ways of TLS polymerase recruitment independent of Rad18 which may rely on ubiquitination by different ubiquitin ligases or even without the need for PCNA ubiquitination.

INO80 CHROMATIN REMODELING COMPLEX PARTICIPATES IN INTERSTRAND CROSSLINK REPAIR

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Interstrand crosslinks (ICLs) are covalent diadducts that physically link adjacent nucleotides on antiparallel strands within the same DNA molecule. The covalent nature of ICLs presents a major challenge to essential DNA transactions, such as replication and transcription, by preventing the melting of the DNA duplex. As a result ICL inducing agents exert a cytotoxic effect, which is especially pronounced in proliferating cells, a property exploited in the clinic to treat oncological disorders. This work examines the role of the mammalian Ino80 chromatin remodeling complex in the repair of ICL lesions. Using RNA interference to silence the expression of INO80, the catalytic ATPase subunit of the complex, we show that knockdown of INO80 increases sensitivity of HeLa cells to mitomycin C (MMC), an ICL inducing agent used in the treatment of bladder and liver cancer. Furthermore we observed a requirement for INO80 in the recruitment of key ICL repair factor FANCD2 to sites of ICL damage visible cytologically as nuclear foci. Following ubiquitination FANCD2 acts a docking platform for repair factors such as nucleases and polymerases. In line with defective FANCD2 foci formation in INO80 silenced cells we found reduced levels of γ H2AX and pS1981 ATM, markers indicative of DNA double stranded breaks, in INO80-silenced MMC treated cells. In addition downstream events such as recruitment of single-stranded DNA binding protein RPA and activation of the checkpoint kinase ATR are compromised in the absence of INO80 following ICL challenge. Together these findings suggest a role of the Ino80 complex in the detection of ICL lesions and setting of a cascade of downstream repair events.

DEVELOPMENT OF LIVING ORGANISMS FROM POINT OF VIEW OF NON-EQUILIBRIUM AND NEGATIVE TEMPERATURE THERMODYNAMICS

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In the non-equilibrium thermodynamics as criteria for evolution of systems to stationary state, the Prigogine's theorem for minimum of mass-specific dissipation function $\psi = (1/M) dS/dt$ is accepted. In this formula M is the mass and dS/dt is the rate of entropy production of the system. For living organisms the ψ function is equal to the mass-specific metabolic rate of organism $q = P_m/M$ (where P_m is the basal metabolic rate and M is the body mass of organism). During organismal development the dissipation function reaches minimum value at the moment of sexual maturity of the organism. But the minimum value of the dissipation function ψ due to the maximum basal metabolic rate (P) and maximum body mass (M) of organism: $\psi_{\min} = P_{\max}/M_{\max}$. At the same moment the maximum values reaches the chemical energy (G) and the internal energy (U) accumulated in the organism i.e. $G = \max$ and $U = \max$. Once the organism reaches the extreme values of the mass and the basal metabolic rate, the organism reaches a stable steady state with minimum dissipative function ($\psi = q = \min$). Such thermodynamic systems that reach a stable steady state at maximum levels of internal, chemical and metabolic energy can be characterized by negative thermodynamic temperature, accordingly thermodynamics of fantom systems with negative temperature. Thus, the question whether the living systems are thermodynamic systems with negative temperature arises.

DIA2-MEDIATED CONTROL OF CTF4 IN THE CONTEXT OF REPLICATION COMPLEX INTEGRITY

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Dia2 from *S. cerevisiae* is a F-box protein that is associated with the modular ubiquitin ligase SCF (Skp1/cullin/F box) to form an E3 ubiquitin ligase. Two key replication proteins - Mrc1 and Ctf4, are involved in SCFDia2 association to replication complex. They physically interact with Dia2 and this interaction is responsible for destabilization of Mrc1 in a proteasome-dependent manner during S-phase checkpoint recovery. Our previous data demonstrated that the S-phase checkpoint escape (a process named adaptation) is also associated with detachment of Mrc1 from chromatin. As we have also shown, Mrc1 plays a key role in the harmonization of polymerase and helicase in the Replication pausing complex. Moreover, recent data shows that Ctf4 is necessary for the stable Pol α association to chromatin and, alike Mrc1, is important for its connection to DNA helicase.

That is why our aim is to study the Dia2-mediated protein level control of Ctf4 in the context of replication complex integrity. Our results demonstrate that Ctf4 is destabilized in a Dia2-dependent manner after the completion of S-phase. We show that Dia2 is responsible to keep the levels of Ctf4 low during G2/M phase of the cell cycle. As Dia2 deficient strains seem to have difficulties when entering G2/M phase and also exhibit prolonged G2/M phase, it can be speculated that decreasing Ctf4 levels in a Dia2 dependent manner, after completion of DNA synthesis is an important event, related to replication complex disassembly and G2/M phase entry.

PHOSPHOGLUCOSE ISOMERASE DEFICIENCY IS LINKED TO INCREASED SPONTANEOUS MUTAGENESIS IN ESCHERICHIA COLI

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The Maillard reaction (glycation) is a spontaneous chemical reaction between primary amines including DNA and carbonyl compounds such as reducing sugars. Recently, we have demonstrated for the first time that the chromosomal DNA of *Escherichia coli* undergoes glycation under normal physiological conditions and accumulates both early glycation (Amadori) products and advanced glycation end products (AGEs). Thus the reasonable question arose which cellular metabolites are involved in DNA glycation. In contrast to glucose, glucose 6-phosphate has higher affinity towards primary amines and therefore may have higher impact on DNA glycation. To test this hypothesis, we constructed an *E. coli* strain with a deleted *pgi* gene encoding the enzyme phosphoglucose isomerase (PGI). It is expected that such a strain will accumulate glucose 6-phosphate due to the block in the early glycolysis. The knock out of the *pgi* gene was confirmed by polymerase chain reaction and Western blotting, and we indeed measured an order of magnitude higher concentration of glucose 6-phosphate in the PGI deficient strain. Moreover, Δ *pgi* cells demonstrated a statically significant increase in the rate of both exponential and stationary phase spontaneous mutation to rifampicin resistance. Unexpectedly, the amount of glycation adducts (Amadori products and AGEs) was higher in the PGI proficient cells. There are two feasible explanation of this observation: i) enhanced DNA repair in the PGI deficient cells and/or ii) loss of glycated DNA during precipitation of DNA from the PGI deficient cells due to glycation-promoted DNA fragmentation.

DIFFERENTIAL SCANNING CALORIMETRY FOR THE MONITORING OF MULTIPLE MYELOMA PATIENTS AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Differential scanning calorimetry (DSC) on blood serum proteome is rapidly advancing as a novel approach for multiple myeloma (MM) diagnostics. Our research has proved that the method identifies specific thermodynamic features for the different secretory and non-secretory MM types that can well be used for patient's stratification (Todinova et al., *Anal. Chem.* 2011, 83, 7992–7998; Todinova et al., *Anal. Chem.* 2014, 86, 12355-12361). Furthermore, our recent study strongly suggested that DSC can be applied for regular monitoring of the effect of treatment of MM (immunoglobulin M isotype) patients since the calorimetric features strongly correlated with other well established as well as with newly introduced immunological markers of the disease progression (Krumova et al., *Thermochim. Acta* 2015, 615, 23–29). In order to validate the calorimetric approach here we applied DSC to monitor patients diagnosed with different MM types that underwent autologous stem cell transplantation. The serum samples were collected before and after (for a period of 1-16 months) the transplantation. The calorimetry derived parameters were compared with the levels of paraproteins, monoclonal free light and involved heavy/light chains. The results revealed correlations between calorimetric and immunological indicators of disease progression that differed for the secretory and non-secretory MM types. Our work demonstrates that DSC has potential to be incorporated in the clinical practice as a sensitive, non-invasive, fast and cheap monitoring test for MM progression.

IN SITU CHROMATIN BINDING ASSAY IN SACCHAROMYCES CEREVISIAE – A NOVEL APPROACH TO STUDY STABILITY OF ARRESTED REPLICATION FORKS

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The *S. cerevisiae* S-phase checkpoint complex Tof1/Csm3/Mrc1 is responsible for normal fork progression and stable checkpoint fork arrest. It is known to stabilize the replicative MCM helicase when DNA synthesis is prohibited. All three proteins are important to keep the MCM complex intact when replication is disturbed, but just Mrc1 is sufficient to guarantee recovery after fork arrest. That is why in our study we checked for alterations in the nuclear behavior of Mrc1 in the process of adaptation to checkpoint-inducing agent. The adaptation is a process of overcoming the S-phase checkpoint and is noticed to take place when cells are subjected to persistent agent treatment or impossibility to repair specific DNA damage.

In order to develop a whole cell study that can permit direct visualization of chromatin attached proteins, we developed an in situ chromatin binding assay for *S. cerevisiae* cells. This method permitted us to perform simple multichannel fluorescent microscopy and directly to visualize the insoluble by detergent, chromatin bound fluorescently-tagged proteins. By this approach we show that during adaptation to prolonged hydroxyurea treatment, Mrc1 dissociates from chromatin to permit replication continuance. Our results demonstrate that the physical presence of Mrc1 at replication pausing complex is required not only for stable fork arrest, but also for the duration of that stability. It can be suggested that the pausing structure is possible when a “clutch” of Mrc1 on MCM is present. This clutch is required for docking Mec1 and Rad53 to ensure their checkpoint kinase activities, leading to fork arrest.

NITRATO COMPLEXES OF PLATINUM(II) WITH N-3-PYRIDINYLMETHANESULFONAMIDE. X-RAY DIFFRACTION, SPECTROSCOPIC AND QUANTUM-CHEMICAL STUDIES

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The revived interest in Pt(II) complexes with pyridine (imine) type ligands comes from the unusual finding of the cytostatic effect of such complexes with trans-configuration (non-classical platinum cytostatics) which, at the same time, retain their activity against cisplatin-resistant tumors [1]. The antineoplastic effect of sulfonamide derivatives also attracts much attention [2]. In continuation of our systematic studies of N-3-pyridinylmethanesulfonamide (PMSA) and its Pt(II) and Pd(II) complexes [3], here we report the structural and spectroscopic investigation of cis- (1) and trans-[Pt(PMSA)₂(NO₃)₂] (2), complemented by quantum-chemical modeling of their molecules. The structure of complex 1 has been solved by single crystal X-ray diffraction: orthorhombic; Pbc₂a; a = 19.8017(6), b = 9.8042(3), c = 24.0279(8); Z = 8; Rint = 0.044, and compared with the calculated one (HF ab initio level with 6-31++G(d,p)/LANL08-ECP basis sets) The analysis of the X-ray powder pattern of 2 revealed the following unit cell parameters: triclinic; P; a = 16.149(6), b = 19.744(7), c = 7.486(4), α = 96.00(4), β = 94.66(2), γ = 93.55(9); Z = 2. The compounds have also been characterized by IR, ¹H, ¹³C and ¹⁹⁵Pt NMR spectroscopy.

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INTERPLAY BETWEEN DIFFERENT DNA REPAIR PATHWAYS

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Each cell receives tens of thousands of lesions each day. Cells have evolved an impressive array of repair, signaling and bypass mechanisms to restore the structure of DNA and to coordinate repair with the cell cycle progression so that the adverse effects of the lesions are minimized. Defects of these mechanisms can lead to devastating diseases, such as premature aging and cancer. Although dozens of proteins were found to participate in several specific pathways of DNA repair, the kinetics of these pathways is still unclear. The reason for this is in the complex interplay between multiple players which is crucial for the maintenance of the genome integrity. Therefore, our work aims to study interplay between different pathways of DNA repair in order to prevent premature aging and cancer.

EXPLORING INTERACTIONS OF NEUROTENSIN ANALOGUES WITH DOCKING STUDIES

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Neurotensin (NT) is a neurohormone and / or neuromodulator containing 13 amino acid residues. Neurotensin receptors (NTR) are transmembrane receptors that bind neurotensin. The active fragment of neurotensin is 8-13 (RRPYIL). The main drawback in the use of NT or any other endogenous peptide as a drug is extremely short half-life as a result of their rapid degradation by the action of peptidases. To overcome this problem, various neurotensin analogues were synthesized, which include linear peptides, cyclic peptides and non-peptide molecules. Chemical modification of the native peptide, however, may result in a radical change in receptor affinity and specificity. The purpose of this work is using computational methods to identify interactions between neurotensin analogues and neurotensin receptor (NTR1) and to establish the relationship between structure and action. Analyzing the results of docking is apparent that the tested analogues of neurotensin bind in the active site of the receptor, but significantly weaker than NT. Replacement of arginine residue in the second position with canavanine strongly increases the total energy of the ligand-receptor complex, therefore it is more unstable than the complex NT / NTR1.

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PATTERNING OF DIAMOND COATINGS FOR BIO-APPLICATIONS

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In recent times, diamond has attracted considerable interest as a material for applications in biology, biotechnology, and biomedicine. This is, on the one hand, due to the outstanding biological properties of diamond. It is composed solely of carbon, the building element of biological structures; furthermore it is biocompatible and chemically extremely inert and stable. On the other hand, it possesses quite a number of outstanding mechanical, optical and electrical properties which can be utilized in biological devices and sensors. For a number of applications nanostructured diamond surfaces are required which can increase the active area for interaction with biomolecules or enhance the interaction with cells. In the current work we have investigated the patterning of nano- (NCD) and ultra-nanocrystalline diamond (UNCD) films, deposited by hot filament chemical vapor deposition and microwave plasma chemical vapor deposition, respectively. The patterning process consisted of deposition of a hard mask (SiO_2 or gold) on top of the diamond coatings and subsequent inductively coupled plasma reactive ion etching (ICP-RIE) with oxygen. Nanostructures of diamond in form of nanopillars with diameters down to 50 nm were prepared after their definition by electron beam lithography (EBL), followed by O_2 RIE. In addition, random nanostructures were prepared with the application of gold nanoislands. The latter were acquired from a very thin Au film (3 or 5 nm) deposited on the diamond films, followed by rapid thermal annealing for dewetting of the Au films and formation of nanoislands.

SYNTHESIS AND STUDY OF CYTOTOXIC EFFECT OF NOVEL AVPI-RGD CONJUGATES

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The compromised ability of cancer cells to undergo apoptosis is a prerequisite for cancers resistance to conventional radio-chemo-therapeutic interventions. Targeting critical apoptosis regulators is thus a promising strategy for the development of new classes of anticancer drugs. Last decades synthesis of small peptides with pro-apoptotic and anti-angiogenic potency is particularly advantageous. We have focused on the synthesis and study of cytotoxic effect of novel AVPI-RGD hybrids. AVPI is a tetrapeptide sequence of N-terminus of pro-apoptotic Smac protein. AVPI binds to some IAP proteins (XIAP, cIAPs) releasing their apoptosis inhibitory effect. RGD is a peptide known to interact with specific overexpressed proteins on the membrane of cancer cells. Thus the conjugation between different AVPI and RGD analogues meant to increase the cytotoxicity and selectivity of the compounds. All peptides we synthesized using the manual stepwise Fmoc solid-phase method. MTT assay was performed in order to examine cytotoxic effect of the hybrids. It was found that hybrids have weak or none cytotoxic effect when used as single agents. Still that is in concordance with the literature data describing Smac-mimetics mainly as agents sensitizing cells to chemotherapeutics. Nevertheless, we showed that AVPI-RGD hybrids exert relatively higher cytotoxic effect than AVPI, AVHyPI, and modified RGDs. Besides that hybrids showed relatively higher effect on HepG2 cells in comparison to MDA-MB-231 cells. One of the compounds - Ala-Val-HyP-Ile-Agb-Gly-Asp showed relatively high activity on HepG2 cells. We may speculate that this effect is caused by the substitution of Arg amino acid in RGD with its analog Agb.

ENHANCING EFFECT OF L-LYSINE ON GLYCATION OF HISTONE H1 AND BOVINE SERUM ALBUMIN IN VITRO

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Glycation is a non-enzymatic post-translational reaction between primary amino groups of proteins and reducing sugars resulting in the formation of advanced glycation end products (AGEs). This reaction contributes to complications in diabetic patients and to other pathophysiological processes in humans. Therefore, many low molecular weight amino and carbonyl compounds have been studied so far for inhibitory effect on glycation. Data obtained with L-lysine are controversial and it is not yet clear whether L-lysine is an inhibitor or enhancer of glycation. Because glycation is a multistage process, we supposed that L-lysine may exert various effects on the different stages of the glycation reaction. In this study we focused particularly on the effect of L-lysine on the advanced stage of glycation. We found that L-lysine enhances the formation of fluorescent AGEs in rat histone H1 and bovine serum albumin treated with either glucose 6-phosphate or D-ribose. Histone H1 also underwent accelerated covalent aggregation and proteolysis in the presence of L-lysine. Resveratrol was further found to suppress the enhancing effect of L-lysine on AGEs-formation, which implies that this effect is oxidation-dependent. In conclusion, L-lysine appears inappropriate for inhibition of glycation in vivo, where over-dosages might accelerate glycation associated pathophysiological processes.

EFFECT OF RESVERATROL ON PLASMA MEMBRANE PROPERTIES OF CELLS CULTURED IN THREE –DIMENSIONAL MATRIX UNDER OXIDATIVE STRESS

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Resveratrol is widely present in various kinds of plants. It is used as a potent antioxidant, anti-inflammatory drug, for treatment of cardiovascular diseases, neurodegeneration and chemoprevention of cancer. In this study the influence of resveratrol on fatty acid composition and physicochemical properties of plasma membranes of cells cultured in tissue-like conditions (3D) was investigated under oxidative stress. Treatment of 3D cells with H₂O₂ caused changes in levels of plasma membrane fatty acid species. The presence of resveratrol in culture media enhanced unsaturated long chain fatty acids and reduced the saturated ones. Moreover, the oxidative stress under resveratrol further increased the level of saturated fatty acids. The temperature dependence of Laurdan generalized polarization (GP) was used for determination of physicochemical properties of plasma membrane bilayer. Oxidative stress slightly decreased Laurdan GP of membrane bilayer. An additional reduction of this value was observed after the incubation of cells only with resveratrol. In contrast, the cell treatment with both H₂O₂ and resveratrol led to large increase of GP values. Since the level of polyunsaturated fatty acids increased during treatment of cells only with resveratrol, the lowest GP values of plasma membrane lipid extract correspond to the highest disordered membrane. The oxidative stress conducted under resveratrol led to higher degree of fatty acid saturation that correlates to the highest measured GP values. Such a membrane is characterized with a comparatively more ordered lipid bilayer compared to the control conditions and resveratrol treated membranes.

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MAMMALIAN RUVBL1 IN DNA REPLICATION

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Mammalian RUVBL1 in DNA replication Ruvbl ATPases are specific components of INO80-class chromatin remodelers forming a structurally discrete module found in INO80, TRRAP-TIP60 and SRCAP complexes. These proteins are involved in various processes such as transcription, DNA repair, chromosome alignment and segregation in mitosis and their functional diversity is likely due to participation in different complexes or complex sub-assemblies. The potential role of Ruvbl proteins in replication has not been explored. Previously, we have shown that the knock-down of Ino80 and Arp8 subunits of INO80 remodeler led to deficient replication elongation and destabilized stalled replication forks, implying a similar requirement for RUVBL proteins. In line with this expectation, knock-down of RUVBL1 slowed S-phase progression and led to reduced rate of replication elongation as found by DNA fiber labeling. However, we have found that the knock-down of RUVBL1 did not affect the restart of stalled replication forks. Further analyses using indicated that Ino80 and RUVBL1 deficiencies affected fork progression by different mechanisms. These results suggest that INO80-class complexes or their sub-assemblies possess both RUVBL-dependent and independent functions that are required during DNA replication.

CYTOCHROME C ADSORPTION ON MONTMORILLONITE NANOPLATES

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Beside its basic function as electron carrier in the respiratory chain, the hemo-protein cytochrome c [cytC] plays important role in the early phase of apoptosis. In cancer cells its functions are blocked but exogenous cytC (introduced by microinjection, electroporation, etc.) activates the apoptosis which finish with cell destruction. We intend to introduce cytC into cancer cells by phagocytoses of colloid particle. For this aim, as a first step, we study adsorption of cytC on montmorillonite particles (MM, nanoplates having negative pH-independent charge and high surface/mass ratio) using the methods of electric light scattering (particle orientation in sinusoidal electric field) and microelectrophoresis (electrophoretic mobility in direct field). The measured at pH 6.5 (where cytC globules are positively charged) interfacial electric polarizability, surface electric charge and size of cytC-MM particles as function of cytC-concentration shows out that the total (net) charge turns its sign from negative to positive, the isoelectric point appears at 5:3 mg/mg cytC/MM, saturated protein adsorption is reached at additional two-fold increasing of cytC/MM ratio and the suspension is stable at low and high protein concentrations; at intermediate ones (where the total charge is near to zero) aggregation arises. Reference: [1] Sv. Hristova, A. Zhivkov, B. Atanasov, Electrostatics of horse heart Cytochrome C and Montmorillonite monolamellar plate, *Biotechnol. & Biotechnol. Equipment* 23 (2009) 568-571. [2] S.H. Hristova, A.M. Zhivkov, Adsorption of cytochrome c on montmorillonite nanoplates: protein concentration dependence, *J. Colloid Interface Sci.*, 446 (2015) 252-262.

MRC1 REGULATES THE STABILITY OF S-PHASE CHECKPOINT ARREST

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Hydroxiurea (HU) is an inhibitor of Ribonucleotide reductase – the enzyme that catalyzes the process of free dNTPs synthesis in living cells. Treating cells with HU causes diminishment of the nucleotide pool. As a result, single-stranded DNA regions are generated, which leads to S-phase checkpoint activation. The progression of replication forks is blocked and the completion of DNA replication is prevented. This results in S-phase cell arrest.

Nevertheless, our results demonstrate that after prolonged HU treatment, the *Saccharomyces cerevisiae* cells seem to escape the arrest and continue the progression of their cell cycle. We show that when cells re-enter the cell cycle, Mrc1, but not Ctf4 is detached from chromatin. Our data also shows that meanwhile, Rad53 checkpoint activity is diminished in order to allow S-phase checkpoint escape and completion of the cell cycle. Moreover, cells not only continue the cell cycle, but steadily surmount in the presence of HU. All this data indicates that cells have made the decision to compromise S-phase checkpoint and to adapt to the novel environmental conditions in order to survive.

As both Mrc1 and Ctf4 are known to be responsible for polymerase and helicase harmonization during replicative arrest, our data indicates that Mrc1 has a more specific role in the process of adaptation. Our data demonstrates that Mrc1 is a leading protein to regulate the stability of S-phase checkpoint arrested replication forks.

GLOBAL GENOMIC STUDIES OF INFLUENZA A VIRUSES: IDENTIFICATION OF CONSERVATIVE SEQUENCES FOR DEVELOPMENT OF WIDE SPECTRUM VACCINES AND GENE THERAPY DRUGS

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Influenza virus is highly infectious and causes worldwide flu epidemics and pandemics. The annual flu epidemics result in between three to five million cases of severe illness and between 250,000 and 500,000 deaths around the world (WHO EB111/10). There are 18 different types Influenza A virus, according to the structure of their major surface antigens hemagglutinin (H) and neuraminidase (N). H (H1 to H18) and 11 of the N (N1 to N11) antigens. All combinations between the two are possible. Influenza viruses H17N10 and H18N11 were discovered in 2013. Besides the great variety in H and N antigens in nature, only H1, H2 and H3, and N 1 and N2 are found in humans. The progress in whole genome sequencing after the year 2000 paved the way for comprehensive investigation of the flu virus variations under the Influenza genome sequencing project (IGRP). It is initiated in 2004 by the National Institute of Allergy and Infectious Diseases (NIAID) and presently operates under The Venter Institute. The present study aims to shed more light on the changeability, evolution and adaptation of the influenza virus A genome in order to design new anti-flu vaccines and anti-flu drugs. To this end we employed the publicly available NCBI GenBank (<http://www.ncbi.nlm.nih.gov/genomes/FLU/>) database and the ClustalW_MPI software package adapted to the Bulgarian BlueGene/P supercomputer for identification of conservative domains in the influenza virus A genome that code for immunogenic peptides or representing unique siRNA target sites.

HMGB1 PROTEIN AFFECTS THE EXOGENOUS RAGE TURNOVER AND INCREASES THE MOTILITY OF THE INVA- SIVE MDA-MB-231 CELL LINE

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HMGB1 protein and its receptor RAGE are considered as a ligand–receptor pair that plays an important role in regulating the invasiveness of tumor cells. We hypothesized that increased basic expression and/or post-translational ligand dependent stabilization of RAGE in the presence of HMGB1 in the extracellular medium influence the motility of tumor cells and promote invasiveness and meta-static potential.

We tested the endogenous level of RAGE as mRNA and protein synthesis in breast cancer cell lines (MCF-7 - noninvasive, MDA-MB-231 - invasive) compared to normal one (MCF-10). The RAGE amount was elevated more than two times in the invasive cell line while in the noninvasive MCF-7 cells the increase was lower. The endogenous level of RAGE was least in MCF-10, which have normal epithelial morphology. We studied the changes in the exogenous RAGE expression in the presence of HMGB1 and its truncated form HMGB1 Δ C. The GFP RAGE signal strongly diminishes up to 4 times after 72h in all tumor cells. Upon addition of HMGB1 and HMGB1 Δ C a stabilization of GFP RAGE was detected in MDA-MB-231 and the strongest effect was detected for the truncated form. One possible explanation is the formation of a stable HMGB1/RAGE complex that blocks the proteolytical activity of metalloproteinases, which does not hold true for MCF7.

Next we tested the influence of HMGB1 and HMGB1 Δ C on cell motility of breast cancer cells. HMGB1 increase significantly only the motility of MDA-MB-231 while HMGB1 Δ C barely influence the process. Both proteins had no effect on MCF-7 nor on MCF10 cells.

INTRA- AND EXTRACELLULAR PRODUCTION OF RECOMBINANT HUMAN INTERFERON-GAMMA AND ITS MUTANT IN INSECT CELL LINES

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The expression systems employed for manufacturing of recombinant proteins include bacterial, yeast, plant, insect and mammalian cells. Their choice depends on numerous factors as protein properties, intended applications, yield and cost. *Escherichia coli* is often preferred due to its low cost and simplicity of cultivation. The over expression of the recombinant protein, however, frequently leads to increased aggregation and formation of "inclusion bodies". Human interferon gamma (hIFN γ) is an immunoregulatory cytokine that has been expressed in *E. coli* for therapeutic uses. The eukaryotic hosts, though, offer the possibility to express recombinant hIFN γ that is structurally similar to the one circulating in the blood stream, due to the naturally occurring glycosylation. Our previous attempts to express the wild type hIFN γ and some hIFN γ mutants in human cells led to unsatisfactory low yields and reduced cell viability. For this reason hIFN γ and its highly prone to aggregation and less stable mutant K88Q were expressed in insect cells. Both targets proteins were cloned in baculovirus expression vectors as fusion proteins with a FLAG sequence, which significantly improves its detection and purifications when used with highly specific anti-FLAG antibodies. Cell line Sf9 was used for intracellular expression whereas High Five $\text{\textcircled{R}}$ was employed for secretory expression. Two-step (affinity and size-exclusion) chromatography was carried out for protein purification and the FLAG tag removal was optimized. The comparison between both expression systems showed an advantage of the secretory expression in High Five $\text{\textcircled{R}}$ cell line leading to higher yield of both proteins comparing to the intracellular expression.

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MODULATING ACTION OF VITAMIN C ON THE CYTOTOXIC EFFECT OF MYOSMINE

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Myosmine (3-(1-pyrrolin-2-yl)pyridine) is an alkaloid found in tobacco as well as in various foods, fruits and vegetables. Myosmine has recently been suspected to be a tobacco-independent carcinogenic source connected to the increase in incidence of esophageal adenocarcinoma. It has been shown that reactions of nitrosation and peroxidation of myosmine take part in its metabolic activation. Increased cancerogenic potential of myosmine may be expected when the oxidative stress in the cell is enhanced. In order to examine the relationship between the oxidative status of the cells and the inhibitory action of myosmine on the cell growth we studied the combined effect of the antioxidant ascorbic acid (vitamin C) and myosmine on cell proliferation as well as on the cell cycle of murine erythroleukemia (MEL) cells. Protective effect of vitamin C was found at incubation times of 48 hours. It was clearly observed that vitamin C had reduced the toxicity of myosmine in a wide range of concentrations. In order to see how cell cycle is affected in the presence of vitamin C and myosmine we conducted flow cytometric analysis. A combination of vitamin C with 100 μM myosmine resulted in a histogram, almost identical to that of the myosmine control. This result showed that the antioxidant had reduced the influence of myosmine on the cell cycle. When myosmine concentrations are higher (200 and 300 μM) addition of vitamin C reduced the amount of hipoloid cells almost in half. Conclusion: Vitamin C may protect cells from the cytotoxic effect of myosmine.

GENERATION OF A MONOCLONAL SCFV ANTIBODY TO STUDY PROTEIN GLYCATION IN EMBRYOGENIC SUSPENSION CULTURES OF DACTYLIS GLOMERATA L. UNDER SALINITY STRESS

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Glycation of proteins results in the formation of advanced glycation end products that irreversibly modify proteins and thereby impair their functions. Various abiotic stresses in plants lead to the overproduction of reactive oxygen species, which are known to accelerate glycation. Despite this fact, protein glycation in plants under stress conditions has not been studied yet. In this study, poly-L-lysine was glycated with D-glucose, and absorbance and fluorescence spectroscopy ($\lambda_{ex}=300$ nm, $\lambda_{em}=440$ nm) were applied to confirm glycation. Glycated poly-L-lysine was used as an antigen for the generation of a single-chain fragment variable monoclonal antibody (scFv mAb) by the phage display technology. *D. glomerata* L. suspension cultures, subjected to moderate (0.085 M NaCl) or severe (0.170 M NaCl) salt stress, were studied for protein glycation using the isolated scFv mAb. We found a negative correlation between the level of protein glycation and the presence of NaCl in the suspension cultures of *D. glomerata* L., which points to the protective role of salt stress against glycation.

INFLUENCE OF GLUTAMATE ON BESTROPHIN-1 INTERACTION WITH POPC IN LANGMUIR–BLODGETT FILMS

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Bestrophin-1 (Best1) is a transmembrane multifunctional protein, expressed in the plasma membrane of retinal pigment epithelium. Best1 has been identified as Ca²⁺-activated chloride channel that may act also as regulator of ion transport or voltage-gated Ca²⁺ channels. The specific interactions with plasma membrane lipids are absolutely necessary for its conformation, oligomerization and expression of different functional activities. Recently, it has been proposed that Best1 plays important role in the transport of glutamate and gamma-aminobutyric acid. We have been focused on the influence of glutamate on Best1 interactions with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in Langmuir monolayers since it is the most abundant phospholipid of animal cell membranes. The π/A (surface pressure/area) isotherms and compression/expansion isocycles of Best1, POPC and mixed Best1/POPC monolayers were recorded in absence and presence of glutamate in the subphase. The effect of glutamate on the morphology of monolayers was observed by Brewster angle microscopy (BAM). Our study shows that the incorporation of glutamate in the subphase does not change the shape of the π/A isotherms of the mono-component (hBest1, POPC) and mixed hBest1/POPC monolayers but slightly increases the mean molecular area of the protein monolayers. BAM morphology images clearly show that the presence of glutamate induces the formation of Best1 macromolecular aggregates during monolayers compression. We assume that glutamate may play a key role for the interaction of Best1 with POPC at physiological conditions in the cell.

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DO C-TAIL AND POSTSYNTHETIC ACETYLATION MATTER FOR THE MOBILITY OF HMGB1 IN LIVING CELL

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High-mobility group box 1 protein (HMGB1) is a highly conserved nuclear protein, that facilitates DNA bending, stabilizes nucleosome formation, and modulates the interactions of regulatory molecules with their targets. By doing this it is involved in practically all chromatin dependent cellular processes. These properties of HMGB1 were shown to be modulated by the acidic C-terminal domain of the molecule as well as by some postsynthetic modifications such as acetylation. As HMGB1 shows low if any sequence specificity we hypothesized that protein mobility in the nucleus is an important feature for its complex functions. We have used Fluorescent Recovery After Photobleaching (FRAP) to determine the protein mobility and to examine if there is any difference in the mobility of its modified forms. We constructed EGFP fused recombinant HMGB1 variants: full length and truncated (lacking C-tail) HMGB1 proteins as well as forms mutated in two known sites for acetylation – Lys 2 and Lys 81. Our experiments showed that the truncated protein has significantly lower rate of mobility compared to the full length HMGB1, interestingly the mobility of the truncated form was even further decreased when mutations in the sites for acetylation were introduced. Full length HMGB1 with mutation in the acetylation site Lys 2 exhibited velocity closer to that of the truncated form. FRAP analysis revealed that HMGB1 is an extremely mobile protein which probably scans rapidly the whole chromatin matrix in order to find the places and the processes where it is needed. As the velocity of the protein decreases when the sites for postsynthetic acetylation are mutated one can presume that acetylation took an important place in the tuning of the mobility of HMGB1.

CHEMICAL STABILITY OF NEW ANALOGUES OF OCTREOTIDE

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Somatostatin (SST-14 H₂N-Ala-Gly-c(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)-COOH), is a hormonal cyclopeptide produced by neuroendocrine neurons of the hypothalamus, gastrointestinal, immune cells, as well as by certain tumours. It has a broad range of biological action that includes inhibition of growth hormone (GH), insulin, and glucagon and other hormones secretions, suppression of gastric and pancreatic exocrine secretion, gut motility, cell proliferation and also plays a role as a neurotransmitter. Some modified octapeptide analogues of somatostatin with the following structure D-Phe-c(Cys-Phe-D-Trp-Xxx-Yyy-Cys)-Thr-NH₂, where Xxx is Lys or Orn and Yyy is Aib (α -aminoisobutyric acid), Ac5c (1-aminocyclopentanecarboxylic acid) or Ac6c (1-amino cyclohexane carboxylic acid) have been synthesized [1]. We evaluate cytotoxic potential of the parent compound and new analogues in cultured non-tumour Lep-3 cells and panel of tumour HepG2; HT-29; MDA-MB-23 and HeLa cell lines by the MTS assay also. The new synthesized analogues have shown improved growth-inhibitory effects on HT-29 cells. The purpose of this study was to evaluate the stability of the synthesized analogues in different values of pH, available physiologically.

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GROWTH CONDITIONS INFLUENCE THE EXPRESSION OF RFB E GENE RESPONSIBLE FOR SYNTHESIS OF LPS IN E. COLI O157

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Introduction: *Escherichia coli* O157 is a foodborne pathogen causing severe disease in humans worldwide. Healthy cattle are natural reservoir of *E. coli* O157 and people can be infected via consuming contaminated food and water. During the transmission cycle the bacteria are passing through different environmental conditions. This study observed the possible effects of culture conditions (different temperature and media) on the expression of the *rfbE* gene responsible for O157 LPS antigen.

Materials and Methods: *E. coli* O157:H(-), A2CK SS was cultivated on nutrient agar or in nutrient broth at 37°C or 20°C. We applied different methods as qRT-PCR, immuno-gold labelling, immunofluorescence, whole-cell ELISA. **Results:** Lectin-gold and immunofluorescence experiments showed that, in the same bacterial culture variable phenotypes were present according to degree of access at the surface. The results of whole-cell ELISA tests showed that the strongest binding of polyclonal serum was performed in bacteria cultured at 37°C on agar. The expression of the *rfbE* gene was tested by qRT-PCR and was calculated as a percentage of "control group" 37°C agar compared with gene expression in other culture conditions (20°C agar, 20°C broth and 37°C broth). The percent of gene expression of the *rfbE* gene in the test groups was reduced compared to the control. **Conclusions:** The experiments confirmed that the cultivation on agar at 37°C promoted expression of *rfbE* gene which supports the data obtained by ELISA tests. The localization of O-specific LPS demonstrated by gold-labeling and fluorescence technic showed variations between individual cells. This phenomenon may be a part of adaptation of whole population at the host environments.

SPECTROSCOPIC ANALYSIS OF PLANT LECTIN-PORPHYRIN INTERACTIONS FOR TARGETED DRUG DELIVERY

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Based on the known property of some plant lectins to agglutinate preferentially cancer cells, lectins could be applied as specific drug targeting molecules. Many metal-based porphyrin compounds are considered as promising anticancer agents; therefore they appear to be an appropriate alternative to the chemotherapeutic drugs. Recently it was found that similar to some anticancer drugs, porphyrins cannot recognize selectively tumor tissues. Considering this we have focused our studies on the capability of metal-based porphyrins to be recognized by two plant lectins: wheat germ agglutinin (WGA) and Concanavalin A (Con A). The present report summarizes spectroscopic data about the binding activities of Con A and WGA towards porphyrin compounds with anticancer activities. Steady-state fluorescence showed conformational rearrangements upon porphyrin binding. We calculated dissociation constants that suggested high affinity binding of the porphyrins to WGA and Con A. The hyperbolic binding curves suggest the presence of one site for the most porphyrin compounds. However, the sigmoid shape of the curve of Con A-Pd porphyrin complex reveals more than one class of binding site with positive cooperativity, confirmed by the Hill slope. Also, using time-resolved phosphorescence we studied the photophysical properties of Pd photosensitizer, bound to Con A and found that the photosensitizer remained a good producer of singlet oxygen in the complex that could find potential application in photodynamic therapy (PDT) of cancer. In conclusion, WGA and Con A interact with porphyrins, possessing anticancer activities which presume their utility in targeted drug delivery.

MRNA ABUNDANCE OF DLG1 AND CAPT, GENETICALLY INTERACTING WITH DFMRP IN DROSOPHILA BRAIN

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Fragile X syndrome (FraX) is an inherited disease, caused by the transcriptional inactivation of the gene FMR1 and the loss of its protein product FMRP. It is an RNA-binding protein involved in the transport, stability and posttranscriptional expression of key neuronal mRNAs. To further understand the molecular and cellular mechanisms underlying the disease, we used the *Drosophila* FraX model and investigated a not well studied role of dFMRP in alternative splicing of neuronal mRNAs to which it binds via a G-quartet sequence. By means of qRT-PCR we established the relative abundance of some isoforms of the gene *dlg1*, resulting from alternative exon skipping nearby a G-quartet and an exonic ESE-sequence, both acting as exonic splicing enhancers. We also investigated the relative mRNA-abundance of all *capt* – isoforms and the pre-mRNAs of both genes. We proposed a possible involvement of dFMRP in alternative splicing of genes, interacting with *dfmr1*. In the absence of dFMRP in larval and pupal brains, we found a change in the mRNA-level of one of the studied isoforms of *dlg1* and of its pre-mRNA.

PHYLOGENETIC DIVERSITY OF BACTERIOPLANKTON INHABITING THE HIGH ALTITUDE OKOTO LAKE, SEVEN RILA LAKES

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Bacterial community diversity in Okoto Lake bacterioplankton assemblages were assessed using 16S rRNA gene clone libraries construction. Okoto Lake is the deepest glacial lake (37.5 m) on the Balkan Peninsula, situated on the territory of Rila National Park at 2 440 m.a.s.l. with surface area of 6.8 ha, water volume of 860 000 m³, and watershed area of 36 ha. Water samples were collected from 0.5 m depth under the surface in July and September 2006 and July 2007. In total, 297 clones from the libraries were selected and the dominant groups of them were partial sequenced. Bacterial 16S rRNA gene sequences exhibited relatively high phylogenetic diversity. Representatives of Proteobacteria (beta and gamma subdivisions) were the most dominant in the samples, followed by Bacteroidetes and Actinobacteria. Temporal shifts in the bacterial diversity were observed with the domination of Betaproteobacteria (35 - 48 %) and Gammaproteobacteria (20 - 26 %) during the July, 2006 and 2007, and Bacteroidetes (23 %) in September 2006. Actinobacteria comprised from 10% in September 2006 to 14% in July 2007 and were absent in July 2006. The bacterial abundance and diversity were high in July 2006/07 due to the spring peak in the development of phytoplankton and still low abundance of the zooplankton. The principal components analysis revealed that the concentrations of ammonia, phosphates and dissolved oxygen, the abundances of phytoplankton (chlorophyll a) and zooplankton, as well as the share of Bacteroidetes, Actinobacteria, and Gammaproteobacteria were the main factors determining the temporal variability of Okoto Lake.

ANTICANCER ACTIVITY OF NEW FERROCENE DERIVED (+)-CAMPHOR DERIVATIVES BEARING SUBSTITUTED SULFONAMIDE GROUPS

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Organometallic chemistry and biochemistry have been merged in the last two decades into a new field - bioorganometallic chemistry. Ferrocene compounds play the main role in this field, especially since the first ferrocene derivate with anticancer activity was reported in 1984 by Köpf-Mayer et al. A very important class of compounds with strong antiproliferative effects are ferrocifen and its derivatives, recognized as successors of clinically used anticancer agent tamoxifen (against breast cancer). A series of new conjugate ferrocene derivatives (+)-camphor and (+)-camphor sulfonamides were synthesized, purified and characterized. All new compounds were examined for their anticancer activity.

IMMUNOGENIC PEPTIDES IN SARCOMAS

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Ewing sarcoma family of tumours (ESFT), Synovial sarcoma, Clear cell sarcoma, and Desmoplastic small round cell tumor are aggressive, malignant bone and soft tissue tumors that predominantly affect children and young adults. The structural disorder of fusion proteins in sarcomas is essential to the oncogenic function, and the vicinity of the breakpoint is highly disordered. The disorder of fusion proteins could be used to design immunogenic peptides. The functional regions of fusion proteins are related to high IPD and probability of PBRs formation. The entire sequences of fusion genes in sarcomas need to be studied to identify antigenic peptides. Investigated is the immunogenic property of peptides derived from sarcoma-specific fusion genes (EWS-FLI1, SYT-SSX, PAX3-FKHR and others), where predominantly peptides spanning the fusion regions. The findings indicate the safety of some studied junction peptides in the use of vaccination. Some specific peptides displayed potent killing of multiple ESFT types in vitro and show that they are promising antigens for immunotherapy of sarcomas, and warrants further clinical development. A peptide may significantly improve clinical outcomes for patients with ESFT undergoing immunotherapy. Some patients receiving immunotherapy against sarcomas experienced minimal toxicity and favorable survival. Chimeric genes identification provided a unique opportunity to examine the immunological response against the tumor-specific chimeric sequences, which serve as putative antigenic peptides. There is a need to increase efficacy by using more immunogenic antigens and more potent dendritic cells. The trials may be useful for the development of novel immunotherapies for sarcoma patients with proved chromosomal translocation.

THE EXPRESSION OF RECOMBINANT HMGB1 PROTEIN IN E.COLI IS MODULATED BY ITS C-TERMINAL DOMAIN

Jordana Todorova

High-mobility group box-1 (HMGB1) as a DNA binding protein plays important role in essential cellular processes as: repair and replication of DNA, remodeling and gene expression. HMGB1 consists of two DNA binding motifs called HMG boxes and an acidic C-tail comprising a continuous array of 30 acidic amino acid residues. The C-terminal tail was proved to affect the biological activity of HMGB1 protein. In several recent studies it was shown that HMGB1 has an antibacterial activity. The DNA of full length HMGB1 (HMGB1) and HMGB1 without C-terminal domain (HMGB1 Δ C) were cloned by PCR technique in pET28a+ vector by the appropriate primers and expressed in E coli. The protein expression level differed for both constructs as well as for the ratio between the soluble and insoluble protein fractions. We conclude that the acidic C terminus shows strong inhibitory effect on the protein expression especially after 15 hours when the recombinant protein is accumulated and displays substantial antibacterial activity. Our findings demonstrate some new regulatory properties of the non-structural acidic C tail of HMGB1 that must be taken under consideration when the recombinant protein is produced in bacteria and used for further investigations.

Keywords: High-mobility group box-1 (HMGB1), C-terminal tail, antibacterial activity

AN ATTEMPT TO DEGLYCATE HUMAN INTERFERON-GAMMA WITH ESCHERICHIA COLI FRLB AMADORIASE

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The Maillard reaction (glycation) is a non-enzymatic reaction between primary amines and carbonyl compounds. The participation of proteins in this reaction results in protein structural alterations, proteolysis and covalent aggregation. Pharmaceutical proteins modified with advanced products of the glycation reaction (AGEs) are immunogenic, have lower biological activity and reduced therapeutic efficacy. Therefore, approaches are needed to either prevent glycation or "repair" glycated proteins. Here, we describe an attempt to deglycate *Escherichia coli*-derived human interferon gamma (hIFN γ) by treatment with the enzyme FrlB amadoriase encoded by the host bacterium. The FrlB enzyme is known to catalyze the decomposition of Amadori products (AGEs-precursors) formed on lysine residues, and our previous studies have shown that FrlB may have broader substrate specificity. To test the FrlB applicability for removal of APs from hIFN γ , fresh hIFN γ preparations were treated with the FrlB enzyme added either in solution or in membrane-immobilized form. To our surprise, treatment with FrlB resulted in rapid hIFN γ fragmentation and oligomerization, especially when the enzyme was immobilized. We further found that in the reaction mixture of hIFN γ with FrlB, hydrogen peroxide and glucosone were formed. In conclusion, the FrlB amadoriase appears to degrade APs formed on proteins by oxidative mechanism and thus is inappropriate for deglycation of therapeutic proteins.

ERUFOSINE CAUSES CELL CYCLE ARREST IN BREAST CANCER CELLS AND DOWN REGULATES KEY CELL CYCLE REGULATORY MOLECULES

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Breast cancer is the second leading cause of cancer deaths among women. The cytotoxic activity of the alkylphosphocholine erufosine - a synthetic phospholipid analogue with antineoplastic activity was assessed on two breast cancer cell lines MCF-7 – non invasive and MDA-MB-231 – invasive. A 24h exposure to erufosine significantly inhibited the growth of both cancer cell lines, with IC₅₀ values ranging from 20 to 40 mM and induced accumulation of cells in the G₂/M phase in a dose-dependent manner. Cyclin-dependent kinases (CDK1, CDK2 CDK4 and CDK6) that play a key role in the regulation of the cell cycle progression and some of specific cyclins (B1, E1 and E2) have been tested by RT-qPCR at three time points (24, 48 and 72 hours) and with three drug concentrations (IC₂₅, IC₅₀ and IC₇₅). All CDKs except CDK6 were down regulated at 24 and 48 hour of the treatment. Cyclin B1 oscillate over the course of the cell cycle reaching a peak just prior to mitosis. We found this cyclin strongly down regulated at 24 hour then slightly increase but still is down regulated at 48 and close to the normal level at 72. Same profile was detected for cyclin E2 but not for cyclin E1, which keeps a normal level in MCF-7 and is even up regulated in MDA-MB-231. The difference could be explaining with the different sensitivity of cyclin E1 and E2 to induction by E2F transcription factors cyclin E2 have been shown inherently more sensitive.

NANOPARTICLES CONTAINING A COPPER CHELATOR: A POSSIBLE INSTRUMENT FOR RADIATION PROTECTION

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Nanotechnology is considered as an emerging technology with great potential in a wide range of applications. In addition to various industrial uses, several innovations are foreseen in biotechnology, medicine and medical technology. The application of nanotechnology to medicine also provides an innovative approach that can enhance the effectiveness of radiotherapies or radioprotection. The aim of the present study was to investigate the radioprotective effect of neocuproine-nanodiamond nanoparticles on gamma irradiated human hepatocellular carcinoma cells. We revealed a significant increase in the radioprotection efficacy against gamma irradiation of HepG2 cells pre-exposed to nanodiamonds or nanodiamonds-neocuproine nanoparticles. The results showed that the protective effect of nanoparticles is connected with decreasing free radicals released during the irradiation and with cytokinesis-blocked micronucleus formation, thus protecting cells from cyto- and genotoxic effect of gamma irradiation.

MAMMALIAN INO80 CHROMATIN REMODELER IS REQUIRED FOR REPLICATION AND RECOVERY FROM REPLICATION STRESS

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A number of yeast studies have implicated the INO80 chromatin remodeler in DNA replication. In S-phase, the complex was shown to be recruited to a significant portion of the yeast autonomous replication sequences and their vicinities and it was necessary for efficient fork progression (Shimada 2008, Papamichos-Chronakis, 2008). The yeast INO80 complex has been implicated to play a role when normal fork progression is impeded. Inhibition of replication induced by hydroxyurea (HU) in Ino80 deletion mutant leads to dissociation of fork components from chromatin, suggesting a crucial role in stabilizing stalled replication forks (Papamichos-Chronakis, 2008). Ino80 mutants treated with HU displayed impaired resumption of DNA synthesis and accumulation of Rad52 foci, suggesting on-going homologous recombination (HR) repair of collapsed forks (Shimada, 2008). On the other hand Falbo et al. (2009) found that INO80 was not necessary for fork restart after HU, but reported its involvement in DNA damage tolerance. So far, the function of mammalian INO80 complex during S-phase remains poorly understood. We have systematically investigated the involvement of the catalytic subunit of the human INO80 complex during unchallenged replication and under conditions of replication stress. We found that INO80 was specifically needed for efficient replication elongation, but was dispensable for replication initiation. Cells depleted of the Ino80 protein became hypersensitive to HU and displayed hyperactive ATR-Chk1 signaling. Using bulk and fiber labeling of DNA, we found that cells deficient for Ino80 and Arp8 had impaired replication restart after treatment with replication inhibitors and accumulated double-strand breaks as evidenced by the formation of Rad51 and gamma-H2AX foci. These data indicate that under conditions of replication stress mammalian INO80 protects stalled forks from collapsing and allows their subsequent restart.

ASSOCIATION OF CD14 C-260T POLYMORPHISM WITH LOW TRIGLYCERIDE LEVELS IN PATIENTS WITH CORONARY ARTERY DISEASE IN BULGARIA

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The purpose of the present study was to evaluate the role of CD14 C-260T single nucleotide polymorphism as a risk factor in the pathogenesis of coronary artery disease (CAD) and to test for any gene-environment interactions which could modify its effects in the context of Bulgarian population. Molecular analysis (PCR/RLFP) of CD14 C-260T SNP was done in 126 age and sex-matched controls and 173 patients with CAD. A binary logistic regression showed that the carriage of the T allele (comparing to CC-genotype) was independent predictor of CAD after adjustment for the conventional risk factors (adjusted OR 2.6, 95% CI = 1.1 to 6.1; $p=0.033$). An analysis of covariance including subjects with obesity and CAD revealed significant difference in marginal means of plasma triglycerides (TG) between TT- and CC-genotype (1.60 mmol/l vs. 2.55 mmol/l, $p = 0.015$), but not between CT- and CC-genotype (1.87 mmol/l vs. 2.55 mmol/l, $p = 0.091$) after Bonferroni adjustment for multiple comparisons. Results from the multiple regression analysis suggest that CD14 C-260T single nucleotide polymorphism, together with cholesterol level and obesity, is a primary predictor of TG level in patients with CAD, and TT-genotype correlated with lower TG level ($\beta = -0.194$, $p = 0.042$) compared with the carriers of CC-genotype. In conclusions, the CD14 -260T allele is independently associated with an increased risk of CAD after adjusting for classical risk factors for atherosclerosis in Bulgarian cohort.

CYTOTOXIC EFFECT OF ALOE-EMODIN ON HUMAN BLOOD-BRAIN BARRIER CELLS

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PURPOSE: The aim of this work was to investigate the mechanisms of toxicity of aloe-emodin (AE) on human brain endothelial cell line hCMEC/D3 and to assess the cellular response in early stage of treatment. **METHODS:** The brain endothelial hCMEC/D3 cells were used to assess the antiproliferative effect of AE. The cell viability was determined by MTS assay. The cellular adenosine triphosphate (ATP) levels were evaluated by CellTiter-Glo® luminescent assay. The intracellular reactive oxygen species (ROS) were determined by 2',7'-dichlorofluorescein (CM-H2DCFDA) fluorescence assay. The effect of AE on the acidic organelles was analyzed flow cytometrically after LysoTracker Red staining. The mitochondrial membrane potential was assessed using tetramethylrhodamine methyl ester (TMRM) staining, while Fluo-4 was used to measure the intracellular free Ca²⁺ concentrations inside living cells, analysed by High Content Analysis using the Arrayscan VTI 740. **RESULTS:** Twenty-four-hour treatment of hCMEC/D3 cells with AE in concentrations between 5 and 200 µM decreased the cell viability as well as the intracellular ATP levels in a dose-dependent manner. Mitochondrial damage and increased production of ROS have also been detected. Notably, AE at a concentration greater than 5 µM dramatically increased intracellular calcium. The slight increase in the intensity of LysoTracker Red signals indicated that AE might trigger autophagy as one of the mechanisms of AE-induced cell death. **CONCLUSION:** These results together suggest that the cytotoxic potential of AE on hCMEC/D3 cells is associated with elevated levels of intracellular calcium, mitochondrial damage and production of reactive oxygen species.

THE EFFECT OF HMGB1 PROTEIN ON THE REPAIR OF CIS-PLATIN DAMAGED PLASMID DNA IN HUMAN LUNG CANCER CELL LINES

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Elucidation of the cellular components responsive to chemotherapeutic agents rationalizes the strategy for anticancer chemotherapy. The most common drug used is cisplatin (cis-Pt). The removal of the cis-Pt /DNA lesions gives the chance to the cancer cells to survive and compromises the chemotherapeutical treatment. The repair efficiency is of crucial importance for the positive clinical outcome. High mobility group box 1 (HMGB1) is non-histone nuclear protein involved in the removal of such lesions. We investigated the role of HMGB1 and the effect of its post-synthetic acetylation at Lys 2 and the C-tail on the repair of DNA damaged with cisplatin at the cellular level. Two non-small lung cancer cell lines different in their p53 tumor suppressor status: H1299 (p53^{-/-}) and A549 (p53^{+/+}) were used. The repair capacity was assessed by host cells reactivation (HCR) assay-method based on the restored fluorescence of the marker gene in damaged with genotoxins plasmid DNA. HMGB1 was down-regulated by esiRNAs and the repair of cis-Pt plasmid DNA was measured in presence of ectopically expressed HMGB1 forms. Increased levels of DNA repair was registered after HMGB1 silencing only in p53 defective cells. Introduction of functional p53 or full length HMGB1s led to DNA repair inhibition whereas the tailless form had no effect on the repair capacity. We conclude that in lung cancer cell line H1999 with higher invasive potential, the repair capacity of cis-platinated DNA strongly depends on the expression level of HMGB1. In A549 - lung cancer with better prognosis, the repair of cis-Pt DNA adducts is determined by a complex action of HMGB1 and p53. Those facts should be considered when chemotherapy is applied for cancer treatment.

INFLUENCE OF ZN/AG AND ZN/AU COMPLEXES WITH SCHIFF BASES SALAMPY AND SALDMEN ON VIABILITY AND PROLIFERATION OF VIRUS-TRANSFORMED RAT SARCOMA CELLS

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The aim of our study was to evaluate the effect of Zn/Au and Zn/Ag complexes with Schiff bases Salampy and Saldmen on viability and proliferation of virus-transformed cancer cells. The permanent cell line LSR-SF-SR, established from a transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Rupin, was used as experimental model. The LSR-SF-SR cells carry the viral oncogene v-src. Investigations were performed by cytological, cytotoxicity and molecular-biological methods such as thiazolyl blue tetrazolium bromide (MTT) test, neutral red uptake cytotoxicity assay, crystal violet staining, double-staining with acridine orange and propidium iodide, single cell gel electrophoresis (Comet assay) and colony forming method. The compounds were applied at concentrations of 0.05; 0.1; 0.5; 1; 5; 10 and 20 $\mu\text{g/ml}$ for 3, 6, 24, 48 and 72 h. The results obtained revealed that they decreased significantly the viability and proliferation of treated cells as well as their ability to grow as 3D colonies in a semisolid medium in a time- and concentration-dependent manner. The investigated metal complexes were found to induce cytopathological changes typical for early and late phases of apoptosis as well as DNA damages. Zn/Au complexes with Salampy and Saldmen were found to be more pronounced cytotoxic/cytostatic agents as compared to Zn/Ag complexes of the same ligands and to be more effective than commercially available antitumor agent cis-platin.

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