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HEALTHY AGEING RESEARCH CENTRE'S VIII WORKSHOP 2016

8th HEALTHY AGEING RESEARCH CENTRE (HARC) WORKSHOP

"Nucleic Acids in Medicine: perspectives for new diagnostic and therapeutic tools"

Book of abstracts

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HARC

MEDICAL UNIVERSITY OF LODZ

1.

SOME RECENT ADVANCES IN CHEMICAL SYNTHESIS OF NUCLEIC ACIDS AND THEIR COMPONENTS

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In the last few years acetal and acetalester derivatives of formaldehyde have been studied as protecting groups of 2'-OH function for RNA synthesis. Such groups have numerous advantages and the most significant one is their small spatial hindrance in the coupling reaction during RNA synthesis. A new method of introduction of acetal and acetalester groups protecting of 2'-hydroxyl function in ribonucleosides for RNA chemical synthesis was developed. It consists of the reaction of appropriately protected ribonucleoside derivatives with thioacetal- or thioacetalester blocking reagents in the presence of tin (IV) chloride at low temperature.

Nucleic acids components such as nucleosides and nucleobases are recognized as natural compounds and their skeletons can be further modified on chemical routes leading to bioactive analogs of large medicinal potential. Nucleosides derived from cytosine and adenine nucleoside analogs were described to posses inhibitory activity towards DNA and histone methyltransferases. The possibility to influence the biological activity of these enzymes could be employed in cell biology and therapy procedures referring to anti-cancer and epigenetic effects of their specific inhibitors.

NOTE:
Chemical synthesis of RNA, DNA and its components and analogues; chemistry of protecting groups; combinatorial chemistry of oligonucleotide analogs

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2.

MODIFICATION WITH BORON CLUSTERS – NEW FORAYS INTO THERAPEUTIC NUCLEIC ACIDS DESIGN

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Natural and modified nucleic acids and oligonucleotides find applications as tools in molecular biology, medical diagnostics, and as prospective biotherapeutic agents. Emerging technologies such as nanotechnology and biosensing show that synthetic DNA and RNA have implications beyond biology and medicine, as components for nanoconstruction, in the field of DNA biocomputing, and others.

Unmodified oligonucleotides are readily available, however, they often do not satisfy requirements necessary to perform designed functions, and hence modified oligonucleotides are frequently used. There is an array of DNA- and RNA-oligonucleotide modifications designed and synthesized for specific applications.
Boron cluster containing oligonucleotides are one of the recently developed and versatile types of oligonucleotide modifications. Their potential applications range from use as boron-rich carriers for Boron Neutron Cancer Therapy (BNCT), therapeutic nucleic acids, molecular probes for molecular diagnostics and molecular biology to new materials for nanotechnology. DNA/RNA modified with boron cluster such as carboranes ($C_2B_{10}H_{12}$) form junction between bioorganic chemistry of nucleic acids and inorganic chemistry. Some aspects of the chemistry, physicochemical and biochemical characteristics of this versatile oligonucleotide modification and their application as antisense anti-IRS-1 and BACE1 targeted siRNA oligomers will be presented.

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NOTE: Chemistry of nucleic acids and their components, boron clusters, modified DNA/RNA-oligomers for diagnostics and therapeutics, nucleoside conjugates as antivirals and anticancer agents, modulators of purinergic receptors and boron carriers for BNCT.

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3.

BORON CLUSTERS AS REDOX LABELS FOR ELECTROCHEMICAL DETECTION OF DNA-PROBES.

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Electrochemical detection of nucleic acid is an attractive alternative to fluorescence and others optical coding technologies in medical diagnostics based on DNA hybridization [1]. Electrochemistry is a simple and versatile method well suited for rapid detection of specific DNA sequence combining high sensitivity, low cost and compatibility with microfabrication technology of transducers. Despite an extensive research and number of available redox labels, the electrochemical coding of DNA still requires improvements [2].

To this end we proposed boron clusters and their complexes with metals, (metallacarboranes), as redox labels for DNA. Several chemical approaches for the synthesis carborane/metallacarborane bearing nucleosides and boron cluster labeled DNA-oligomers were developed [3,4]. Electrochemical properties of the nucleosides and DNA-oligomer conjugates containing carborane/metallacarborane label have been studied [3] and two prototype DNA-electrochemical sensors were proposed. The first design is based on the electrochemically active 7,8-dicarba-nido-undecaborate group [5], and the second format utilizes metallacarborane [3-iron bis(dicarbollide)] as redox label [6]. The sensor bearing metallacarborane redox label was used for detection of DNA sequence derived from and Avian Influenza Virus, type H5N1 (Fig. 1) [6].

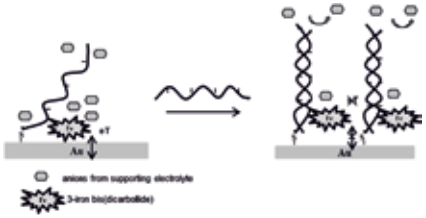

Fig. 1. Schematic illustration of mechanism signal generation of 3-iron bis(dicarbollide) sensor [6].

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Acknowledgements: This study was supported in part by the National Science Centre (grant N N204 531739), and by an internal grant from IBM PAS.

NOTE: Author's interests include chemistry and application of nucleosides, nucleic acid, DNA intercalators, boron clusters (carborane, metallacarborane) as modifying entity for these molecules.

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4.

DNA TOOLS TO SELECT ANTI-BACTERIAL GLYCOCLUSTERS

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Usually, oligonucleotides are used as therapeutics (antisense, siRNA, microRNA…),[1] or for diagnostic applications. In the current project, we have used oligonucleotides to design a DNA-based microarray allowing the detection of protein-glycocluster interactions. Indeed, carbohydrates are involved in many important biological events and especially in infections by bacteria.[2] Our goal is to design glycoclusters able to compete with the natural carbohydrate on the cell surface in order to gain anti-adhesive compounds. Since monovalent carbohydrate interaction with carbohydrate-binding protein (lectins) is generally weak (Kd in the µmolar range), it is necessary to enhance the binding using multivalency.[3] For this purpose, we synthesized libraries of new DNA--glycoclusters exhibiting up to 10 saccharidic residues with different topologies. Their synthesis was performed mainly on solid support using phosphoramidite or H-phosphonate DNA chemistries in combination with Cu(I) catalyzed Azide Alkyne cycloaddition (CuAAC) click reaction.[4] Thanks to DNA Directed Immobilization of glycoclusters on the microarray, we determined their affinity (IC50 and Kd) against Pseudomonas aeruginosa lectins (LecA and LecB) using only tens of picomoles of them.[5] Among the numerous glycoclusters synthesized,[6,7] two of them exhibiting high affinities for LecA were selected and synthesized in solution, without the DNA tag, at hundreds of milligram scale for their biophysical properties (HIA, ELLA, SPR and ITC) and for anti--biofilm investigation. Their high affinity for LecA was confirmed (Kd of 157 and 194 nM). They were found to decrease of the biofilm formation of PA.[8]

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NOTE: Synthesis of modified oligonucleotides and their conjugates using new phosphoramidites and solid supports for therapeutic or diagnostic applications. Design and synthesis of neoglycoclusters using nucleic acid and Click chemistries.

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5.

TRNA DAMAGE IN OXIDATIVE STRESS: DESULFURATION OF WOBBLE 2-THIOURIDINES

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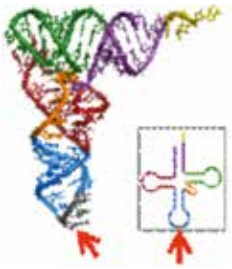
Under conditions of a cellular homeostasis, a reactive oxygen species (ROS) are formed at a low, safe level and play a positive role as regulators of many cellular processes. Oxidative stress occurs in the cells due to imbalance between production of ROS and their removal by the antioxidant system. Oxidative environment results in consequent damage of the proteins, lipids or nucleic acids and induces a number of cellular dysfunctions. There is a close relationship between the presence of the oxidative stress and the pathogenesis of diseases, in particular resulting from the process of aging, like neurodegenerative, cardiovascular and cancer diseases. This effect is the result of weakening of repair mechanisms or systems, which are responsible for degradation of defective molecules in the senescent cells. Although, the transfer nucleic acids (tRNAs) are stabilized by their tertiary structure and the numerous chemical modifications, also these molecules undergo dynamic changes under oxidative stress conditions. For example the activation of some intracellular nucleases during stress leads to the hydrolysis of the tRNA at the anticodon loop (see Figure). Such half-tRNAs play the role in cellular response by regulation of gene expression at the transcription or translation level.



The tRNA molecules containing 2-thiouridine (S2U) or its 5-substituted derivatives (X5S2U), situated at the first position of anticodon (position 34, wobble), play an important role in tuning the translation process through the codon-anticodon interactions. These sulfur-containing tRNA components may be the primary site of attack of the reactive oxygen species. Recently, we have shown that 2-thiouridine (alone or incorporated into an RNA chain) under in vitro conditions mimicking an oxidative stress in the cell is transformed predominantly to 4-pyrimidinone riboside (H2U), and not to uridine [1].

The process is pH- and concentration-dependent [2,3]. The resulting major lesion offers entirely changed donors/acceptors pattern changing the A to G codon reading preference [4,5]. This hypothesis, although already confirmed by in vitro experiments, has not been proven in the cell, and studies focused on the effect of S2U-tRNA damage on cellular oxidative stress are presently being performed in our laboratory.

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NOTE: Professor of CMMS PAS, author or co-author of ca 140 research papers, ca. 1300 - citations, H=20. Point of interest - application of nucleic acid models for investigation of biological processes (gene silencing, enzyme inhibitors, oxidative damage of tRNA).

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6.

PROANGIOGENIC ACTIVITY OF NUCLEOSIDE 5'-O-PHOSPHOROTHIOATE ANALOGUES UNDER HYPERGLYCAEMIC CONDITIONS

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Di- and triphosphates od adenosine and uridine (ATP, ADP, UTP and UDP) are known to bind to specific P2Y nucleotide receptors and activate numerous intracellular signaling cascades, including proliferation, migration, differentiation and cell death. We have also shown that, contrary to a widely held opinion, nucleoside 5'-O-monophosphorothioate analogs, containing a sulfur atom in a place of one nonbridging oxygen atom in a phosphate group, act as ligands for selected P2Y subtypes. Especially interesting appeared to be thymidine 5'-O-monophosphorothioate (TMPS) which acts as a specific partial agonist of the P2Y6 receptor (P2Y6R).

Moreover, recent studies have also implicated a critical role of P2X/P2Y nucleotide receptors in dermal tissue regeneration and maintaining vascular homeostasis. These effects seemed to be especially important for wound healing under hyperglycaemic conditions because new vessel generation and keratinization process are decreased in diabetic patients. We determined whether nucleoside 5'-O-phosphorothioate analogues might accelerate vascular endothelial growth factor (VEGF) production as well as the growth and migration of human keratinocytes under hyperglycaemic conditions. We also investigated the expression pattern of P2X/P2Y receptors in human keratinocyte HaCaT cells. We evidenced that nucleoside 5'-O-phosphorothioate analogues are better candidates to overcome hyperglycaemia-induced impairment of angiogenesis as compared to their unmodified counterparts. The greatest potency for VEGF release and stimulation of cell migration by thiophosphate analogues of ATP and UTP correlates with the highest P2Y2 receptor expression by HaCaT cells. We also found that UTPαS significantly increased the viability and proliferation of the HaCaT cells. These findings suggest that thiophosphate analogues of nucleotides could serve as potential therapeutic agents for promoting impaired angiogenesis under diabetic conditions.

NOTE: Prof. Maria Koziolkiewicz is full professor and the Dean of the Faculty of Biotechnology and Food Sciences at the Lodz University of Technology Technical University of Lodz. She is the author or co-author of ca. 85 papers devoted to antisense oligodeoxyribonucleotides, modified nucleotides and lysophospholipids acting as membrane receptor ligands.

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7.

FROM CHARGE TRANSFER TO DNA DAMAGE

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By definition clustered DNA damage consists of "two or more single lesions within one or two helical turns of DNA (Deoxyrybo Nucleic Acid) helix". This type of lesions can be generated in oligonucleotides as a consequence of exposure to ionisation radiation, as a product of an external attack of free radicals or as a consequence of charge migration through ds-DNA. In the natural environment the most widespread radical is HO• (hydroxyl radical) which can be generated during aerobic cell metabolism, Fenton-type reactions or in ambient conditions, in the presence of photosensitizers. Most of the DNA damages are removed via BER (Base Excision Repair) mechanism due to the action of specific enzymes like UDG (Uracil Glycosylase) (dU-deoxyuridne–specific enzyme), OGG1(DNA Glycosylase 8-oxo-guanine specific) (dGoxo- 8-oxo-7,8-dihydro-2'--deoxyguanosine), which initiate the repair process. Another repair mechanism, however much more complex, is the NER (Nucleotide Exision Repair) pathway. Thus complex damage such as 5',8-cyclo purine nucleosides, pyrimidine dimmers or clustered lesions can be removed from native DNA as part of the released oligomers that contain at least 20-mers.

Moreover, depending on the type of damage the following mechanisms may be also operative
- Mismatch repair
- Direct Repair, being of the main mechanism for elimination of modifications of the pyrimidine dimers type in prokaryotes (photolyases)
- Repair mechanisms of double-strand DNA breaks: Homologous recombination and Non-homologous end joining
- Cross-link Repair implying the XPF•ERCC1 complex (structure specific endonuclease involved in NER).

NOTE: Investigation of charge transfers through ds-DNA, oligonucleotide structure analysis, electronic properties by theoretical approach and experimental mode. Efficiency of DNA repair system, especial BER pathway, in different types of cells by biochemical assay.

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8.

ABERRANT NOTCH PATHWAY SIGNALING IN CANCER

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Cancer is a most common human genetic disease. In its initiation, promotion and progression stages numerous mutations and transcriptional deregulation lead to accumulation of cellular differentiation and metabolic abnormalities causing destructive effects on tissues and whole organism. Notch signaling pathway regulates stem cell function and is critical for tissue differentiation and organ modeling. Therefore, aberrant Notch signaling is associated with cancer development and progression.

We have examined gene expression data of 5272 samples from patients of breast, ovarian, prostate, kidney, lung, colon and brain tumors. Analysis differential expression of 20 Notch pathway members and tumor recurrence showed that disease free survival is strongly associated with Notch aberrant signaling resulted from deregulation of transcription of Notch receptors, ligands and mediators. Gene expression of different Notch pathway members has a contrary effect on DFS in different cancers and its subtypes. Specifically, NOTCH1 receptor lowered gene expression is associated with better DFS in all examined tumors. However, elevated gene expression is a good prognostic value for disease recurrence in ER+ breast cancer for NOTCH2, colon cancer for NOTCH3 and colon and ovarian cancer for NOTCH4. Similar distinct associations were found for other Notch pathway members and tumors. To find, how aberrant Notch signaling influenced cellular and tissue differentiation we performed GSEA analysis using molecular signatures databases for canonical pathways, transcription factor binding motifs and gene ontology groups. We found many distinct associations of differential Notch signaling with tumor cell and tissue architecture and metabolism.

Our results suggest that differential gene expression associated with aberrant Notch pathway signaling is strongly associated with cell and tissue remodeling in carcinogenesis and tumor progression. Moreover, distinct Notch signaling signatures can be used to split tumor subtypes into groups of good and bad prognosis of disease recurrence and progression.

NOTE: Our team is using cellular modeling and bioinformatics to study differential gene expression in cancer. We are particularly interesting in WWOX gene as a global gene expression regulator.

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9.

UNDERSTANDING TOLERANCE TO PROTEIN MISTRANSLATION AND AGGREGATION

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To investigate how organisms mitigate the deleterious effects of protein synthesis errors and protein aggregation, which are intrinsically associated with aging related diseases, a mutant yeast strain was engineered to produce high levels of aberrant proteins. Laboratory evolutionary experiments revealed that fitness loss due to accumulation of aberrant proteins can rapidly be mitigated. Genomic analysis demonstrated that adaptation was primarily mediated by large-scale chromosomal duplication and deletion events, suggesting that errors during protein synthesis promote the evolution of genome architecture. Evolution increased the level of tolerance to aberrant and aggregated proteins through acceleration of ubiquitin-proteasome mediated protein degradation and protein synthesis. As a consequence of rapid elimination of erroneous protein products, evolution reduced the extent of toxic protein aggregation. However, there was a strong evolutionary trade-off between adaptation to aberrant protein synthesis and survival upon starvation: the evolved lines showed fitness defects and impaired capacity to degrade mature ribosomes upon nutrient limitation. Moreover, as a response to an enhanced energy demand of accelerated protein turnover, the evolved lines exhibited increased glucose uptake by selective duplication of hexose transporter genes. We conclude that adjustment of proteome homeostasis to accumulation of aberrant proteins evolves rapidly, but this adaptation has several side-effects on cellular physiology. Our work also indicates that translational fidelity and the ubiquitin-proteasome system are functionally linked to each other and may, therefore, co-evolve in nature.

We are grateful for the financial support by the European Union Framework Program 7 (EUFP7) Sybaris Consortium Project 242220 (M.A.S.), the Portuguese Science Foundation through Project ANR/IMI-MIC/0041/2012 (M.A.S), FEDER and COMPETE2020 programs.
in NER).

NOTE:
Our main research interests focus on the role of RNA modification and RNA modifying enzymes on protein aggregation and its relevance for aging and aging related diseases

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10.

IDENTIFICATION OF CONSERVED TRNA DERIVED FRAGMENTS IN ZEBRAFISH REVEALS THEIR POTENTIAL AS GENE EXPRESSION MODULATORS AND DISEASE BIOMARKERS

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Small non-coding RNAs (sncRNAs) are a class of transcripts implicated in several eukaryotic regulatory mechanisms, namely gene silencing and chromatin regulation. tRNA derived fragments (tRFs) constitute a novel class of sncRNAs produced by specific cleavage of certain tRNAs. Despite significant progress in their identification by next generation sequencing (NGS) their function is still not completely understood. We have identified tRFs by NGS of the sncRNA fraction of the vertebrate model zebrafish. These tRFs are 18-30 nt long and are derived from specific 5' and 3' processing of mature tRNAs. Our data show that tRFs are differentially expressed during development and in differentiated tissues, being barely expressed at early stages, but highly expressed in adult fish, as determined by northern blot analysis. We further show that these tRFs are conserved among vertebrates and that a highly expressed tRF (5'tRF-Pro(CGG)) has silencing capacity, indicating that it can enter the RNAi pathway.
Since tRFs are conserved among vertebrates, we have perfomed a computational search of publicly available datasets and found that specific tRFs are differentially expressed in disease conditions, namely during infection and colorectal cancer, suggesting their involvement in the mechanisms underlying diseases and their potential use as disease biomarkers. This work demonstrates that tRFs constitute a class of conserved regulatory RNAs in vertebrates and may be involved in mechanisms of genome regulation in normal and disease conditions.

We are grateful for the financial support by the Portuguese Science Foundation (FCT) through Project ANR/IMI-MIC/0041/2012 (M.A.S), FEDER and COMPETE2020 programs and grant SFRH/BPD/77528/2011.

NOTE: Current research interests are the contribution of translation errors and deregulation of translation quality control mechanisms for the development and progression of aging related diseases and the role of sncRNAs during development and disease.

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11.

IN VITRO AND IN VIVO METABOLOMIC ANALYSIS OF ARGININE DEPRIVATION THERAPY IN MALIGNANT PLEURAL MESOTHELIOMA

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Arginine is a critical amino acid for tumour cells lacking the key enzyme, Argininosuccinate synthetase 1 (ASS1), required for its biosynthesis. Arginine deprivation strategy using pegylated arginine deiminase (ADI-PEG20) was effective against many types of tumours in vitro and in vivo. In this study, we determined the metabolic changes induced by ADI-PEG20 in a panel of malignant pleural mesothelioma (MPM) cell lines with promoter methylation-dependent silencing of ASS1. We confirmed these metabolic changes in plasma of patients with MPM treated with ADI.

Methods: MPM, ASS1-negative (H2591, MSTO and JU77) and ASS1- positive (H28) cell lines were used. All cells were treated with 750 ng/ml of ADI-PEG20 for 24 hours. In addition, MPM plasma samples (29 patients treated with ADI-PEG20 and 6 control patients over a 9 wk period) were also analysed. All metabolomic profiles were assessed using LC-MS technique.

Results: ADI-PEG20 induced marked metabolomic changes in cell lines and patient plasma, which clearly discriminated treated from untreated samples using multivariate statistical approaches. Arginine depletion was noted in all treated cell lines and patient plasma. Citrulline, n-a-acetylcitrulline, and glutamine were upregulated. A reduction of the thymidine nucleotide pool was noted in treated cell lines and linked to suppression of thymidylate synthetase and dihydrofolate reductase, and paradoxically, reduced uptake of 3H-FLT. In patient plasma, ADI--PEG20 increased thymine, carnitine and proline while decreasing isoleucine. A 2-fold increase in plasma glutamine and glutamate was detected in non-responder patients, reaching a maximum by wk 5 of ADI-PEG20 treatment.

Conclusion: This study has identified several ADI-PEG20 induced metabolic changes in cells and plasma that can serve as potential biomarkers optimizing the efficacy of ADI-PEG20 in the treatment of MPM. However, further validation is required and is ongoing in a phase triplet combination trial of ADI-PEG20, pemetrexed and cisplatin in patients with mesothelioma and other arginine-dependent cancers.

NOTE:
Peter Szlosarek is a clinical senior lecturer and principle investigator in Barts Cancer Institute. Peter translational lab program focuses on aberrant tumour metabolism and inflammation and is closely allied to his clinical interests in the treatment of mesothelial, lung and skin cancers.

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12.

NOVEL NUCLEOTIDE ANALOGUES THAT OVERCOME THE KEY CANCER DRUG RESISTANCE LIMITING PATIENT SURVIVAL

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Nucleoside analogues such as cytarabine, 5-flourouracil (5-FU) and gemcitabine are frequently used in treating patients with cancer. They constitute the backbone for several chemotherapy regimens intended for treating solid and haematological malignancies. However, these analogues are prodrugs relying on active cellular uptake and subsequent intracellular phosphorylation for their conversion into the active cytotoxic metabolites. Mutations in cancer cell involving either the uptake process through specific nucleoside transporters or the phosphorylation mechanism can eventually lead to development of resistance against these anti-cancer agents. In addition, excessive plasma degradation of the nucleoside analogues leads to shorter plasma half-life and reduced tissue accumulation limiting their therapeutic efficacy. Therefore, a new generation of nucleotide analogues has been recently developed in order to overcome the key cancer resistance mechanisms associated with the nucleoside analogues. The addition of a phosphoramidate motif to the nucleoside analogues protected them from plasma degradation and subsequently increased their tissue accumulation and activation. In preclinical studies, NUC-3373, a phosphoramidate analogue of 5-FU, demonstrated 330x greater cytotoxic activity compared to 5-FU in multiple cancer cell lines and maintained activity in conditions mimicking drug resistance such as thymidine kinase inhibition. NUC-3373, unlike 5-FU, was resistant to dihydropyrimidine dehydrogenase (DPD) mediated degradation. Moreover, NUC-3373 achieved 363x higher intracellular accumulation of the active agent 5-fluorodeoxyuridine monophosphate, the active metabolite of 5-FU. In phase I clinical trial, NUC-1031, a phosphoramidate analogue of gemcitabine, has demonstrated impressive clinical activity with durable disease control rate in a wide range of patients with advanced and rapidly progressing disease. A Phase III clinical studies in ovarian, pancreatic and biliary cancers and combination studies with paltinum compounds are currently recruiting. These encouraging pre-clinical and clinical results strongly supports the phosphoramidate approach to produce promising new anticancer agents.

NOTE: Dr Essam Ghazaly is a post-doctoral researcher and a clinical pharmacologist with an interest in clinical pharmacokinetics and biomarker studies in drug development and early phase clinical trials. He has advanced expertise in liquid-chromatography-mass spectrometry based assays for detection and quantification of peptides and small molecules in complex matrices.

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13.

180° TURN - HOMPURINE ([ALL-RP-PS]-DNA OLIGOMERS FORM STABLE PARALLEL COMPLEXES WITH HOOGSTEEN PAIRED NUCLEIC ACIDS

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In 2007, Lianrong Wang and co-workers from Shanghai Jiaotong University found a phosphorothioate d(GPSA) segment in bacterial DNA [1]. In the same 2007 we showed that synthetically obtained homopurine phosphorothioate analogs of DNA, possessing all phosphorus atoms of RP configuration ([All-RP-PS]-DNA), when interact with Hoogsteen paired RNA or (2'-OMe)-RNA templates, form parallel triplexes [2].
If the sequence is non-palindromic, stable parallel duplexes are mostly formed [3].
The more recent results show that T-LNA or 5-Me-C-LNA units introduced into the parallel Hoogsteen-paired (2'-OMe)-RNA strands (up to four units in the oligomers of 9 or 12 nt in length) stabilize the parallel complexes [4]. At neutral pH, dodecameric parallel duplexes have Tm values of 62-68°C, which are by 4-10°C higher than Tm for the reference duplex (with no LNA units present), while for corresponding triplexes, Tm values exceeded 85°C. In experiments of reverse transcription RNA⁄DNA (using AMV RT) we observed that the formation of parallel triplexes (consisting of an RNA template, [All-RP-PS]-DNA nonamer and Hoogsteen--paired (2'-OMe)-RNA strands containing four 5-Me-C-LNA units) led to efficient inhibition (94%) of the process.

To enhance the A conformation of the parallel duplexes, we introduced LNA units into the PS--DNA strand. The LNA-OTP monomers were synthesized, separated into P-diastereomers and used in synthesis of P-stereodefined PS-(DNA/LNA) chimeras. Melting and CD measurements indicate that the PS-LNA units stabilize the Watson-Crick paired duplexes with DNA and RNA matrices, compared to the reference P-stereodefined duplexes containing no LNA units [5]. Stability of the parallel complexes is being studied, but preliminary data indicate that PS-(DNA/LNA) chimeras adopt the A conformation, which is extended on DNA Hoogsteen paired matrices giving rise to the formation of so far unknown, parallel PS-(DNA/LNA)/DNA duplexes.

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(5) K. Jastrzębska, et al., Org. Biomol. Chem.- 2015, 13, 10032

NOTE:
Associate professor at CMMS PAS; author or co-author of 72 papers and 6 patents, CI=911, h=17. Works on nucleic acids chemistry (especially stereochemical aspects of modifications at phosphorus atoms) and on structural aspects of interactions of PS-DNA with other biomolecules.

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14.

THERMODYNAMIC PROPERTIES OF COMPLEXES FORMED BY STEREODEFINED PS-OLIGOS CONTAINING LNA UNITS WITH COMPLEMENTARY DNA TEMPLATES

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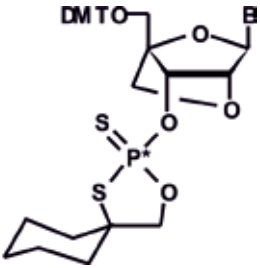

LNA - locked nucleic acids are nucleic acid analogs with the ribose ring "locked" in the C3'-endo conformation by a methylene bridge connecting the 2'-O and 4'-C atoms (1), (2). LNA oligonucleotides and complementary DNA and RNA matrices form complexes of high thermal stability.

The in home synthesized LNA nucleosides (B=AdeBz, CytBz, GuaiBu, Thy) (1) were transformed into corresponding 3'-O-(2-thio-'spiro'-4,4-pentamethylene-1,3,2-oxathiaphospholane) derivatives. The P-diastereomers were separated by silica gel HPLC and used for solid-phase synthesis of stereodefined [All-RP-] and [All-SP-PS]-oligomers (PS-DNA/LNA, 11-mers containing 2-3 LNA units). Then, conformational characteristics (by Circular Dichroism, CD) and thermal stability studies (Melting Temperature, Tm) of antiparallel duplexes formed by stereodefined oligomers PS-DNA/LNA with the Watson--Crick paired DNA and RNA templates were executed. It was found that the duplexes containing oligomers with incorporated LNA monomers are thermally more stable than the reference PS--DNA/DNA and PS-DNA/RNA duplexes, and the increase in duplex stability of 6-9°C per LNA unit was noted. The CD spectra have shown that even only two LNA units incorporated into the PS-DNA/LNA oligonucleotides significantly move the duplex conformation towards the A-type (an RNA-like) (3).

It is known that P-stereodefined [All-RP-PS]-DNA and RNA or 2'-OMe-RNA matrices form parallel duplexes and triplexes (stabilized by Hoogsteen's interactions), which are thermally more stable than the corresponding DNA/RNA structures (4). It is hypothesized that this increased stability is related to a more profound C3'-endo conformation of the 2'-OMe-RNA matrices (compared to the corresponding RNA analogs), which is extended on the [All-RP-PS]-DNA components. If so, the structures with the [All-RP-PS]-DNA analogs containing inserted PS-LNA units should gain further increase of thermal stability. Therefore, [All-RP-PS]-homopurine oligomers containing inserted LNA units were synthesized. Preliminary thermal stability studies indicate that so far unknown, parallel PS-(DNA/LNA)/DNA duplexes are formed.

This work was supported by National Centre of Science, Poland, decision DEC-2011/03/B/ST5/0267

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NOTE: Synthetic organic chemistry of nucleosides, nucleotides and oligonucleotides with particulate interest in stereodefined P-chiral analogs of DNA. Stability of complexes of stereodefined P-chiral analogs of DNA/LNA with DNA, RNA templates.

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15.

COMPREHENSIVE PROTECTION VIA THERMOLABILE GROUPS FOR BIOCONJUGATES SYNTHESIS

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To fully understand the properties of biologically active biopolymers being tested as unconventional drugs or diagnostic tools, one needs a simple and efficient way of their chemical synthesis. Special tools are necessary for this purpose and the use of well-suited protective groups is especially important. Thermolabile protecting groups (TPGs) provide an innovative solution for the protection of a molecule's reactive sites during the chemical synthesis. The unique characteristic of TPGs is their ability to be removed in neutral conditions in response to an increase in the temperature. This particular means of deprotection prevents pH-sensitive molecules, such as marked oligonucleotides, peptides or their bioconjugates, from degradation under acidic or basic conditions which are commonly used in the synthesis. Such a unique approach eliminates the need for hazardous reagents used to unmask other protecting groups, and complies with the principles of green chemistry. Till now 2-pyridynyl TPGs have been used for the hydroxyl and phosphate groups of oligonucleotides. Moreover it is possible to modulating the stability of 2-Pyridinyl TPG (2-PyTPG) via the "chemical switch" approach. The main advantage of this approach is the possibility of changing the nucleophilic character of pyridine nitrogen using different switchable factors, which results in an increase or decrease in the thermal deprotection rate. This methodology make it possible to explore the issue of thermolabile protecting group on carboxyl or amine functionality and extend it to the protection of wider range of biomolecules.

NOTE:
Broad experience in the synthetic organic chemistry of nucleosides, nucleotides and oligonucleotides, both in DNA and RNA series and their analogues. Design and development of thermolytic group for DNA, RNA , peptide synthesis and their functionalization for different application.

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16.

RNA NANO-OBJECTS AS PROGRAMMABLE THERAPEUTICS

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Nucleic acids, often overlooked, seem to be a perfect material for smart therapeutics. Due to the intrinsic programmability, biocompatibility, specific recognition potential and predictable folding, the DNA/RNA molecules are of the interest for scientists. We can imagine creating responsive DNA or RNA nano-particles, which would be able to find and destroy misbehaving cells in human organism. The RNA fragments can be programmed to regulate expression of specific oncogenes via RNA interference (RNAi). Such assemblies have been constructed and successfully applied in model animal studies.

Here we will view the methodology leading towards design, synthesis and analysis of structurally stable RNA nano-objects. Multimolecular nano-particles, containing specific RNA fragments targeting selected genes are designed in-silico, like LEGO pieces, using library of structurally stable RNA motifs. Controlled folding process ensures stability of entire RNA nano-particles. Such particles, supplemented with delivery agent can be applied in cellular studies in time used for personalized medical therapies. Nucleic acids based nano-structures are getting more attention in the perspective of their use in Medicine.

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17.

RNA TRIMER AS A GENE REGULATING NANO-PARTICLE IN A MODEL GFP SYSTEM

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Central dogma of molecular biology describes the route from the genes content to proteins that build the living organisms. It also shows the level of complexity of the control mechanisms governing genes expression regulation in the cell. The RNA interference (RNAi) is one of phenomena naturally occurring in the cell to regulate the gene expression. Despite the fact that transcript is created the translation process is inhibited, mRNA is specifically degraded and does not result in protein production.

The RNAi can be triggered by a number of artificial RNA structures that can be designed, synthetized and engineered to target a chosen gene, leading to the expression inhibition in the treated cells. Our approach is to create structurally stable RNA triangular nano-object utilizing RNA architectonics rules and then examine their potential to silence reporter gene in cellular model. This methodology involves the cellular RNAi machinery, especially nucleases processing sncRNAs, resulting in release of siRNA and triggering interference. Herein the multistep synthesis leading from the in silico design to structure confirmation will be explained. The results, from the cellular model with the green fluorescent protein (GFP) reporter gene, are promising. This methodology presents its way towards biomedical applications and possible personalized therapies.

NOTE: Dominika Jędrzejczyk – Lodz University of Technology graduate in Molecular Biotechnology and Technical Biochemistry, currently PhD Candidate at the Centre of Molecular and Macromolecular Studies PAS. Present scientific interests in RNA nanotechnology and the potential of artificial RNA for gene expression regulation.

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18.

ANTI-AGING PROPERTIES OF N4-FURFURYLCYTOSINE

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Natural low molecular weight compounds have been a major source of new drugs for centuries. Their chemical diversity have provided numerous applications e. g. in pharmacy, cosmetology. There are evidences that naturally occurred compounds may exert modulatory effects through selective actions on different components of the signaling cascades vital for cellular functions such as growth, proliferation and apoptosis. In contrast to non-natural nucleic acids derivatives, characterized by high toxicity and unexpected side effects, naturally modified nucleic acids do not show such adverse reactions and are devoid of nonspecific toxicity.

N4-furfurylcytosine (FC) is a derivative of cytosine identified as a natural component of DNA in calf thymus. It was shown to possess anti-aging properties in normal eukaryotic cells. Our data present that FC mildly influenced proliferation in normal eukaryotic MRC-5 and HaCaT cell lines without any cytotoxic effect up to 1mM studied by xCelligence.

It was shown pro-proliferative properties of FC in the eukaryotic cells using several cytometric analyses. Even 1 mM FC didn't affect cell cycle neither induce apoptosis or necrosis confirmed also by confocal microscopy analysis. It has not been noticed any epigenetic effect of FC by measuring changes in global 5mC level in DNA. FC possesses biological properties that might have excellent anti-aging effect.

NOTE:
Activity of small molecular compounds in the eukaryotic cells, interactions between nucleic acids, catalytic nucleic acids, activity of L-RNA and D-RNA hammerhead ribozymes.

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19.

EXOSOME-TRANSPORTED MIRNAS MAY CONTRIBUTE TO CANCER IMMUNOSUPPRESSION

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Exosomes are nano-particles released from all cell types. They transport proteins, lipids, mRNAs, and miRNAs and function as intercellular communication vehicles. Cancer cells secrete exosomes at higher rates than healthy cells. Secreted exosomal miRNAs can be detected in biological fluids such as plasma and can reflect the physiological status of the cells they originate from. They potentially serve as predictive and prognostic biomarkers for diseases including various kinds of cancer. Exosomes can also be used for innovative therapeutic applications. Loaded with therapeutic nucleic acids exosomes can be employed as drug delivery systems to target cancer cells. Exosomes derived from mature dendritic cells find applications in cancer immunotherapies.

In the patient, cancer cell-released exosomes assist tumors by shaping the microenvironment, interfering with drugs, and evading the response of the immune system. In general, tumor-induced impairment of antigen presentation is considered as one of the major strategies of cancer-induced immunosuppression. We found some evidence that miRNA in cancer derived exosomes interfere with efficient antigen presentation. Transfer of melanoma derived exosomes to myeloid cells resulted in down-regulation of co-stimulatory molecules and of cytokine production. Our results support the existence of a hitherto undescribed immunosuppressive mechanism mediated by miRNAs transported by cancer derived exosomes.

NOTE: Prof. Dr. hab. Markus Düchler is leading a NCN project (grant no. 2012/05/B/NZ2/00574) aiming at the elucidation of a possible influence of cancer cell derived miRNAs transported through exosomes on immunosuppression. The project is realized at the CBMM/PAN in Łódź in collaboration with the Medical University Łódź.

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POSTER SESSION

1.

ANTIOXIDANT ACTIVITY OF RED DRY WINES AND PORTER BEERS

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The interaction of free radicals with cellular macromolecules: nucleic acids, proteins, lipids and carbohydrates, leads to variety of damage: DNA breakage, mutations and chromosomal aberrations. An excess of oxygen free radicals is harmful to cellular and tissue structures. DNA changes may induce the pathological cell proliferation and carcinogenesis process. It is also documented that free radicals are the reason of civilization development of many diseases, such as atherosclerosis, diabetes, cataract, Parkinson's disease, Alzheimer's disease. Alcoholic beverages produced from fruit, seeds, grains or leaves are rich in phenol anti-oxidant. These biologically active substances are represented mainly by phenolic acids, flavanones, flavones and flavonols, anthocyanidins, flavan-3-ols and proanthocyanidins. All of these substances in different proportions are present in the alcoholic beverages and influence on their anti-inflammatory, and cardioprotective properties. They play also protective role against brain degenerative processes. Moderate alcohol consumption in conjunction with a healthy diet and physical activity is considered as beneficial in the protection of health.

AIM:

Dry red wines of Cabernet sauvignon and beer porters were tested for antioxidant activity, total polyphenols and other compounds affecting the health benefits of these beverages.

MATERIAL AND METHODS:

Seven wines from different wine regions and 29 beers porter were purchased in local stores. We used in vitro method of measuring the antioxidant activity and compared to percentage of DPPH radical scavenging by the compounds contained in the test wines and beers. The study of total polyphenol content and individual polyphenols used spectrophotometric methods.

RESULTS AND CONCLUSIONS:

Both wine and beer, characterized by a good ability to scavenger free radical DPPH. Studies have also shown the relationship between the level of the antioxidant activity and the content of total polyphenols, flavonoids and anthocyanins. The results suggest that red wines and porter beer could be sources of bioactive compounds in human diet.

NOTE: My current research activity is focused on the search for bioactive components in foods, the study of the antioxidant activity of their potential impact on the human body. Additional an important element of my work is the study of the authenticity of the composition of dietary supplements, determination of contamination and adulteration of food.

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2.

LONG NONCODING RNAS EXPRESSION IN BLADDER CANCER

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3 Max Delbruck Center of Molecular Medicine, Berlin, Germany.

INTRODUCTION: Bladder cancer (BC) is the fourth commonest male malignancy and one of the most expensive human cancers to manage. The majority of tumors are urothelial carcinoma in histologic type. Although many reports detail genetic events in urothelial cancer, alterations of epigenetic gene regulation are also important in this disease. Epigenetic gene regulation may occur directly or indirectly through noncoding RNA (ncRNA) species. To date, few data have reported the role of long noncoding RNA (lncRNA) in urothelial cancer and little is known of their function.

MATERIAL AND METHODS:
The expression of 17112 lncRNAs and 22074 mRNA was determind using microarrays in 83 normal and malignant urothelial samples (discovery step) and selected RNAs with qPCR in 138 samples (validation step). Significantlly differentially expressed RNAs were identified and stratified according to tumour phenotype. siRNA knockdown, functional assays, and whole genome transcriptomic profiling were used to identify potential roles of selected RNAs.

RESULTS:
We observed upregulation of many lncRNAs in urothelial cancer that was distinct to corresponding, more balanced changes for mRNAs. In general, lncRNA expression reflected disease phenotype. We identified 32 lncRNA with potential roles in disease progression. Focusing upon a promising candidate , we implicate upregulation of AB074278 in apoptosis avoidance and maitenance of a proliferative state in cancer through a potential interaction with EMP1, a tumour supressor and a negative regulator of cell proliferattion.

CONCLUSION:
We have identified many lncRNAs significantly altered in urothelial cancer and associated with disease progression and tumor subtypes.

NOTE: Edyta Borkowska is a researcher of the Medical University in Lodz. In 2011 she was awarded a grant from the Polish Ministry of Science and Higher Education and worked in United Kingdom at the Universities of Oxford and Sheffield. Her research interest are clinical genetics, cancers, DNA, noncoding RNA changes.

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3.

POTENTIAL GROWTH INHIBITORY EFFECT OF OENOTHERA PARADOXA EXTRACT AGAINST HUMAN MALIGNANT PLEURAL MESOTHELIOMA CELLS

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Malignant pleural mesothelioma (MPM) is recognized as a relatively rare but very aggressive tumor of mesothelial cells arising in response to prolonged inflammatory processes in respond to asbestos fibers exposure. Due to the delayed and very often ambiguous misdiagnosis MPM brings poor prognostic outcomes and similarly to most solid tumors becomes a serious therapeutic challenge due to the resistance to conventional treatment. A modern treatment strategy considers L-arginine deprivation, using arginine deiminase and its pegylated modification (ADI--PEG20), in argininosuccinate synthase deficient MPM cancer cells (ASS-1 negative tumor) which show L-arginine degradation sensitivity. In the light of observed severe side effects after standard chemotherapeutics it is still relevant to looking for a new supportive treatment ways, including natural dietary interventions. Plant-derived supplements are considered as multitargeted protectants which reveal strong antioxidant properties in normal cells, enhancing scavenging of toxic reactive oxygen species forms and improving immune system defense. On the other hand, numerous studies have proved that phytochemicals, including polyphenols compounds, reveal potential pro-oxidative and pro-apoptotic activity against tumor cells simultaneously increasing cytotoxicity of standard chemotherapeutics. Extracts obtained from Oenothera sp. have documented high polyphenols content including gallic acid and penta-O-galloyl-D-glucose (PGG) compounds which display potential antitumor activity against several cancer types including melanoma, breast and prostate cancer as well as for hepatocellular carcinoma.

The aim of our studies is to investigate the potential cytotoxic effect of evening primrose extract (Oenothera paradoxa, EPE) obtained in alcoholic extraction method validated in Department of Structural Biology against malignant pleural mesothelioma cells. Cells growth inhibition was monitored by mitochondrial dehydrogenases activity (formazan crystals formation by viable cells) as wells as crystal violet dye uptake.

All investigated MPM-derived cancer cells reveal sensitivity to izopropanolic EPE.

The growth inhibitory effect of studied extract was time- and dose-dependent.

We believe that the pro-apoptotic activity of EPE can be associated with excessive ROS generation as it was established that both EPE and its active compound, PGG may reveal dual action. The pro-oxidative activity of EPE as well as metabolomic changes in MPM cells after EPE administration are currently studied in our laboratory.

This study is supported in part by the Healthy Ageing Research Centre project (REGPOT-2012-2013-1, 7 FP) and research project no. 502-04-028 from the Medical University of Lodz, Poland.

NOTE: Current research interests are metabolomic profiles analysis of cancer cells, particularly malignant pleural mesothalioma cells, upon the conditions of new therapeutic treatment strategies with parallel qualitative and quantitative determination of metabolites implicated in targeted metabolic pathways using microLC-QTOF-MS system.

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4.

UNEXPECTED ROLE OF CYTOCHROME C IN OXIDATION OF SULFUR-CONTAINING PYRIMIDINE NUCLEOSIDES AND SULFUR-CONTAINING RNAS

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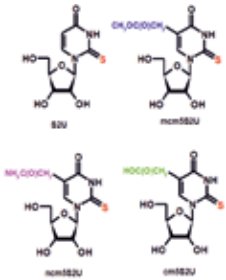
b Institute of Organic Chemistry, Lodz University of Technology, Lodz, Poland.

It has been shown recently that in the oxidative stress (defined as a disturbance in the balance between the production and removal of reactive oxygen species (ROS)), damage of various biomolecules, including RNA, takes place. The sulfur-modified nucleosides may be a primary site for the attack of ROS. Our earlier studies have shown that in the presence of hydrogen peroxide oligoribonucleotides containing 2-thiouridine (S2U-RNA) are particularly susceptible to oxidative desulfuration leading predominantly to a product containing 4-pyrimidinone nucleoside (H2U). The process depends on the concentration of the oxidant, pH of a buffer and on a substituent at C5 (1,2,3).



Recently, it was demonstrated that cytochrome c in the presence of H_2O_2 catalyzes oxidation of guanosine units in an RNA chain (4). Moreover, the resulting RNAox is prone for the formation of a covalent complex with cyt c. Since the desulfuration of S2U and the oxidation of guanosine undergo in the presence of H_2O_2, we combined these pieces of information and asked the question whether cyt c, to any extent, affects the process of S2U→H2U transformation. In the reported studies, treatment of 2-thio-modified substrate with H_2O_2 in the presence of cytochrome c in a HEPES buffer (pH 7.4), resulted in predominant formation of the H2U-containing product. Our current research demonstrates that cytochrome c catalyzes oxidative desulfuration of S2U unit at the nucleoside (2-thiouridine (S2U) and its 5-substituted derivatives (R5S2U, Figure 1)) and oligonucleotide (S2U-RNA) levels under conditions that mimic oxidative stress in the cell. The reaction proceeded at low concentrations of hydrogen peroxide and was much faster than the control reaction carried out in the absence of cytochrome c.
The role of cytochrome c in the RNA oxidation is of particular importance in the context of recent discoveries of a significant role of damaged forms of RNA in pathogenesis of various diseases, especially neurodegeneration and cancer.

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5.

SEQUENCE-SPECIFIC RNASE

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RNA is one of the leading actors in almost all life processes. Its catalytic prowess, biological importance and ability to form complex structures have made this molecule an important subject of research in the recent years. However, currently available methods for obtaining RNA molecules in the laboratory have many limitations, including high cost and labor intensity. It would be very useful to be able to produce various RNAs by cleaving and ligating other RNA molecules in a sequence-specific manner, similar to the manipulation of DNA with the use of restriction enzymes.

Currently, there are **no enzymes available for the purely sequence-dependent fragmentation of RNA**. A research group in IIMCB, led by prof. Janusz M. Bujnicki have created prototypic 'RNA restriction enzyme' based on BsMiniIII nuclease – a member of the RNase III family. The engineered RNase III-like enzyme BsMiniIII is the first known RNase, capable of **cleaving a long dsRNA substrate in a sequence-specific manner**. We introduced a new class of enzymes which has a broad spectrum of potential applications, and gives advantages over the existing solutions for RNA molecules production and manipulation. This innovative technology can be applied as a **diagnostic tool** e.g., for RNA cleavage, detection and characterization of specific RNA molecules, and in **RNAi research field** (siRNA and shRNA production).

NOTE: I am working at the IIMCB in the Laboratory of Bioinformatics and Protein Engineering as a researcher. Having good experience in molecular biology and protein biochemistry, I am interested in RNase technology and RNA-protein interactions. In order to investigate it, I employ various techniques, like protein engineering, crystallization and gene silencing.

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6.

DETERMINATION THE INTERLEUKIN-12 IN DENDRITIC CELLS IN RESPONSE TO TUMOR-DERIVED EXOSOMES

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Cancer is a multifactorial disease involving complex interactions between normal cells and cancer. Recent studies have indicated the possibility of using immunotherapeutic methods, which leads to enhanced immune response of the host against the cancer. These strategies include the use of cell-activating cytokines involved in immune response, such as Interleukin-12 (IL-12). The main sources of IL-12 in humans are activated antigen presenting cells such as dendritic cells as well as the phagocytic cells (macrophages). Interleukin 12 plays an important role in regulating both the innate and acquired immune response (high capacity to activate T cells and NK cells - natural killer). IL-12 alone can induce potent antitumor activity or act synergistically with several other immunoregulatory cytokines enhancing the antitumor activity of the body. One activity of the immune system is to recognize and destroy tumor cells. Tumors on the other hand by cell--cell contact and factors secreted into the extracellular space fight against the immune system to support the progression of cancer. It was shown that there is a mechanism of intercellular communication through exosomes. Exosomes contain miRNAs. These particles may impair the target cells by inhibiting the expression of certain genes. The result is a low level or complete loss of the protein. This kind of process may occur with the miRNA binding to targeted mRNA of IL-12, which can lead to the inhibition of further translation process. Therefore, exosomes secreted by tumor cells may be involved in the development of immunosuppression as a line of defense against the body's immune system response. However, the mechanisms underlying these processes are poorly understood.
We determine the level of interleukin 12 in the culture medium of cells of the immune system such as macrophages and immature and mature dendritic cells. The cells during activation for the production of IL-12 were treated with exosomes isolated from a melanoma cell line (A375). Determination of IL-12 in the culture medium of cells was performed by the method of Cytometric bead array (CBA). The results were processed using FlowCytomix Pro BD.
The results showed that incubation of activated cells with not incubated exosomes increased level of cytokines produced by the cells to the cell medium relative to the positive control (induction of IL-12). On the other hand, incubation with previously incubated exosomes exhibit an increased IL-12 level relative to the non-incubated exosomes and the positive control. Therefore, lowering the level of IL-12 may be a possible mechanism by which tumor cells acquire control of the immune system through suppressing the production of cytokines.

NOTE:
Cancer-derived exosomes and microvesicles in intercellular communication and immunosuppression research.

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7.

AN EVALUATION OF KNOWLEDGE IN SELECTED GROUPS REGARDING DIETARY SUPPLEMENTS CONTAINING POLYUNSATURATED FATTY ACIDS

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Long-chain ?-3 polyunsaturated fatty acids (LCn-3PUFA) in fish are the key nutrients for the benefits and are important for cardiovascular disease, anticancer prevention. The American Heart Association recommends eating fish at least 2 times a week. In the Polish population LCn-3 PUFAS deficits are strongly linked with food habits.

The aim of this study was the assessment of omega-3 and omega-6 supplements intake by adults (females and males). Four age groups i.e.: I: 18 – 30, II: 31 – 50, III: 51-64 and IV: > 65 have been examined using a questionnaire. Omega-3 and omega-6 fat acids play an important role in the metabolism, growing and functioning of the human body. The results of the study have shown that the knowledge about omega-3 and omega-6 application among the respondents is very good. However, most consumers do not pay attention to diet types in context of supplements intake. Interestingly, population knowledge about omega-3, omega-6 supply and demand is rather small. The information about diet supplements was obtained by the respondents via: friends, television, radio and the internet. Most of females and males have bought the mentioned products in pharmacies. Additionally, the intake of omega-3 and omega-6 diet supplements has been assigned as seasons dependent. Surprisingly, a higher supplements consumption has been observed in the autumn and winter seasons than in summer. From our point of view: it is important to inform the whole population about the cons and pros of omega-3 and omega-6 fat acids supplements, especially to pregnant and breast-feeding women.

NOTE: My current research activity is focused on the search for bioactive components in foods, their antioxidant activity, research of the relationship between the various components of food and human health. Additional an important element of my work is the study of the authenticity of the composition of dietary supplements.

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8.

CONJUGATED OLIGOELECTROLYTES – FLUORESCENT MEMBRANE DYES

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Biological fluorescence imaging is one of the most important, interdisciplinary field of research, involving chemistry, biology, life science and biomedicine, that has been widely used for various in vitro and in vivo applications. Bioimaging allows for understanding of some of cellular processes such as the cell-cell interactions and can lead to development of treatment for various human diseases, including cancers.

The good fluorescent agent should have properties such as excellent photostability, high quantum efficiency, tunable absorption-emission wavelengths, biocompatibility, low cytotoxicity, good solubility and potentially simple bio-conjugation for energy transfer capability. Many kinds of extensively employed fluorescent probes suffer from sensitivity to photobleaching or significant cytotoxicity. Given these limitations, search for better fluorescent probes is still an open challenging task.

One of the best candidates to meet the challenges for suitable fluorescent dyes are Conjugated OligoElectrolytes (COEs). This class of compounds possess a defined number of hydrophobic π-conjugated repeat units with delocalized electrons and are amphipathically functionalized by ionic pendant groups.

The representatives of conjugated oligoelectrolytes investgated by us, COEs based on the phenylene-vinylene (PV-COEs) or styrylo-naphthalene (SN-COEs) could be applied as a fluorescent membranous-specific dyes.

NOTE:
Scientific interests: bioimaging, cell biology, cell cultures

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9.

CHARACTERIZATION OF RECOMBINANT TRNA 2-SELENOURIDINE SYNTHASE (SELU) FROM ESCHERICHIA COLI

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The C5 substituted 2-thiouridines (X5S2U) present in tRNAGlu, tRNAGln and tRNALys can be converted into corresponding 2-selenouridines (X5S2U→X5Se2U) (1,2) or S-geranyl-2-thiouridines (X5S2U→ X5geS2U) (3) in the reactions catalyzed by tRNA 2-selenouridine synthase (SelU). In the known biological examples, the enzyme utilizes the same (c)mnm5S2U substrate, but chemically these two reactions are remarkably different and to date neither a single nor a dual coherent catalytic mechanism was proposed. Our considerations brought a hypothesis that SelU actually catalyzes two consecutive reactions X5S2U→X5geS2U→X5Se2U, so the C5 substituted S-geranyl--2-thiouridine is the intermediate in this conversion. We undertook efforts to prepare active SelU in amount sufficient to verify this idea.

The selU gene was amplified from the total bacterial RNA and cloned into a pET28c expression vector. The overexpression of SelU protein was performed in the E. coli BL21 Star DE(3) strain using two methods of induction: (1) by IPTG and (2) auto-induction system. The enzyme was purified using the HisPure Cobalt Agarose resin that resulted in a >90% pure preparation (~1.5 mg/l of culture medium). The purified SelU protein (containing the C-terminal His6 tag) exhibited expected enzymatic activity being able to convert oligoRNA-S2U→ oligoRNA-geS2U with ~11% efficiency (confirmed by RP-HPLC and MALDI-TOF mass spectrometry analysis). Additionally, we found an essential dependence of the SelU enzymatic activity on the presence of magnesium ions in the reaction environment.

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10.

OCCURRENCE AND RECURRENCE OF UROTHELIAL CELL CARCINOMA IN THE LIGHT OF MUTATION ANALYSIS

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INTRODUCTION & OBJECTIVES
We are still searching an appropriate markers for monitoring bladder cancer recurrence and response to treatment. The aim of our study was to determine the genetic diversity of bladder cancer and to evaluate the usefulness of the proposed molecular markers panel to bladder cancer patients monitoring.
MATERIALS & METHODS
Genetic diversity of the bladder cancer was evaluated in 185 tumor DNA samples by the mutations prevalence in TP53, HRAS, FGFR3 and WWOX genes. Mutations has been detected using the PCR-MSSCP and DNA sequencing methods. Assessment of the suitability of the selected markers as a non-invasive test was performed in 38 cases of urinary sediment collected from patients enrolled in the earlier studies in tumor tissue.
RESULTS
Mutations were detected in 62.2% of the tumor samples. The most fre¬quently mutated genes were FGFR3 (49.7%) and TP53 (16.2%). No mutation was observed in WWOX gene. FGFR3 mutations, contrary to TP53, correlated with lower tumor stage and grade, as well as multiple tumors. The risk of death was significantly higher in patients with mutations in TP53 gene (HR=3.12; 95%CI: 1.14-7.27; p=0.006) in opposite to patients with mutations in FGFR3 gene (HR=0.36; 95%CI: 0.15-0.87; p=0.0022). None of the investigated genes were an independent predictor of disease--specific survival, recurrence-free survival or progression-free survival. The sensitivity of urine sediment examination using the proposed panel of molecular markers was 39.3%.
CONCLUSIONS
The results confirm the existence of two alternative pathways of bladder cancer, but
a high percentage of wild type variants in the higher stages of the disease suggesting the existence of another pathway of molecular changes leading to tumor development. Implementation of molecular analy¬sis may have prognostic value and may facilitate the classification of patients in their own appropriate forms of treatment - especially in the case of patient with T1 tumor (in each grade were observed different mutational pattern).

NOTE:
Magdalena Traczyk-Borszyńska PhD, MSc, laboratory diagnostician. She joined the Department of Clinical Genetics staff at Medical University of Lodz in 2008. Her research focuses on molecular biology of cancer - especially molecular mechanisms of bladder cancer development.

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11.

THE RELATIONSHIP OF INDUCIBLE NITRIC OXIDE SYNTHASE (NOS2) AND SURFACTANT PROTEIN D (SP-D) WITH GESTATIONAL DIABETES MELLITUS (GDM)

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OBJECTIVE
The linkage of the immune system with metabolism has been recognized in gestational diabetes mellitus (GDM), but molecular factors underlying this relationship still are awaiting identification. The purpose of this study was to investigate whether there are changes in the expression of the two well-known immune-related genes such as NOS2, encoding inducible nitric oxide synthase, and SFTPD, encoding surfactant protein D, in leukocytes obtained from patients with GDM at the third trimester of gestation and, moreover, whether these alterations are related to clinical characteristics of patients.
RESEARCH DESIGN AND METHODS
Leukocytes were isolated from the blood of normal glucose tolerant (NGT; n = 38) and GDM (n = 87) pregnant women between 24 and 33 weeks of gestation. Leukocyte NOS2 and SFTPD mRNA expression was determined by quantitative real time PCR (qRT-PCR). Univariate correlation analyses were performed to investigate associations of the expression of NOS2 and SFTPD with clinical characteristics of patients.
RESULTS
Compared to NGT pregnant controls, leukocyte NOS2 and SFTPD mRNA expression was significantly higher in hyperglycemic GDM patients ($p<0.05$). In the entire study group, there were significant positive associations of leukocyte NOS2 and SFTPD mRNAs with C-reactive protein. Additionally, transcript level of SFTPD also correlated positively with fasting glycemia and insulin resistance ($p<0.05$).
CONCLUSIONS
This study demonstrates that an impaired glucose metabolism in GDM may be predominant predictor of leukocyte NOS2 and SFTPD overexpression in diabetic patients. Furthermore, alterations in the expression of these genes are associated with glucose metabolism dysfunction and/or inflammation during pregnancy. In addition, these findings support the utilization of leukocytes as good experimental model to study a relationship between immune-related genes and metabolic changes in women with GDM, as well as to assess the potential mechanisms underlying these alterations.

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