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The road to Stockholm: from 2-4D to kuru prions

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In 1939 at the Boyce Thompson Institute in Yonkers, New York, a 16-year-old high school student was hired as a summer helper by a plant physiologist, Percy Zimmerman. The helper, who was paid 40 cents/hour, successfully synthesized 2 – 4D, described in Chemical Abstracts as a possible cure for athlete's foot. There was no record made of the name of the summer helper, who left after four weeks and was forgotten. Zimmerman applied 2 – 4D to broad-leafed plants. They grew so rapidly that they collapsed and died. The first weed killer was thus discovered. It was patented by Zimmerman and the license sold to Dupont Co. in Wilmington, D.E. When I visited Zimmerman in 1954, I inquired how he got the idea of using 2 – 4D. When he mentioned the forgotten summer helper, I decided to search and find what happened to this bright student. It took me many years, before Irene Dobroscky, a former associate of my mentor L.O. Kunkel, visited me at Rockefeller University and told me that the summer helper of Zimmerman was her nephew, the famous virologist D. Carleton Gajdusek. In 1970, I met Gajdusek and when I greeted him as "the discoverer of 2 – 4D", he thought that I confused him with someone else. I reminded him of his first employment at the Boyce Thompson Institute. He requested that I send him the names of the scientists who worked there in 1939.We became close friends. In 1976, when Gajdusek received the Nobel Prize in Stockholm, he told about his aunt and his synthesizing of 2 – 4D, of which I had reminded him a few years earlier.

Gajdusek received his Nobel Prize for the discovery of the infectious agent causing the kuru disease of fore people in the highlands of New Guinea. Although cannibalism was banned there years earlier, brains of deceased fore family members were smeared on the faces of women and children, then cooked, and eaten. Gajdusek send sampled of kuru brains to his laboratory at the National Institutes of Health in the United States. After two years, chimpanzees inoculated with kuru brain extracts developed signs closely resembling human kuru. The disease in people and chimpanzees is always fatal. Gajdusek called the infectious agent a "slow virus", resembling the infectious agents of Creutzfeldt-Jacob human dementia and scrapie of sheep. In 1974 Stanley Prusiner started to work with scrapie of sheep and found that "slow viruses" contain neither DNA nor RNA and consist of self-duplicating, twisted

protein. Using the first letters of his name, he coined the word “prion” for “slow viruses”. Prusiner won the Wolf Prize in 1996 and the Nobel Prize in 1997.
Gajdusek brought the first New Guinea aborigines to the United States. One, of the Anga tribe, arrived barefoot, with a stick through his nose. A total of 56 children from Micronesia were adopted by Gajdusek. He put his adopted sons through schools, colleges, and a few through medical schools, using his Nobel award and his own salary for their support. In 1996, Gajdusek was accused of child molestation by one of his sons, at that time a 3rd year chemistry student at College Park, MD, who testified that, some 20 years earlier, Gajdusek abused him on his native island. Sentenced and jailed for one year, the 74 year old brilliant scientist left permanently the United States for Europe where he was received with open arms.
He lived during summers in Amsterdam, and winters beyond the Arctic Circle in Tromse, Norway. He said that when it was dark during the 24-hour days, he could do more writing. In addition to more than 600 refereed papers, he wrote his diaries and donated the hard-bound mimeographed volumes to his close friends and a few libraries. He died in Tromse on December 12, 2008.

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Cardiovascular disease (CVD) risk factors for women a Life Events-Course Perspective

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Cardiovascular disease (CVD) in women is the most common cause of death and in 2009 accounted for one third of all deaths. The purpose of this paper is to present what conditions during pregnancy and during the pre-menopause period lead to a greater risk of CVD. The early recognition and the application of interventions may decrease this risk. To emphasize this point we have taken a "Life Events-Course Perspective". Current data suggests that genetic predisposition to disease in conjunction with behavior and environmental factors during fetal life is related to permanent changes in fetal-placental-maternal physiology and function, resulting in fetal programming characterizing the phenotype of the child which may persist into adulthood. Longitudinal studies have identified biological, behavioral and environmental factors related to childhood diseases such as hypertension, insulin resistance and mental health disorders. Gender differences have been identified and animal studies have suggested that estrogens in women are protective and when the risk of CVD in men is considered, the risk in women is delayed by 10 years. Thus, a normal pregnancy may be protective and reduce the risk of CVD in women. However, hypertension developing in women before or during pregnancy is a significant risk factor for women and diabetes further increases this risk of CVD, as does smoking. It is very clear that an "intervention action plan" must be developed. It is the current opinion of the authors that this action plan must be implemented early in life to decrease the risk for the development of CVS in women.

Vitamin D – a novel role in pregnancy

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Vitamin D regulates placental development and function. It is a potent regulator of the immune system-stimulating antimicrobial responses while suppressing inflammation. Its deficiency has been linked to increased risk of serious chronic and inflammatory diseases. Vitamin D deficiency during pregnancy increases susceptibility to infection and inflammation, leading, in turn, to outcome like preterm birth or preeclampsia. Pregnant women with darker skin pigmentation are more likely to be vitamin D deficient, particularly when living in regions with low exposure to sunlight. It is possible that during pregnancy, a primary non-infectious inflammatory process is activated by vitamin D deficiency. Combined assessment of vitamin D deficiency and inflammatory markers in early pregnancy or during different stages of pregnancy may facilitate the recognition of the risk of complications.

Vitamin, mineral and iron supplementation in pregnancy: cross-sectional study

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Aim: To assess the use of vitamin, mineral and iron supplements during pregnancy in Zagreb and Novi Sad. **Methods**: The study was conducted by use of a structured standardized questionnaire consisting of two parts, i.e. data obtained by maternal interview and hospital records. It is designed as a cross-sectional study in two countries (Croatia and Serbia). The study included 893 pregnant women from Zagreb and 6099 pregnant women from Novi Sad. **Results**: In Zagreb, pregnant women reported highest utilization of vitamin-mineral supplements (n=508; 56.9%), whereas in Novi Sad these supplements ranked third (n=408; 20.3%), following tocolytics and iron supplements. There was no statistically significant difference in the prevalence of congenital malformations between neonates at in utero exposure to vitamins, minerals and iron supplements and those without such exposure in either Zagreb or Novi Sad arm, with the exception of iron and calcium supplementation in the Zagreb arm. **Conclusions**: In spite of certain study limitations, the results obtained pointed to the unreasonable and potentially harmful use of these supplements in pregnant women from Zagreb.

Amniotic fluid Pentraxin 3 as a new marker of subclinical chorioamnionitis in women with preterm premature rupture of membranes

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Pentraxins are a superfamily of proteins that are phylogenetically highly conserved in evolution. Based on the primary structure of subunits, pentraxins are divided into short and long pentraxins. C-reactive protein and serum amyloid P component are classic short pentraxins. The prototype of the long pentraxin family is pentraxin 3 (PTX3) which is rapidly produced and released by several cell types upon activation, in particular, by macrophages, dendritic cells, fibroblasts, and endothelial cells in response to proinflammatory signals. In addition, PTX3 is expressed in amniotic epithelium, chorionic mesoderm, trophoblast terminal villi, and perivascular stroma of placentas. The purpose of this study was to evaluate amniotic fluid concentration of PTX3 in patients with preterm premature rupture of the membranes and to determine whether amniotic fluid PTX3 concentrations are of value in the identification of patients with subclinical histological chorioamnionitis. Forty pregnant women with PPROM between 24 and 36 gestation weeks without (n = 21) and with (n = 19) histological chorioamnionitis (PPROM group) and 42 women between 16 and 20 gestational weeks (mid-trimester group) were included in the study. We compared amniotic fluid PTX3 levels in the PPROM group with versus without histological chorioamnionitis, and between the PPROM and the mid-trimester groups. Patients with subclinical histological chorioamnionitis had a significantly higher median amniotic fluid PTX3 concentration than patients without the histological signs of chorioamnionitis (3.69 ng/mL, 0.51 – 106.8 versus 0.8 ng/mL, 0.36 – 121.0; p = 0.015). Patients in the PPROM group reached a significantly higher median amniotic fluid concentration of PTX3 compared with those in the mid-trimester group (1.0 ng/mL, 0.36 – 121.0 versus 0.67 ng/mL, 0.4 – 2.8; p = 0.007). Subclinical histological chorioamnionitis is associated with a significant increase of amniotic fluid PTX3 levels.

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TIEG1 and TWIST1 integrate proinflammatory and BMP effects on the skeleton

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Impaired bone homeostasis contributes to development of osteopenia, osteolysis and joint erosions during the rheumatoid arthritis (RA). Bone morphogenetic proteins (BMP) are crucially important regulators of osteogenesis. Activation of specific BMP receptors (BMPR) leads to activation of the major BMP signaling pathway, namely intracellular Smad proteins, as well as other, Smad-independent, pathways. Using *in vitro* tissue culture approaches we show that activation of NF-κB pathway with proinflammatory cytokines IL-1β and TNFα inhibits osteogenic differentiation of pluripotent mesenchymal precursor cells through Smad7-independent inhibition of Smad1/5 transcriptional activity. Immunoblot and EMSA experiments show that neither Smad1/5 phosphorylation by BMPR-Is, nor direct Smad1/5 binding to DNA into BMP target genes promoters are affected by the activation of NF-κB pathway with TNFα, or by the overexpression of NF-κB signaling components. Nevertheless, Smad1/5 transactivation and, consequently, transcription of BMP target genes is greatly reduced upon activation of NF-κB signaling. Neither ectopic expression of Smad1/5, nor CBP/p300 can rescue the negative effect of NF-κB pathway activation. We used Real time PCR to analyse BMP and TNFα target genes mRNA induction in the presence of protein synthesis cycloheximide and found that negative effect of NF-κB activation requires new protein synthesis due to the induction of BMP signaling inhibitor expression. Futhermore, we found two distinct TNFα target genes that are novel potent inhibitors of BMP signaling. One of them, TWIST1 is a transcriptional target of NF-κB and has been implicated into repression of RUNX2 driven osteogenesis. Another one, KLF10/TIEG is induced by TNFα in NF-κB-independent manner. shRNA-mediated knockdown of the expression of each of these BMP signalling repressors results in partial rescue of BMP-Smad-driven transcription from inhibition by TNFα. We generated crosses of BMP reporter mice with p65/RelA knockout mice and found that NF-κB (most likely, via TWIST1) controls the intensity and the duration of BMP signals *in vivo* already during the embryogenesis. Thus, our data demonstrate TIEG1 and TWIST1 as transcriptional repressors of BMP-Smad signalling and as the central candidates responsible for proinflammatory control of osteogenic program possibly also involved in the development of osteolysis and joint erosions during the RA.

Oxidative stress, advanced glycation end products and residual renal function in the rat model of unilateral ureteral obstruction: effects of Phlogenzym and losartan

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Aim: Oxidative stress plays a role in the pathogenesis of ureteral obstruction. **Methods**: We studied parameters of oxidative status, levels of advanced glycation end products (AGEs), and contralateral (CL) kidney function in the rat model of unilateral ureteral obstruction (UUO). The effects of Phlogenzym (12 mg/d orally); losartan (20mg/l in drinking water), and their combination was studied. **Results**: In placebo-administered UUO rats AGEs and malondialdehyde levels were higher than in the sham operated controls. Function of the CL kidney was slightly impaired, its collagen content and protein/deoxyribonucleic acid ratio (P/DNA) in the glomeruli increased. All treatments prevented the rise in collagen content, P/DNA ratio, and improved CL kidney function. Phlogenzym ameliorated lipid peroxidation and AGE levels. **Conclusions**: In the model of UUO systemically increased oxidative stress may play a role in development of tubulointerstitial fibrosis and in the functional impairment of the CL kidney. Suppression of the oxidative stress and blockade of angiotensin-1 receptors might mitigate the progression of obstructive uropathy.

The quest for the ganglioside functions; what did we learn more from 'evo-devo' or signaling of long-term maintenance?

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Gangliosides are characteristic extracellular-facing plasma membrane determinants in vertebrate brain. The four major gangliosides (GM1, GD1a, GD1b and GT1b) dominate among more than one hundred glycolipid structures in nervous tissue. During brain development the expression of simple gangliosides shifts toward more complex ones, accompanied by a multiple increase in their total amount. The shift is precisely regulated and some specific structures represent well established neurodevelopmental milestones.
From the evolutionary perspective, the ganglioside content in fish and amphibian brain is significantly lower than in mammalian brain, but the general variability is greater. More-polar structures, abundant in Antarctic fishes, are rare in higher vertebrates or expressed only in a narrow developmental frame. Reptiles, birds and mammals share identical common structures expressed in similar patterns with minor interspecies differences. On the contrary, fish and amphibian brains show significant interspecies differences in amount, structure and expression patterns.
The initial assumption of evolutionary studies was that the variations in lipid content, particularly the glycolipid content, during temperature adaptations in ectothermic and hibernating heterothermic animals, represent an efficient molecular mechanism of the membrane function preservation. Studies of ordered lipid domains in the last decade verified the ganglioside-mediated regulation of membrane proteins (receptor kinases, neurotransmitter receptors and ion channels) as well as receptor-ligand interaction important for cell signaling.

Functional expression of ion channels in developing human dendritic cells

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Modulation of the expression and activity of plasma membrane ion channels is one of the mechanisms how immune cell can regulate their intracellular Ca2+ signaling pathways required for proliferation and/or differentiation. Dendritic cells (DCs) function as professional antigen presenting cells and participate in the initiation of adaptive immune response. Human monocyte-derived DCs have two developmental phenotypes: immature DCs (IDCs) take up and process foreign antigens, while mature DCs are able to trigger T cells in the lymph nodes. Our study aimed at the identification and characterization of ion channels expressed during the course of human DC differentiation.

Human myeloid DCs were generated from monocytes isolated from peripheral blood mononuclear cells by positive selection with anti-CD14-coated magnetic beads. To generate IDCs cells were cultured in the presence of IL-4 and GM-CSF. Maturation of IDCs into MDCs was induced by an inflammatory cocktail containing TNF-α, IL-1β, IL-6, and GM-CSF. Ion currents of IDCs and MDCs were recorded using the whole-cell patch-clamp technique. The biophysical, pharmacological and molecular biological properties of the channels were determined and used for the classification of the expressed channels.

We report here the first time that IDCs express voltage-gated Na+ channels in their plasma membrane. The parameters characterizing voltage-dependent gating (activation and inactivation) and the TTX sensitivity of the current and PCR-based cloning revealed the presence of Nav1.7 channels in human IDCs. Transition from the immature to the mature state, however, was accompanied by a down-regulation of Nav1.7 expression concomitant with the expression of a K+ current having biophysical characteristic of a voltage-gated Kv1.3 current. The expression of Kv1.3 channels by MDCs was confirmed by high affinity block of the current by margatoxin, a selective inhibitor of Kv1.3 channels, and by PCR-based cloning. The presence of Kv1.3 channels seems to be common for immune cells; hence, selective Kv1.3 blockers may emerge as candidates for inhibiting various functions of mature DCs that involve their migratory, cytokine secreting and T-cell activating potential.

Synthetic biology and gene therapy (in Weigl's footsteps)

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My own concepts of the Synthetic Biology and Gene Therapy evolved gradually between the 1940's, 1970's and beyond. It all started when I was born in 1921 in Lwow, and was influenced by my teachers and the pioneering biological research of Professor Rudolf Weigl, also being trained as a chemical engineer at the Politechnika Lwowska. It would be difficult for me to recall all the lectures and seminars where I have introduced, described and used my novel term of Synthetic Biology, but some examples were found and preserved by various reviewers and also by the Editors of Wikipedia, since under the entry, "**Synthetic Biology**" it says:

"In 1974, the Polish geneticist Waclaw Szybalski introduced the term "synthetic biology"[1], writing: Let me now comment on the question "what next". Up to now we are working on the descriptive phase of molecular biology. ... But the real challenge will start when we enter the synthetic biology phase of research in our field. We will then devise new control elements and add these new modules to the existing genomes or build up wholly new genomes. This would be a field with the unlimited expansion potential and hardly any limitations to building "new better control circuits" and finally other "synthetic" organisms, like a "new better mouse". I am not concerned that we will run out of exciting and novel ideas, in the synthetic biology, in general. When in 1978 the Nobel Prize in Physiology or Medicine was awarded to Arber, Nathans and Smith for the discovery of restriction enzymes, Waclaw Szybalski wrote in an editorial comment in the journal Gene: The work on restriction nucleases not only permits us easily to construct recombinant DNA molecules and to analyze individual genes, but also has led us into the new era of ***synthetic biology*** *where not only existing genes are described and analyzed but also new gene arrangements can be constructed and evaluate[2].*

If we use 1974 as the start point, the Synthetic Biology is presently 35 years old".

Let me list here several examples of experiments performed by myself or in my laboratory, which at that time, I thought, would represent various modes of chemical organic or enzymatic DNA synthesis DNA in association with the living organisms. These included various method of transferring biologically active DNA, necessary biological and other assays, physical mapping and sequencing of DNA, cutting and splicing of genomes, and modifying or creating of novel organisms.

1) We chemically modified DNA by replacing thymine with halogenated analogues, and we were first to prove that such DNA retains its biological transforming activity. That convinced me that DNA could be chemically manipulated, even by an organic chemist, like myself, or by my collaborators, late Stefan Zamenhof. Zofia Opara-Kubinska and Erela Elizur.

2) Another chemical manipulation, the total enzymatic synthesis of DNA, was first described by the late A. Kornberg, but there was no proof that synthesis in ***highly purified*** system leads to a 'life-like' DNA. Thus, in cooperation with the late Rose Litman and using a more ***crude*** enzymatic system, but in conjunction with very sophisticated, at that time, method of quantitative separation of the template from the newly synthesizes DNA, I and the late Zofia Opara Kubinska we were able to prove that DNA synthesized by Rose was biologically alive, as determined by the transforming activity. Probably, it was the first proof for the synthesis of life in the test tube.

3) To be able to test DNA not only in bacteria, but also in eukaryotic systems, we have designed a very novel selective system, named by my wife HAT, which for the first time permitted us to transfect the human cells with purified DNA. To do that, we produced deletions in the *HPRT* gene, which corresponded to the "synthetic Lesch-Nyhan syndrome", which incidentally, was not discovered until 1964. Transfection with the $HPRT^+$ DNA yielded the $HPRT^+$ transfectants, while this operation corresponded to the in vitro gene therapy of the cells with a "synthetic" Lesch-Nyhan syndrome.

(4) Ultimately, our HAT section procedure has permitted us also to develop hybridoma cells by the fusion of my own $HPRT^+$ epithelial cells and our laboratory bone marrow D98/AH cell line that was $HPRT^-$. Ultimately, that led others to the new field of monoclonal antibodies (mAbs), that could be also considered as a **synthetic** product.

(5) The restriction endonucleases provide a powerful tool to engineer DNA *in vitro* leading to assembly various combination of genes. My laboratory was quite active in this area, also inventing new tricks and creating novel '**synthetic**' enzyme activities.

(6) That led to a new huge field of recombinant DNA (reDNA) and new kinds of the biotechnological industries.

(7) Among most important of our tools for physical mapping the synthetic genomes were electron microscopy of DNA heteroduplexes supplanted later by the outright DNA sequencing.

As an aside, we attempted in 1962 to synthesize new kind of 'viruses', which mimic the properties of the scrappie-like viruses that are an example of the infectious protein. As model, we used termination (***t***)/anti-termination (***nut***) elements of our DNA phage lambda and the N protein.

The field of the Synthetic Biology (SB) is now well established with various attempts to produce synthetic organisms almost totally from scratch, whereas Gene Therapy has still long way to go as to become a standard clinical procedure.

Materials for rational nanomedicine design and enginnering

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Applications of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems has recently been referred to as “nanomedicine” by the National Institutes of Health. Research into the rational delivery and targeting of pharmaceutical, therapeutic, and diagnostic agents is at the forefront of projects in nanomedicine. These involve the identification of precise targets (cells and receptors) related to specific clinical conditions and choice of the appropriate nanocarriers to achieve the required responses while minimizing the side effects. Mononuclear phagocytes, dendritic cells, endothelial cells, and cancers (tumor cells, as well as tumor neovasculature) are among the key targets. Today, nanotechnology and nanoscience approaches to particle design and formulation are beginning to expand the market, particularly for the anticancer drugs, and are forming the basis for a highly profitable niche within the industry, but some predicted benefits are hyped. This lecture will highlight rational approaches in design and surface engineering of nanoscale vehicles and multifunctional entities for site-specific drug delivery and medical imaging after parenteral administration. Potential pitfalls or side effects (e.g., cytotoxicity and adverse immune reactions) associated with nanoparticles and their constituents will be discussed at the molecular level.

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Metabolic engineering of microorganisms for construction of the efficient producers of pharmaceutically important metabolites and proteins

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Currently, industrial microbial producers of pharmaceutically important metabolites and heterologous proteins are isolated by methods of metabolic engineering. This will be illustrated by examples based on works carried out in Institute of Cell Biology. Thus, efficient producers of riboflavin (vitamin B2) and flavin nucleotides FMN and FAD have been constructed based on metabolically engineered yeast *Candida flareri* (*Candida famata*). The stable riboflavin overproducers were constructed by means of amplification of the gene encoding the positive regulator of riboflavin synthesis SEF1, structural genes of riboflavin biosynthesis pathway RIB1 and RIB7 and gene of purine nucleotide interconversion IMH3 into selected earlier *C. flareri* flavinogenic strains AF4. Overproducers of FMN and FAD de novo have been isolated for the first time by overexpression of genes FMN1 and FAD1 coding for riboflavin kinase and FAD synthetase, respectively, in the mentioned riboflavin producer C. flareri AF4. The medium composition and cultivation conditions were optimized for maximal accumulation of riboflavin, FMN and FAD. Microbial producers of anticancer enzyme arginine deiminase (ADI) from pathogenic bacterium *Mycoplasma hominis* have been constructed in *Escherichia coli*. To construct efficient producer of ADI, the corresponding gene was isolated from total DNA of M. hominis. The codon optimized gene was expressed in Escherichia coli cells. ADI expression level consisted of at least 25% of the total bacterial proteins. The homogenous recombinant ADI with specific activity of 18 U/mg protein were obtained. Besides, the producers of human arginase (hARG1) have been constructed in the methylotrophic yeast *Hansenula polymorpha*. For the first time, overproduction and efficient secretion of catalytically active hARG1 were achieved in yeasts. Optimization of fermentation protocols and development of simple purification procedure for hARG1 were carried out. As anticancer enzymotherapy based on arginine deprivation is currently been vigorously developed, we expect the growing demand in preparations of recombinant arginine-degrading enzymes in near future, both for laboratory and clinical studies. In parallel, the work is in progress on the design of novel efficient combined therapies that utilize recombinant hARG1 on different tumor models.

Novel functionalized nanocomposites: molecular design, synthesis, and biomedical application

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Novel functionalized nanocomposites (NC) were developed as a result of tight collaboration between the Department of Organic Chemistry at LNPU and the Department of Regulation of Cell Proliferation and Apoptosis at the ICB. In order to create the basic platform for these NC, the polymeric surface-active oligoelectrolytes were designed and synthesized [1]. The developed technology permits to control: 1) the quality and quantity of structural blocks of the NC, and the unimodality of their size (1.5-6.0 kDa); 2) branching of the polymer chain at specific sites (if necessary); 3) providing the polymer with specific chemical groups (hydroxyl, carboxyl, amino, aldehyde, epoxy, others); 4) covalent conjugation of specific bio-targeting molecules via these groups. The following molecules were used as bioactive element of the developed NC: a) specific anticancer drugs, antibiotics, alkaloids; b) DNA and siRNA; c) immunoglobulins and lectins; d) low molecular compounds (lipids, amino acids); e) polyethylene glycol. To make these NC detectable and measurable, fluorescent and other dyes [2], as well as super-paramagnetic core were utilized. Biocompatible NC possessing low toxicity towards mammalian cells *in vitro* and *in vivo* (experimental mice) were selected. These NC were applied in the form of micelle materials or nanoparticles for delivery of: 1) drugs (ex. doxorubicine and levomycetin) during chemotherapy *in vitro* and *in vivo*; 2) DNA (transfection of mammalian, yeast and bacterial cells) and siRNA (blocking gene expression *in vivo*); 3) protein antigens at animal immunization [3]. Novel technologies were also developed for detecting and measuring apoptotic (dying) mammalian cells via recognizing specific cell surface glycoprotein(s) by means of specific lectin conjugated with the NC used in the form of nanoparticles [4]. Similar NC were applied for targeted action towards apoptotic human cells (cell isolation and destruction). Apoptosis supports cell homeostasis in norm and is responsible for tissue damage in pathology. It is also involved in chemotherapy and radiotherapy. Thus, the developed nanotechnologies can be important in diagnostics and monitoring of treatment.

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Multifunctional magnetic nano/microparticles for bioapplications

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Rapidly growing interest in magnetic nano- and microparticles is determined by the progress in nanotechnology in medical diagnostics and treatment including magnetic targeting, drug delivery and hyperthermia. For example, superparamagnetic iron oxide nanoparticles (~ 10-30 nm in size) were developed to label the transplanted cells in order to non-invasively visualize their survival, migration, homing and fate in magnetic resonance imaging (MRI) which is of key importance for success of cell therapy and regenerative medicine. The efficacy of iron oxide nanoparticles depends mainly on their physicochemical properties, particularly on their size and surface chemistry. Examples of successful surface modification of iron particles for labeling of various types of cells will be given. On the other side, magnetic microspheres (~ 1-3 μm in size) play important role in immobilization of proteins, peptides and enzymes, bioseparation applications, biosensors and so on. In the design of magnetic microspheres suitable for the detection of circulating tumor cells in peripheral blood, we have developed magnetic poly(2-hydroxyethyl methacrylate)-based microspheres containing carboxyl groups suitable for subsequent attachment of biomolecules. Requirements laid on such microspheres include also complete encapsulation of iron oxide to avoid its contact with the environment, high iron oxide content to get high magnetization, no particle aggregation in physiological media, monodisperse size to have uniform physicochemical properties, low non-specific adsorption of proteins and low autofluorescence. Such particles will be described in more detail.

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Structure – anticancer activity relationships among 4-azolidinone-3-carboxylic acids derivatives

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The **aim** of present research was investigation of anticancer activity of 4-azolidinone-3-carboxylic acids derivatives, and studies of structure-activity relationships (SAR) aspects. **Methods**: organic synthesis; spectral methods; anticancer screening was performed according to the US NCI protocol (Developmental Therapeutic Program). **Results**. The data of new 4-thiazolidinone-3-alkanecarboxylic acids derivatives in vitro anticancer activity were described. The most active compounds which belong to 5-arylidene-2,4-thia(imida)zolidinone-3-alkanecarboxylic acids; 5-aryl(heteryl)idenerhoda-nine-3-cuccinic acids derivatives were selected. Determination of some SAR aspects which allowed to determine directions in lead-compounds structure optimization, as well as desirable molecular fragments for design of potential anticancer agents based on 4-azolidinone scaffold were performed. 5-Arylidenehydantoin-3-acetic acids amides were identified as a new class of significant selective antileukemic agents. Possible pharmacophore scaffold of 5-ylidenerhodanine-3-succinic acids derivatives was suggested. **Conclusion**. The series of active compounds with high anticancer activity and/or selectivity levels were selected. Some SAR aspects were determined and structure design directions were proposed.

Identification and characterization of tumor-associated antigens for cancer diagnostic and therapy

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The discovery of genes that are potential cancer-associated antigens and markers provides valuable insight into cancer biology and might lead to the development of more effective treatment strategies for combating this disease. In our study we applied several approaches for the identification novel tumor markers. Ovarian cancer is the most common gynecologic malignancy that usually becomes far advanced before it is diagnosed. So far, only few tumor-associated markers and antigens specific for ovarian cancer have been identified. MX35 antigen is one of them. MX35 specific Mabs developed against ovarian cancer showed homogeneous reactivity with approximately 90% of human ovarian epithelial cancers and with a limited number of normal tissues by immunohistochemistry. Although mAb MX35 has been used in a number of clinical trials in ovarian cancer, the molecular identity of MX35 was unknown. I our study we have received strong evidence that antigen recognizes by mAb MX35 corresponds to the sodium-dependent phosphate transport protein 2b (NaPi2b). This conclusion is based on several lines of experimental evidence, including 1) the identification of SLC34A2, the gene coding for NaPi2b, by immunoscreening an ovarian cancer cell line cDNA expression library with mAb MX35; 2) mass spectrometry sequencing of peptides obtained from mAb MX35 affinity-purified antigen; 3) selective down-regulation of SLC34A2 gene expression by RNA interference and the resulting loss of mAb MX35 binding to MX35-expressing cells; and 4) the demonstration of specific mAb MX35 reactivity with recombinant fusion proteins and with synthetic peptides of the putative largest extracellular loop of NaPi2b. We believe that membrane transporter molecules, such as NaPi2b, represent a new family of potential cell surface targets for the immunotherapy of cancer with monoclonal antibodies.

The role of adaptor/scaffold protein Ruk/CIN85 in carcinogenesis

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The adaptor/scaffold protein Ruk/CIN85 was implicated in carcinogenesis by influencing a number of processes such as cell adhesion, motility and apoptosis. Although Ruk/CIN85 appears to modulate tyrosine kinase receptors and PI3 kinase signalling, the exact molecular mechanisms by which Ruk/CIN85 affects carcinogenesis are largely unknown. Therefore, we investigated the oncogenic potential of Ruk/CIN85 by overexpressing the full-length isoform in weakly invasive MCF-7 breast adenocarcinoma cells. The Ruk_l/CIN85 overexpressing cells showed a slower growth rate, decreased cell adhesion, and an enhanced anchorage-independent growth in soft agar. Further, overexpression of Ruk_l/CIN85 also affected EGF-dependent signalling: activation of both Akt and ERK1/2 was faster than in the control cells and both kinases remained in their active state for up to 30 min after EGF treatment. Transwell migration and wound healing assays revealed that Ruk_l/CIN85 overexpressing cells possessed increased motility. The EGF-induced motility was attenuated in Ruk_l/CIN85-overexpressing cells but could be restored upon knock-down of Ruk_l/CIN85 with specific shRNA. Together, these findings suggest that high levels of Ruk_l/CIN85 can modulate EGF-dependent signalling and contribute to the conversion of breast adenocarcinoma cells into a more malignant phenotype.